Assessment of skin microcirculation and inflammatory markers of metabolic syndrome in a rat model

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Abstract: Analysis of the literature reveals that metabolic syndrome is invariably linked to microvascular disturbances, such as abnormalities in arteriolar reactivity, capillary recruitment, permeability, and hemorheology. The aim of this study was to assess skin microcirculation under baseline conditions and maximum skin hyperemia in response to heating (vasodilatory capacity) in control rats and in the rat model of metabolic syndrome. Twenty four young female rats were randomly assigned into control group (CG) fed on standard rat show & fructose induced insulin resistance group (FG) fed on fructose enriched show (60% of caloric intake) for 2 months. The skin microcirculation was assessed in the hairless ear of rat by Laser Doppler Flowmetry to measure skin blood flow, frequency of vasomotion waves, (frequency 1: 1-3 cycles/min (endothelial activity), frequency 2: 3-5 cycles/min (sympathetic activity), frequency 3: 5-20 cycles/min (vascular myogenic Activity)) & the Power of vasomotion (in perfusion units PU) in relation to the recorded frequencies . All the parameters were measured at 30^0 C and after local heating of the skin to 44^0C.The results demonstrated a significant increase in body mass index, serum glucose & insulin levels ($P<0.05$), systolic blood pressure, total cholesterol, low density lipoprotein cholesterol & triglycerides ($P<0.05$) in addition to a significant increase in nitric oxide, high sensitivity C reactive protein & tumor necrosis factor alpha ($P<0.05$), in FG compared to CG. So it can be claimed that use of fructose in diet for at least 2 months could be a model for experimentally studying the pathophysiological changes in the metabolic syndrome. Regarding parameters of microcirculation, there was a significant decrease in the % change in blood flow between blood flow at 30^0C and that after local heating of the skin to 44^0C ($P<0.05$) in FG compared to CG indicating impaired maximum skin hyperaemia induced by heating of the skin (vasodilatory capacity). Also FG showed a significant lower frequency values in the mid- range of frequency (frequency-2 i.e sympathetic dependent) at 30^0 C ($P<0.05$) and in the mid and high range frequencies (frequency-2 &frequency- 3 i.e sympathetic and myogenic dependent) at 44^0C ($P<0.05$) in addition to a significant decrease in the power of vasomotion (PU) at all frequency ranges (power-1, 2, and 3) after local heating of the skin to 44^0C in comparison to the CG ($P<0.05$). The microvascular dysfunction is a hallmark in our results that may be a potential factor explaining the clustering of several components of the metabolic syndrome & associated cardiovascular complications. Our results strongly suggest that targeting micro vascular and endothelial dysfunctions in patients with metabolic syndrome might help to prevent cardiovascular morbidity in those patients.

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1. Introduction

The metabolic syndrome is a multifaceted clinical entity resulting from the interaction of genetic, hormonal, and lifestyle factors. Over the past two decades, the number of people diagnosed with the syndrome has steadily increased and is associated with the global epidemic of obesity and diabetes[1].

National Cholesterol Education Program's Adult Treatment Panel III report (ATP III), [2] suggests a working definition of the metabolic syndrome that includes the presence of at least three of the following characteristics: abdominal obesity, hypertension, insulin resistance± glucose intolerance, dyslipidemia, pro-inflammatory and prothrombotic states. The pathophysiological basis of the metabolic syndrome is multiple and complex.

There is increasing evidence that microvascular dysfunction is a potential factor explaining the clustering of several components of the metabolic syndrome such as hypertension, obesity, and insulin resistance. Also, microvascular defects play an important role in the end-organ damage associated with the metabolic syndrome and may contribute to macrovascular dysfunction[3].

In a recognized experimental model of metabolic syndrome, the perfusion of multiple tissues has been shown to be compromised[4]. The direct mechanism of this decrease in perfusion, found in both humans and rats seems to be multi-faceted: a combination of altered responsiveness to vasodilator

and vasoconstrictor mechanisms, changes to the mechanical properties of the perfusing arteries, or a limit in the density/number of available microvessels to supply the tissue[5].

