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Original Article

# Comparison of anthropometric, cardiovascular, autonomic, baroreflex sensitivity, aerobic fitness, inflammatory markers and oxidative stress parameters between first degree relatives of diabetes and controls

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ABSTRACT

*Aim:* We aimed at assessing cardiovascular risk of first degree relatives of diabetes (FDRD).
*Methods:* A cross sectional study involving 90 apparently healthy normoglycemic volunteers aged between 15 and 50 years (45 FDRD and 45 FDRs of non-diabetics). We measured anthropometric parameters, baroreflex sensitivity, heart rate variability, cardiac autonomic function tests, and aerobic capacity, fasting blood glucose and insulin, lipid profile, inflammatory markers, nitric oxide and oxidative stress markers.
*Results:* FDRD had significantly higher hip circumference and BF%. Blood pressure, total peripheral resistance and cardiac output were comparable. FDRD had higher HR and rate pressure product. There were no significant differences in cardio-respiratory fitness (VO2max) and physical activity level. Time and Frequency domain parameters were comparable except for reduced NN50 and total power. Baroreflex sensitivity, 30:15 ratio and E: I ratio were significantly less in FDRD. Fasting glucose was comparable. Fasting Insulin, HOMA IR, HOMA %B and HOMA AD were higher while HOMA %S and QUICKI index were lower in FDRD. Lipid profile or lipid derived parameters were comparable except for higher non-HDLc in FDRD. Adiponectin was lower while Leptin and Leptin/apidonectin ratio was higher in FDRD. IL-6, hsCRP, TNF- alpha and MDA were significantly higher in FDRD, while TAS and nitric oxide were significantly lower in FDRD.
*Conclusion:* Higher body fat percentage, with insulin resistance, deranged cardiac autonomic function, higher oxidative stress and inflammation, lower adiponectin and nitric oxide levels places FDRD at higher cardiovascular risk and necessitates early lifestyle modification/intervention.


## 1. Introduction

Diabetes is the most common metabolic disorder with an increased prevalence worldwide. ICMR-INDIAB national study census had shown that 62.4 million T2DM and 77.4 million prediabetic subjects are in India [1]. Asian Indians are shown to be more susceptible to diabetes due to the genetic predisposition and unhealthy lifestyle. Nowadays, the incidence of diabetes is becoming more common among young individuals particularly in developing countries like India. This is considered to be due to the heritable nature of the disease. Further, the first-degree relatives/offsprings of the diabetes tend to share the same socio economic and cultural background which are shown to precipitate the disease in a genetically predisposed individual. Diabetes and its complication at an early age influence their quality of life with adverse consequence in a long run. The increasing incidence of

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diabetes mellitus also increases the risk for cardio vascular disease as well. Unlike, diabetic patients, first-degree relatives of diabetics (FDRD) do not manifest with signs of symptoms and their risk factors are often overlooked. However, studies have documented altered autonomic modulation and cardiovascular risk in FDRD [2,3]. Early assessment of high-risk individuals such as first-degree relatives of diabetes for the risk factors, becomes vital to curb the disease progress by early intervention. Considering the multifactorial pathophysiology of diabetes, we have looked into the several risk factors such as body composition, autonomic function, aerobic fitness, lipid profile, inflammatory and oxidative stress in the first-degree relatives of diabetes of Indian origin.

## 2. Materials and methods

**Study design and setting:** This observational case-control study was conducted by Department of Physiology in collaboration with Departments of Medicine and Biochemistry, JIPMER, Puducherry between 2013 and 2015. The study was approved by JIPMER scientific advisory committee and Ethics committee for human studies.

**Subjects:** Apparently healthy first-degree relatives (Either parents, brother or sister of the subject should be diabetic) in the age group of 18–50 years who were accompanying the diabetic patients (in and around Pondicherry district) to Diabetic clinic in JIPMER, Pondicherry were considered for study (n = 115). Subjects on medication for any medical illness which prevents subjects from doing submaximal exercise, and subjects involved in any form of regular physical exercises including yoga and other biofeedback techniques were excluded (n = 20). Candidate who showed willingness to enroll in the study were included (n = 45). Apparently healthy individuals with no family history of diabetes or first/second degree relatives with diabetes were considered for control from JIPMER staffs and their relatives (n = 150). After matching for age and gender (n = 90), Forty-five subjects who volunteered first were included as control. Informed and written consent was obtained from all the participants.

### 2.1. Parameters recorded

Participants were requested to have sound sleep before the day of recording and to refrain from caffeinated beverages, exercise, alcohol and nicotine 24 h prior to recording. Participants were asked to report to the Department of Physiology in the morning after overnight fasting (at least 8 h of caloric restriction was recommended) [4]. The fasting venous blood sample (5 mL) was drawn in sodium fluoride tubes from median cubital vein for biochemical analysis as soon as they reported to lab. They were then oriented to research lab and explained about the procedures that will be done on subsequent day. Participants were evaluated for undergoing submaximal exercise test using Physical Activity Readiness -Questionnaire (PAR-Q) [5] and general examination. The physical activity of the participants was recorded using Global Physical Activity Questionnaire (GPAQ) [6]. Participants were habituated to treadmill. Participants then reported to the lab 2 h after light breakfast with comfortable clothing (suitable for exercise), empty bowel and bladder between 9 a.m. and 11 a.m. The temperature of the lab was maintained between 24 and 26 °C. The room was lit with dim light. Recordings were done in the following order.

**Anthropometric measurements:** Measurements were made by International Society for the Advancement of Kinanthropometry [7] certified investigator. A wall mounted stadiometer (V M Electronics Hardware Ltd) accurate to the nearest 0.1 cm was used to measure height. Weight was measured using digital weighing scale (Charder Electronic Co. Ltd, Taiwan) accurate to the nearest 0.1 kg. The waist (WC) and hip (HC) circumference were measured using non-stretchable measuring tape. (CESCROF Sports Equipment Limited, Brazil) and Waist to hip ratio (WHR) was calculated.

**Cardiovascular parameters**: Blood pressure (BP) and heart rate (HR) were measured after 10 min of rest in sitting position [8]. HR was assessed manually from the radial artery. The BP (mm hg) was recorded from right arm using mercury sphygmomanometer (Model: Diamond, Industrial Electronic & allied product, India). BP recordings were taken thrice with 2 min rest intervals and the average was taken [9,10]. All measurements were taken by the same investigator.

**Body fat percentage (BF%):** After removing any metals, participants were made to lie supine on the couch. Body fat electrodes were placed in a tetrapolar manner and body fat percentage (BF%) was measured using bio impedance method (Quadscan 4000 R, UK). After removing BF% electrodes, ECG electrodes were placed.

**Short-term heart rate variability:** We followed guidelines formulated by Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology [11]. After 5 min of supine rest, lead II electrocardiography (ECG) was recorded for 5 min. The conversion of analog to digital signal was done using 16 bit, 16- channel data acquisition system with Acqknowledge 3.8.2 software (Biopac MP100, USA). The sampling rate was 500 Hz and Band pass filter of 2 Hz–40 Hz was used. From the RR tachogram both frequency and time domain measures were computed using Kubios 1.0 software (Bio-signal analysis Group, Finland) using Fast Fourier Transformation (FFT) and RR trend respectively. The frequency domain indices - Very Low Frequency (VLF; 0.003 Hz - 0.04 Hz), Low frequency (LF; 0.04–0.15 Hz) and High Frequency (HF; 0.15 Hz- 0.4 Hz) both in absolute powers ($ms^2$) and in normalized unit (nu), Total power (TP) $ms^2$ and LF/HF ratio was calculated. Time domain measures - Standard deviation of all NN intervals (SDNN), sum of the squares of differences between adjacent RR intervals (RMSSD), adjacent RR interval differing more than 50 ms (NN50) and its percentage (pNN50).