Thus, the microcirculation may present a promising future therapeutic and preventative target in the metabolic syndrome. Hence, clarification of pathophysiological pathways that contribute to microvascular dysfunction is essential.

Insulin resistance and endothelial dysfunction are characterized by elevated circulating markers of inflammation[6]. C-reactive protein (CRP), an inflammatory biomarker that has proven to be a strong, independent predictor of both incident diabetes and incident cardiovascular disease[7]. Tumour necrosis factor alpha (TNF-α) is another circulating marker of inflammation that has been associated with obesity. TNF-α was shown to be constitutively expressed by adipose tissue, to be hyperexpressed in obesity, and to mediate insulin resistance in the major animal models of obesity[8].

Aim of work:

The present study was designed to assess skin blood flow and capillary vasomotion by using Laser Doppler Monitor over hairless ear of the rat in addition to metabolic parameters and rat tail arterial blood pressure in fructose- induced insulin resistance rat as a model of metabolic syndrome.

2. Material & Methods

Experimental animals:

Twenty four female rats (150-250 grams) approximately 6 weeks old belonging to the local strain were used in this study. Veterinary care was provided by the laboratory animal house unit of Kasr Al-Aini Faculty of Medicine, Cairo University. Throughout the study period, the animals had free access to food and water all through the daytime with deprivation from food at night. Each rat was bred and housed individually in his own wire mesh cage at room temperature with normal light and dark cycle. Animals were allowed to acclimatize to their environment for 1 week before start of experiments. The animals were divided randomly according to the diet type into two groups of 12 rats each, control group (CG) fed on standard rat chow containing 75% of its caloric intake as carbohydrates & fructose induced insulin resistance group (FG) fed a fructose-rich diet contained 60% fructose, 21% proteins, 5% fat and 8% cellulose[9]for the entire study duration for 2 months**.**

Experimental procedures

Body mass index (BMI) measurement:

The animals were weighed in grams & the naso-anus length in cm was measured while the rats were anesthetized with ether, to make it easier and accurate. [10]

The obesity index was calculated according to an equation formulated by Dubuis *et al.*[11]:

BMI= cubic root of weight in grams x 1000/naso-anal length in cm

Systolic blood pressure (SBP) measurement:

Systolic blood pressure was measured by Harvard rat tail blood pressure monitor system in conscious animals. Four to six readings were averaged together to obtain a value for systolic blood pressure (MNL 490601 System)

Laser Doppler Flowmetry

Assessment of the skin capillary blood flow in anaesthetized rats was done using Laser Doppler Flowmetry (LDF) periflux 5000 satellite primed made in Sweden. The method involves conducting 2Mega Watt light from a laser system via a fiberoptic light guide to the skin surface using a probe held by a plastic adhesive tape. All measurements were performed in the morning in a quiet room at temperatures of approximately 28°C. The rats were placed on their side and the probe was fixed to the inner surface of the external ear. Local thermal hyperemia was induced using a heating disc surrounding the probe, connected to a heating unit. The probe was attached to the skin using a double-sided sticker. Recordings of the laser Doppler signal were made using PeriSoft for Windows. Baseline skin blood flow was recorded for 3 minutes with the local heating disc temperature set at 30°C[12]. This was immediately followed by rapid local heating to 44 °C which was maintained for 1 minute to obtain maximal vasodilatation[13]. After this, another 3 minutes of recording was then repeated at 30°C to study the microvascular reactivity to heat and maximum skin hyperemia. The data recorded are