**Baroreflex sensitivity (BRS):** After 5 min of rest, the reconstructed brachial pressure from finger pressure recorded using finger cuff was obtained via data acquisition system (Finapres Medical Systems BV, Netherlands) for 10 min. The parameters recorded from reconstructed brachial pressure includes, BRS (ms/mmHg) (monitored by continuous blood pressure variability), left ventricular ejection time (ms) (LVET), cardiac output (L/min), and total peripheral resistance (TPR) ($dyn \cdot s \cdot cm^{-5}$).

Cuffs of BRS were removed and they were explained about the procedures for autonomic reactivity tests. Lead II ECG was monitored continuously for the autonomic reactivity tests using biopac. Epochs for each procedure were marked manually and analyzed separately. BP measurements were done using automated BP monitor (Omron, SEM-1, Japan).

### 2.2. Autonomic reactivity tests

**Forced timed breathing:** Subjects in supine position were asked to breath at the rate of 6 breaths per minute comprising inspiratory and expiratory cycles for 5 s [12] avoiding abrupt inhalation/exhalation/holding breath during the procedure. Deep breathing was synchronized to a paced voice metronome and if necessary guided by hand movement. The ratio between maximal RR interval during expiration (E) and minimal RR interval during inspiration (I) is considered as E: I ratio.

**Orthostatic stress test (OST):** Participants were instructed to stand within 3 s from supine position(13). The ratio of longest RR interval around $30^{th}$ beat and shortest around 15th beat (30:15 ratio) was calculated to obtain HR response to OST. During the

procedure BP was monitored continuously (every 40 s) using automatic BP monitor for 5 min.

**Isometric hand grip test (IHG):** Participants were made to sit comfortably. IHG was evaluated at 30% of their maximal strength for 3 min. We measured BP during the maneuver in the contralateral arm. The vasoconstrictor response was calculated by computing the difference between sitting diastolic blood pressure (DBP) and DBP at the end of 2 min of maneuver [13].

Participants were then connected to a non-invasive automated exercise monitoring system (Cardiosoft and Suntech Tango Exercise BP monitoring system).

**Predicted $VO_2$max:** We followed the guidelines of American college of sports medicine [14]. Participants were asked to stand for 5 min on the automated treadmill (T2100, General Electricals) and their HR and BP was measured. Participants were briefed about the test and were told that they are free to end the test at any point of time and explanation was given about Borg's rating of perceived exertion scale from 6 to 20 [15,16]. Three minutes of warm-up at 1.7 mph and grade 0% was given, followed by Bruce protocol. HR, BP, ECG and perceived exertion was continuously monitored. The requirements were to complete two separate workloads that result in HR values between 110 and 150 bpm. At each 3-min work stage, a steady-state HR is required. If steady state HR is not reached the stage would be continued for another minute. Test termination criteria as given by ACSM guidelines for submaximal testing was followed [17]. However, all the participants were able to achieve two steady state HR without having any of the test termination criteria's. After exercise, HR and BP were monitored for 5 min during recovery.

$VO_{2max}$ is estimated using the equation - $VO_{2max}$ (mL/kg/min) = a ($HR_{max}$ - HR2) + $VO_2$ 2 where a = $VO_2$2 − $VO_2$1/(HR2 - HR1), $VO_2$1 is submaximal predicted $VO_2$ from stage 1 in mL/kg/min, $VO_2$2 is submaximal predicted $VO_2$ from stage 2 in mL/kg/min, HR1 is steady state HR from stage 1 in bpm, HR2 is steady state HR from stage 2 in bpm, $HR_{max}$ = 220 − age [18,19]. $VO_2$ for each stage was calculated using the equation - $VO_2$ (mL/kg/min) = [(0.1 × S) + (1.8 × S X G)] + 3.5; where s (speed) = mph X 26.8 m/min/mph, G (grade) = % elevation/100.

**Biochemical parameters:** The biochemical parameters were analyzed according to the manufacturer guidelines.

**Blood glucose, Insulin resistance and sensitivity:** Fasting blood glucose - glucose oxidase-peroxidase method (Genuine Biosystem). Fasting Insulin - ELISA (Enzyme linked Immuno Sorbent Assay) (DIASOURCE, Germany). Insulin resistance was calculated using HOMA2: the updated HOMA model (i.e., the computer model) [20], HOMA- AD [fasting glucose (mmol/L) x fasting insulin (mU/L)]/[22.5 × fasting adiponectin (μg/ml)] [21,22], and Quantitative insulin sensitivity check index (QUICKI) [24], and HOMA %S was used to calculate insulin sensitivity. HOMA %B was calculated to assess beta cell function [20].

**Inflammatory, anti-inflammatory, endothelial and oxidative stress markers:** ELISA was done to estimate IL6 (Ani Biotech Tiilitle, Finland), hs-CRP (DBC diagnostics biochem Canada Inc, Canada), IL2, TNF-α, leptin (R&D Systems, USA) and adiponectin (Raybiotech), Endothelial function was estimated by nitric oxide (Cayman) measurement. Oxidant-antioxidant status was measured by quantification of malondialdehyde (Cayman) and total antioxidant status (BT-Lab) using ELISA.

**Lipid profile:** Total cholesterol (TC) was estimated by Colorimetric, enzymatic methods with cholesterol oxidase peroxidase by using Diagnostic kit from Agappe diagnostics. High density Lipoprotein (HDL) was estimated by direct enzymatic, colorimetric method with cholesterol oxidase esterase by using Diagnostic kit from Accucare, Lab care diagnostics. Triglycerides (TG) was estimated by Colorimetric, enzymatic method with GPO − PAP − ESPAS by using Diagnostic Kit from Agappe diagnostics using fully automated clinical chemistry analyzer (AU400, Olympus, USA). VLDL was calculated using Friedwalds's formula [3]. Other lipid profile derived parameters were calculated (TC/HDL, TG/HDL, LDL/HDL, atherogenic index).

### 2.3. Statistical analysis

Normality of the data was analyzed using Kolmogorov-Smirnov normality test. Normally distributed data were expressed as Mean ± SD. Non-normally distributed data were expressed in Median (Interquartile range). Differences between the groups were compared using unpaired t-test and Mann-Whitney U −test according to their data distribution. Statistical tests was done using SPSS ver. 19 (SPSS, Chicago, IL, USA). P value < 0.05 was considered to be statistically significant.

## 3. Results

Table 1 shows comparison of anthropometric measurements. Both groups were similar for age, height, weight, WC, BMI and WHR. FDRD had significantly higher HC and BF%.

Table 2 shows comparison of cardiovascular parameters. There were no differences in SBP, DBP, MBP, pulse pressure, stroke volume, LVET, CO, TPR, and physical activity levels. FDRD had higher HR and rate pressure product. Cardio-respiratory fitness ($VO_2$max) was lower in FDRD but not statistically significant.

Table 3 shows comparison of cardiac autonomic function. There were no significant differences in time domain parameters except NN50 (counts). FDRD demonstrated significantly reduced NN50 (count). There was no significant difference in frequency domain parameters except TP ($ms^2$). FDRD demonstrated significantly reduced TP ($ms^2$). Both 30:15 ratio and E: I ratio was significantly less in FDRD. Baroreflex sensitivity was less in FDRD but not statistically significant.

Table 4 shows comparison of glucose, insulin and derived parameters. Fasting glucose was comparable. Fasting Insulin, HOMA IR, HOMA %B and HOMA AD was higher in FDRD. HOMA %S and QUICKI index were significantly lower in FDRD. (Table 4).

Table 5 shows comparison of lipid profile, lipid derived parameters and adipokines. There was no significant difference between the groups in lipid profile or lipid derived parameters except non-HDL. The mean value of non-HDL was significantly reduced in FDRD. Adiponectin was significantly lower in FDRD, while leptin was higher though not statistically significant. Leptin/apidonectin ratio was significantly higher in FDRD. Also, Table 5 shows comparison of inflammatory and anti-inflammatory parameters. IL-6, hsCRP, TNF- alpha were significantly higher in FDRD. The mean value of MDA level was significantly higher in FDRD. TAS was significantly less in FDRD. Nitric oxide level is significantly lower in FDRD.