- Basal skin blood flow at 30^0C in perfusion units (P.U), the percent change between blood flow at 30^0C and blood flow after local heating of the skin to 44^0C to study the maximum skin hyperaemia in response to heating & the slope of this change in milliseconds
- Frequency of vasomotion waves (cycles/minute) at 30^0C and after local heating of the skin to 44^0C. Three frequency ranges were recorded: -Frequency 1: 1-3 cycles/min (endothelial activity), frequency 2: 3-5 cycles/min (sympathetic activity) & frequency 3: 5-20 cycles/min (vascular myogenic Activity).
- Three Power of vasomotion (perfusion units PU) in relation to the recorded frequencies at 30^0 C and after local heating of the skin to 44^0C were

also recorded: Power-1: Increase in blood flow at frequency-1, Power-2: Increase in blood flow at frequency-2 & Power-3: Increase in blood flow at frequency-3

Biochemical analysis

After an over-night fast, blood samples were withdrawn through retro-orbital route and serum was separated and stored at -70°C until used except for the insulin & glucose; which were measured immediately after sampling.

Plasma glucose in blood samples was measured using oxidase- peroxidase method[14].

Plasma insulin levels were analyzed using enzyme-linked immunosorbent assay ELISA (Dako, Carpinteria, CA) according to the manufacturer's instructions[15].

Homeostasis model assessment of insulin resistance (HOMAIR)

HOMA is an indirect method for the assessment of insulin resistance. It depends on relationship between fasting plasma glucose and insulin based on a mathematical model:

HOMA-IR: [fasting plasma glucose (mmol/L) x fasting plasma insulin (uIU/ml)] /22.5[16].

HOMAIR values more than 4.0 are diagnostic of insulin resistance[17].

Measurement of lipid profile

Serum total cholesterol was assayed as described by Siedel *et al.* [18] while the protocols of Gordon and Gordon[19] and Jacobs and VanDenmark[20] were adopted for the determination of HDL-cholesterol and triglycerides (TG). LDL-cholesterol level was determined by calculation using the Friedwald formula[21] as follows:

$$LDL - C = Total\ cholesterol - \frac{TG}{5} - HDL - C$$

Measurement of NO:

Serum NO level was determined indirectly as its metabolic products (nitrate + nitrite ions) spectrophotometrically using a test kit (Boeringher, USA) in which all the nitrate ions in serum were first reduced to nitrite ions by nitrate reductase followed by the reaction between nitrite ions and the Greiss reagent (0.1% naphthylethylenediamine dihydrochloride in distilled water and 1% sulfanilamide in 5% H_3PO_4) to form a blue color solution[22]. Absorbance measurement was done at 540 nm against the reagent blank in which the serum sample was replaced with de-ionized water. The levels of nitric oxide in the experimental animals and control were determined by extrapolation from absorbance-concentration curve of the sodium nitrate standard solution (10–100 µM).

Measurement of hsCRP & TNF- α

Serum hsCRP levels were measured with a Rat C-Reactive Protein ELISA Kit (Alpha Diagnostic International, San Antonio, TX, USA).according to manufacturers instruction[23].

Serum TNF-α was measured by using ELISA (quantikine R & D system USA) according to the manufacturer`s instructions[24].

Statistical analysis:

Data was coded and entered using the statistical package SPSS (version 15). Data was summarized using mean and standard deviation for quantitative variables. Comparisons between groups were done using analysis of variance (ANOVA) and multiple comparisons (Post Hoc test) for quantitative variables while non parametrical (Kruskal-Wallis test) and (Mann-Whitney test) were used for quantitative variables not normally distributed. Correlations were done to test for linear correlations between quantitative variables. P-values < 0.05 were considered statistically significant

3. Results

As shown in table 1 & Fig-1A, BMI was significantly higher in FG than CG (298.3±9.7versus 279.04±2.7) (P<0.05).

These results demonstrated that high fructose diet significantly increased (P<0.05) the levels of serum glucose (mmol/L), serum insulin (uIU/ml) &HOMAIR compared to CG (5.89±.98), (15.32±1.8) and (4.02±.91) versus (3.20±.50),(10.20±.98) and (1.45±.31) respectively (Fig.1B).

Regarding serum lipids, there was significant elevation (P<0.05) in TC , LDL-C &TGs (mg/dl) in FG as compared to CG [(180.13±17.2), (124.7±10.07) & (105.6±8.9) vs (148.8±12.7), (87.7±12.4) & (75.7±7.4) respectively] while HDL-C level is significantly decreased (P<0.05) relative to the control (34.2±3.8)versus (45.5±4.9) (Fig. 1C).