## 4. Discussion

The main objective of this cross-sectional study was to assess cardiometabolic risk in First degree relatives of diabetes (FDRD) as compared to age and gender matched controls. While most of the commonly used screening anthropometric measurements such as BMI, WC, WHR were comparable, only BF% was significantly higher in FDRD. This could be due to the strong heritable nature of body composition [23](increased BF%) which in turn leads to insulin resistance and diabetes [24]. We also observed higher insulin resistance (HOMA-IR & HOMA-AD) and decreased insulin sensitivity (HOMA %S and QUICKI) in FDRD which places them at higher risk of developing diabetes and cardiovascular disease.

**Table 1**
Comparison of anthropometric measurements between first degree relatives of diabetes and non-first degree relatives of diabetes.

| Group | First degree relatives of diabetics (n = 45) | Non-First-degree relatives of diabetics (n = 45) | p value |
|---|---|---|---|
| Parameters | Mean ± SD | Mean ± SD | |
| Age (years) | 29.36 ± 6.07 | 29.20 ± 5.96 | .903 |
| Height (cm) | 164.82 ± 11.96 | 163.42 ± 9.53 | .541 |
| Weight (kg) | 63.60 ± 14.30 | 61.36 ± 12.58 | .433 |
| Waist circumference (cm) | 82.64 ± 12.89 | 78.24 ± 9.87 | .072 |
| Hip circumference (cm) | 97.51 ± 10.05 | 92.42 ± 7.46 | .008 |
| Body fat (%) | 27.10 ± 10.48 | 21.68 ± 6.23 | .004 |
| Body mass Index (kg/m$^2$) | 23.29 ± 4.12 | 22.82 ± 3.24 | .545 |
| Waist-hip ratio | 0.85 ± 0.08 | 0.85 ± 0.07 | .996 |

The values are presented in Mean ± SD. Unpaired 't"test was done between the groups to compare the mean difference. The significance was set at p value < 0.05.

**Table 2**
Comparison of cardiovascular parameters between first degree relatives of diabetes and non-first-degree relatives of diabetes.

| Group | First degree relatives of diabetics (n = 45) | Non-First degree relatives of diabetics (n = 45) | p value |
|---|---|---|---|
| Parameters | Mean ± SD | Mean ± SD | |
| SBP (mmHg) | 116.46 ± 10.32 | 115.70 ± 8.68 | .706 |
| DBP (mm Hg) | 67.52 ± 7.12 | 68.11 ± 4.78 | .649 |
| MBP (mm Hg) | 87.41 ± 7.60 | 87.38 ± 5.91 | .987 |
| Pulse pressure (mm Hg) | 48.94 ± 7.14 | 47.59 ± 6.55 | .354 |
| RPP/100 | 8461.84 ± 920.97 | 7983.58 ± 1117.42 | .029 |
| Heart rate (beats per minute) | 72.82 ± 6.84 | 69.10 ± 9.21 | .032 |
| Stroke Volume (mL) | 88.07 ± 16.75 | 86.14 ± 16.43 | .583 |
| LVET (ms) | 327.24 ± 15.98 | 324.48 ± 15.09 | .402 |
| CO (L/min) | 6.15 ± 1.20 | 6.28 ± 1.14 | .600 |
| TPR (dyn·s·cm$^{-5}$) | 0.89 ± 0.19 | 0.89 ± 0.20 | .872 |
| VO2max mL/(kg·min) | 45.88 ± 8.63 | 48.01 ± 9.15 | .260 |
| Physical activity (METs) | 354.41 ± 380.46 | 325.00 ± 410.75 | .613 |

The values are presented in Mean ± SD Unpaired *t*-test was done between the groups to compare the mean difference. The significance was set at p value < 0.05. SBP-Systolic blood pressure; DBP- diastolic blood pressure; MBP- mean blood pressure, RPP- rate pressure product, LVET- Left ventricular ejection time; CO— cardiac output, TPR-total peripheral resistance, VO2 max —Cardio respiratory fitness.

**Table 3**
Comparison of cardiac autonomic function between first degree relatives of diabetes and non-first degree relatives of diabetes.

| Group | First degree relatives of diabetics (n = 45) | Non-First-degree relatives of diabetics (n = 45) | p value |
|---|---|---|---|
| Parameters | Median (IQR) | Median (IQR) | |
| **Time domain parameters** | | | |
| SDNN (ms) | 40.60 (18.50) | 47.20 (34.10) | .160 |
| RMSSD (ms) | 29.40 (17.70) | 35.90 (32.25) | .199 |
| NN50 (count) | 27.00 (62.00) | 43.00 (105.50) | .051 |
| pNN50 (%) | 6.90 (20.00) | 13.70 (29.90) | .139 |
| **Frequency domain parameters** | | | |
| LF (ms$^2$) | 558.00 (450.50) | 718.00 (896.50) | .436 |
| HF (ms$^2$) | 828.00 (489.50) | 1014.00 (721.00) | .077 |
| TP (ms$^2$) | 1897.00 (1363.50) | 2662.00 (3163.00) | .006 |
| LF (n.u) | 0.44 (0.09) | 0.40 (0.15) | .081 |
| HF(n.u) | 0.56 (0.09) | 0.60 (0.15) | .081 |
| LF/HF ratio | 0.79 (0.29) | 0.66 (0.45) | .081 |
| **Autonomic reactivity test** | | | |
| 30:15 ratio | 1.41 (0.29) | 1.61 (0.27) | .006 |
| EI ratio | 1.37 (0.29) | 1.48 (0.26) | .036 |
| **Baroreflex sensitivity** | | | |
| BRS (ms/mmHg)* | 15.27 ± 5.79 | 17.99 ± 7.72 | .063 |

The values are presented in Median (interquartile range). Mann Whitney *U* test was done between the groups to compare the mean difference. The significance was set at p value < 0.05. SDNN: Standard deviation of all NN intervals; RMSSD: Square root of mean of the sum of the squares of differences between adjacent NN intervals; NN50 count: Number of pairs of adjacent NN intervals differing by more than 50 ms in entire recording. Total power: The variance of NN intervals over the temporal segment; LF: Power in low frequency range (0.04–0.15 Hz); HF: Power in high frequency range (0.15–0.4 Hz); LF norm: LF power in normalized units(LF/(TP-VLF)*100); HF norm: HF power in normalized units(HF/(TP-VLF)*100); LF/HF ratio: Ratio LF (ms$^2$)/HF (ms$^2$). 30 15 ratio: ratio of the longest RR interval after standing to the shortest RR interval. E: I ratio: ratio of longest RR interval during expiration to shortest RR interval during inspiration.* Unparied *t*-test was done between the groups to compare the mean difference. The significance was set at P value < 0.05. BRS- baroreflex sensitivity.

Hyperinsulinemia with normal glucose levels observed in FDRD could be the early compensatory metabolic derangement to combat insulin resistance denoting the defect in peripheral tissue response (due to increased BF%) and a normal beta cell function (higher HOMA %B in FDRD) [25]. Hyperinsulinemia by itself is an independent risk factor for ischemic heart disease even in subjects with normal glucose tolerance [26] and its association with obesity and dyslipidemia is well known [27]. Excess body fat as seen in FDRD

**Table 4**
Comparison of glucose, insulin, insulin resistance and insulin sensitivity parameters between first degree relatives of diabetes and non-first-degree relatives of diabetes.

| Group | First degree relatives of diabetics (n = 35) | Non First degree relatives of diabetics (n = 38) | p value |
|---|---|---|---|
| Parameters | Mean ± SD | Mean ± SD | |
| Fasting glucose (mg/dl) | 80.14 ± 8.25 | 80.58 ± 5.85 | .794 |
| Fating Insulin (μIU/ml) | 6.69 ± 1.57 | 5.55 ± 0.90 | <.001 |
| HOMA-IR | 0.843 ± 0.20 | 0.701 ± 0.14 | <.001 |
| HOMA %B | 111.70 ± 26.24 | 96.76 ± 17.33 | .005 |
| HOMA %S | 124.28 ± 25.06 | 145.73 ± 20.95 | <.001 |
| QUICKI | 0.369 ± 0.01 | 0.378 ± 0.01 | .002 |
| HOMA-AD | 0.229 ± 0.12 | 0.137 ± 0.05 | <.001 |