Also, SBP increased significantly (P<0.05) from 115.8±4.1 in CG to 153.7±9.07 mmHg in FG (Fig.1D).

So rats fed on high fructose diet for 2 months showed the major components of metabolic syndrome, obesity, insulin resistance, high blood pressure & dyslipidemia

NO & inflammatory markers in metabolic syndrome:

Levels of nitric oxide (NO) µmol/l, the inflammatory markers; hs-CRP (mg/l) and TNF-α (ng/ml) were increased significantly (P<0.05) in FG as compared to CG [mean values (10.8±1.4),

(2.3±0.5) and (98.5±11.9) versus (2.4±0.6) , (0.42±0.35) and (61.2±7.9) respectively (Fig. 1D & E).

Table (1): The effect of high fructose diet on body mass index BMI, metabolic parameters , systolic blood pressure SBP, nitric oxide NO& inflammatory markers in young female rats (n=12)

Measured parameters	Control Group	Fructose induced insulin resistance group
BMI (%)	279.04±2.7	298.3±9.7*
Serum Glucose (mmol/L)	3.20±.50	5.89±.98*
Insulin (uIU/ml)	10.20±.98	15.32±1.8*
HOMA	1.45±.31	4.02±.91*
Total Cholesterol (mg/dl)	148.8±12.7	180.13±17.2*
HDL-C (mg/dl)	45.5±4.9	34.2±3.8*
LDL-C (mg/dl)	87.7±12.4	124.7±10.07*
Triglycerides (mg/dl)	75.7±7.4	105.6±8.9*
SBP (mmHg)	115.8±4.1	153.7±9.07*
hs-CRP (mg/l)	0.42±0.35	2.3±0.5*
TNF-α(ng/ml)	61.2±7.9	98.5±11.9*
NO(µmol/l)	2.4±0.6	10.8±1.4*

Results are mean±SD
HOMA:Homeostasis Model Assessment of insulin resistance .
HDL-C: high density lipoprotein .
LDL-C: low density lipoprotein .
hs-CRP :high sensitivity C-reactive protein
TNF-α :tumour necrosis factor -α .
*: significant P as compared to control group (P<0.05)

Parameters of microcirculation:

Table 2 showed that levels of % change in blood flow between blood flow at 30^{0}Cand 44^{0}C and Slope of change (m sec) were decreased significantly (P<0.05) in FG as compared to CG [mean values 29.09±4.3 and 0.49±0.19 versus 51.6±5.6 and 1.2±0.30 respectively] (Fig.2A).

At 30^{0}C the only frequency affected in FG was frequency 2(sympathetic activity) which decreased significantly (P<0.05) from 6.66±1.39 in CG to 5.082± 1.6. After local heating of the skin to 44^{0}C frequency 2 and 3 showed significant decrease (P<0.05) in FG as compared to CG [mean values (4.2±0.63), (44.6±4.0) versus (6.7±1.4), (52.48±8.43) respectively} while frequency 1, showed no significant difference (Fig. 2B).

Regarding vasomotion power, there was a significant decrease (P<0.05) in power 1,2 and 3 in FG in comparison to CG after heating to 44^{0}C [mean values (1.09±0.22), (0.97±0.11) and (0.53±0.05) versus (7.3±1.6), (4.9±0.83) and (4.3±0.62) PU, respectively] while no significant changes was observes at 30^{0}C (Fig. 2C).

Table (2): Mean ± SD of all parameters measured by Laser Doppler Flowmeter in control & fructose induced insulin resistance young female rats.

Measured Parameters	Control Group (n=12)	Fructose induced insulin resistance group (n=12)
Basal Bl Fl at 30^{0}C in P.U.	41.1±7.5	38.4±6.07
% change between Bl Fl at 30^{0}C and 44^{0}C	51.6±5.6	29.09±4.3*
Slope of change in Msec.	1.2±0.30	0.49±0.19*
Frequency. 1 at 30^{0}C in cycles/min	4.0±1.7	4.4±0.98
Power of vasomotion 1 at 30^{0}C in P.U.	0.97±0.42	0.79±0.13
Frequency 2 at 30^{0}C in cycles/min	6.66±1.39	5.08±1.6*
Power of vasomotion 2at 30^{0}C in P.U.	0.56±0.2	0.47±0.11
Frequency 3 at 30^{0}C in cycles/min	47.8±7.8	43.9±8.12
Power of vasomotion 3 at 30^{0}C in P.U.	0.51±0.16	0.41±0.06
Frequency 1 at 44^{0}C in cycles/min	4.5±1.6	4.25±0.7
Power 1at 44^{0}C in P.U.	7.3±1.6	1.09±0.22*
Frequency 2 at 44^{0}C in cycles/mi	6.7±1.4	4.2±0.63*
Power 2 at 44^{0}C in P.U.	4.9±0.83	0.97±0.11*
Frequency 3 at 44^{0}C in cycles/min	52.48±8.43	44.6±4.0*
Power 3 at 44^{0}C in P.U.	4.3±0.62	0.53±0.05*

n: number of rats
Bl.Fl : blood flow
PU: Perfusion unite
*: significant P as compared to control group (P<0.05)

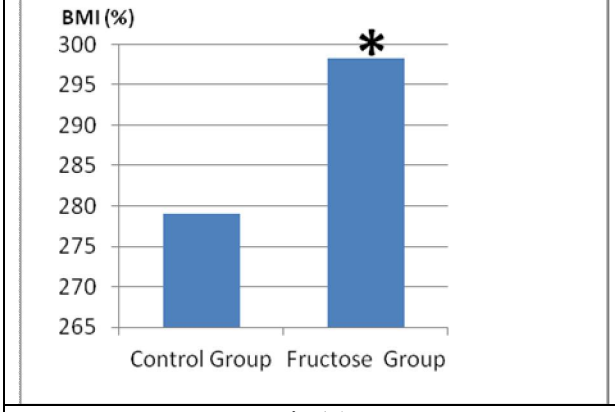
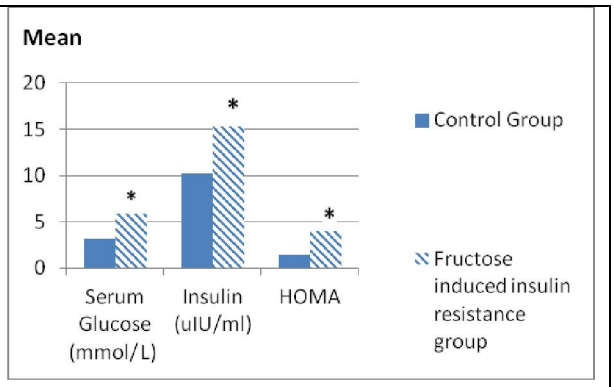


Fig 1A



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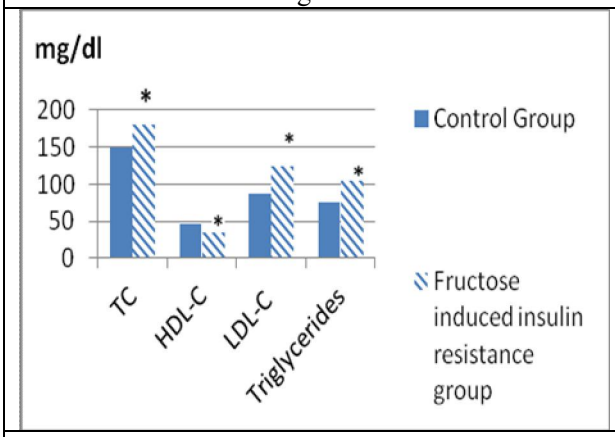