The values are presented in Mean ± SD. Unparied *t*-test was done between the groups to compare the mean difference. The significance was set at p value < 0.05. HOMA-IR: Homeostasis model assessment-Insulin resistance; QUICKI- Quantitative insulin sensitivity check index; HOMA-AD: Homeostasis model assessment- Adiponectin; HOMA %B-beta cell function in percentage; HOMA % S– Insulin sensitivity in percentage.

**Table 5**
Comparison of Lipid profile, lipid derived parameters, inflammatory, oxidative stress and adipokines between First degree relatives of diabetes and Non-first-degree relatives of diabetes.

| Group | First degree relatives of diabetics (n = 35) | Non First degree relatives of diabetics (n = 38) | p value |
|---|---|---|---|
| | Mean ± SD | Mean ± SD | |
| TC(mg/dl) | 170.74 ± 31.05 | 168.89 ± 32.06 | .803 |
| TG (mg/dl) | 113.03 ± 56.90 | 97.34 ± 41.58 | .186 |
| HDL (mg/dl) | 37.18 ± 12.47 | 40.77 ± 12.32 | .221 |
| VLDL (mg/dl) | 22.61 ± 11.38 | 19.47 ± 8.32 | .186 |
| LDL (mg/dl) | 110.95 ± 26.44 | 108.65 ± 30.79 | .732 |
| non HDL | 112.78 ± 55.45 | 99.64 ± 61.09 | .029 |
| TC/HDL | 4.38 ± 2.80 | 3.66 ± 2.96 | .242 |
| TG/HDL | 2.89 ± 2.24 | 2.12 ± 1.80 | .750 |
| LDL/HDL | 2.95 ± 2.27 | 2.46 ± 2.41 | .320 |
| AIP | 0.39 ± 0.30 | 0.28 ± 0.28 | .890 |
| Adiponectin (mg/mL) | 6.63 ± 2.08 | 8.99 ± 2.85 | <.001 |
| Leptin (ng/mL) | 1.69 ± 0.69 | 1.48 ± 1.19 | .351 |
| leptin/adiponectin ratio | 0.292 ± 0.19 | 0.178 ± 0.13 | .004 |
| IL-6 (pg/mL) | 173.08 ± 148.13 | 62.34 ± 91.37 | <.001 |
| hs-CRP (ng/dL) | 46354.27 ± 49385.78 | 19833.17 ± 26151.76 | .005 |
| TNF-α (pg/mL) | 177.94 ± 227.09 | 73.34 ± 96.12 | .011 |
| MDA (μmol/L) | 20.09 ± 5.33 | 8.61 ± 2.25 | <.001 |
| TAS (mmol/L) | 575.48 ± 198.83 | 805.28 ± 185.38 | <.001 |
| Nitric oxide (μmol/L) | 137.50 ± 101.34 | 259.37 ± 233.93 | .005 |

The values are presented in Mean ± SD. Unparied *t*-test was done between the groups to compare the mean difference. The significance was set at p value < 0.05. TC- Total cholesterol; TG- Triglycerides; HDL-High density lipoprotein; VLDL- Very low density lipoprotein; LDL- Low density lipoprotein. AIP – Atherogenic index of Plasma. Non HDL-C was calculated using Fried-Wald formula (TC-HDL-C). IL-6- *Interleukin 6;* hs-CRP- *high sensitivity; TNF-α-Tumor necrosis factor-α; C-reactive protein* MDA-malondialdehyde; TAS- total antioxidant status. The significance was set at p value < 0.05.