Fig 1C

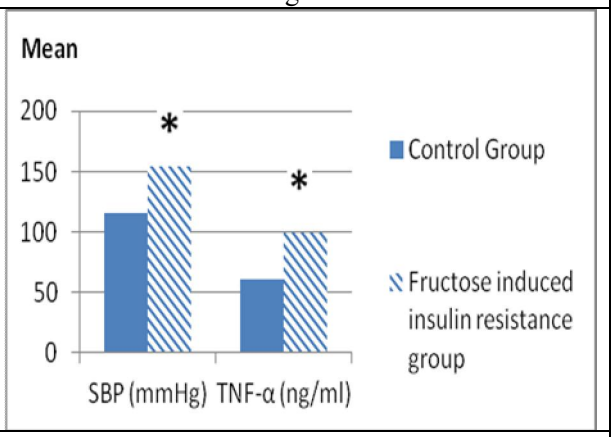

Fig 1D

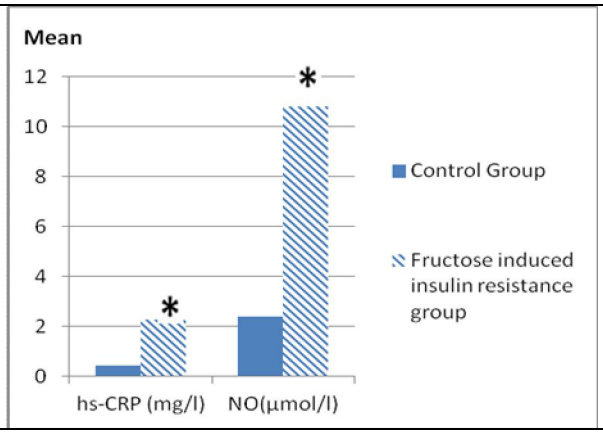

Fig. 1: The effect of high fructose diet for 2 months on body mass index BMI (Fig1A),serum glucose, insulin and HOMAIR (Fig1B), serum lipids (Fig 1C), tumor necrosis factor alpha TNF-α , systolic blood pressure SBP (Fig.1D) , high sensitivity C reactive protein, hsCRP & nitric oxide NO (Fig.1E) in young female rats
*: significant P as compared to control group (P<0.05)