could lead to adiposopathy (ectopic deposition of dysfunctional adipose tissue) that may directly or indirectly cause cardiovascular disease through development of type 2 diabetes, high BP and dyslipidemia [28]. However, in our study we found that the lipid profile was comparable; total cholesterol, triglycerides, VLDL, LDL were only slightly higher in FDRD compared to controls. Of all the predictor of cardiovascular diseases (AIP [29], TC/HDL, TG/HDL [30], LDL/HDL [31] and Non-HDLc [32] only non-HDLc showed significant increase in FDRD. One observation that supports adiposopathy is the paradoxically (even though their BF% is higher) decreased adiponectin levels [33] and higher leptin levels [34] in FDRD that is associated with development of type 2 diabetes [35] and cardiovascular disease [36] respectively. Adiponectin is shown to correlate well with insulin resistance and hyperinsulinemia than with adiposity and glucose intolerance [37] and is in line with our observation that FDRD have significantly higher insulin levels and insulin resistance while their lipid profile and glucose levels were comparable. In contrast BF% is the strongest predictor of leptin levels [38] and higher BF% in our study group could be the cause for observed increase in leptin. Leptin to adiponectin ratio which is shown to be a better indicator of cardiovascular risk than either of them alone was higher in FDRD [39]. Hyperinsulinemia is known to decrease adiponectin levels [40] and there are studies both supporting [41] and against [38] the effect of hyperinsulinemia on increasing leptin levels. Low adiponectin levels lead to insulin resistance [42], which in turn could increase insulin levels leading to a vicious cycle ending in development of diabetes and its complication. Whether hyperinsulinemia causes insulin resistance, or it is a result of insulin resistance is long been argued and is considered both a result and driver of insulin resistance [43].

Increased BF% [44] and hyperinsulinemia [45], is also associated with sympathetic overactivity, decreased parasympathetic activity and higher vascular resistance. In our study, BP or TPR were comparable. Further, we also observed significant derangements in the cardiac autonomic function - Parasympathetic activity (SDNN, RMSSD, NN50, pNN50, Total power, HF power, and HFnu) and reactivity (30/15 ratio and E/I ratio) and sympathetic activity (LF power) was lower and relative sympathetic activity (HR, LFnu, and LF/HF ratio) was higher in FDRD. (Though the values of these parameters have large difference it was not statistically significant. This may be due to higher variability of these parameters and lesser sample size.) Spontaneous BRS is one of the early predictors of autonomic dysfunction in diabetes [46]. Decreased BRS, decreased HRV (total power), decrease in parasympathetic activity and reactivity, and increased sympathetic activity observed in FDRD increases their cardiovascular risk.

Insulin mediated sympathetic vasodilation [47] plays an important role in postprandial glucose uptake [48] and vascular insensitivity leads to insulin resistance [49]. Nitric oxides levels were lower in FDRD, denoting defective synthesis [50] and which in turn could increase central sympathetic outflow. Further the bioavailability could be reduced by increased oxidative stress (higher Malondialdehyde and less total antioxidant status) which was found in FDRD. Oxidative stress can increase inflammation and vice versa [51]. Inflammatory cytokines (TNF alpha, hsCRP, IL 6) were also increased in FDRD which is shown to interfere with insulin signaling (insulin resistance) or beta cell destruction causing hyperglycemia [52]. Which in turn could increase oxidative stress leading to a vicious cycle. Cardiorespiratory fitness (VO2max) and physical activity levels were comparable, which is shown to have an inverse relationship with inflammation and oxidative stress [53].

Considering the primary role played by increased BF%, inflammation and oxidative stress in cardiometabolic risk, FDRD should focus early in their life on the modifiable risk factors such as macronutrient intake (high glucose or free fatty acids), obesity, smoking, mental stress and sedentary life style to reduce their cardiovascular risk.

## Limitations

Biochemical parameters were measured only for 35 subjects in each group.

## Conclusion

Higher body fat percentage, with insulin resistance, deranged cardiac autonomic function, higher oxidative stress and inflammation, lower adiponectin and nitric oxide levels places FDRD at higher cardiovascular risk and necessitates early lifestyle modification/intervention.

## Conflicts of interest

None.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dsx.2018.11.047.

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