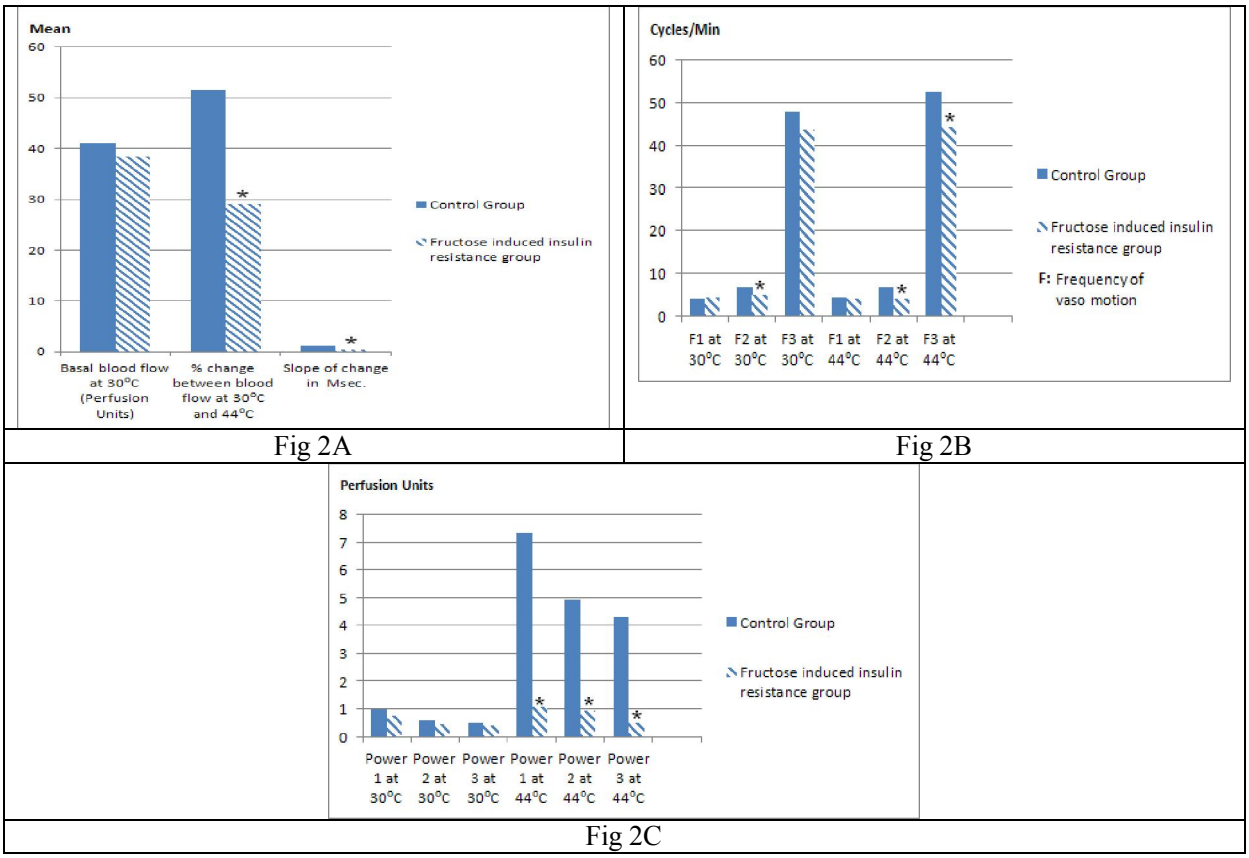

Fig 2A | Fig 2B

Fig 2C

Fig. 2: The effect of high fructose diet for 2 months on basal blood flow at 30C, % change of blood flow at 30^0 & 44^0 C & the slope of change (Fig.2A), frequency of vasomotion (Fig 2B) & power of vasomotion in relation to the recorded frequencies (Fig 2C) at 30^0C & after local heating of skin to 44^0 C in young female rates
*: significant P as compared to control group (P<0.05)

4. Discussion

The metabolic syndrome refers to the co-occurrence of several known cardiovascular risk factors, including insulin resistance, obesity, atherogenic dyslipidemia and hypertension. These conditions are interrelated and share underlying mediators, mechanisms and pathways[25].

In the present study, insulin resistance was clearly revealed in rats fed on high fructose diet (60% of caloric intake for 2 months) in relation to the rats fed on normal standard chow. Our results showed that rats on high fructose diet have significantly increased weight gain and BMI in comparison to the control rats.

Many results showed increased fasting plasma glucose and plasma insulin levels after high fructose consumption in rats[26&27].

On the other side, it was reported that short term intake of dietary fructose, is not a contributor to insulin resistance and hypersecretion in obese adolescents[28].

Increased insulin resistance on receiving fructose may be related to glucose transporter 5 (GLUT5), a fructose transporter that mediates the uptake of substantial quantities of dietary fructose, that was found to have significantly higher expression levels in young obese rats compared to lean controls[29]. Another theory explaining how chronic fructose over nutrition can lead to type 2 diabetes is the hexosamine hypothesis, where hexosamine flux is thought to regulate glucose and satiety-sensing pathways. With overexpression of glutamine, fructose-6-phosphate amidotransferase (the key regulatory enzyme in hexosaminesynthesis), the liver produces excess fatty acids, skeletal muscle becomes insulin resistant, and hyperinsulinemia results. This pathway of excess hexosamine flux leads to long-term storage of energy, and eventually obesity and type 2 diabetes[30]. Moreover, chronic fructose consumption has been reported to reduce adiponectin responses, contributing to insulin resistance[31].

The observation of increased body weight associated with fructose ingestion is of interest. One explanation for this observation could be that fructose ingestion did not increase the production of

the two hormones, insulin and leptin, that have key roles in the long-term regulation of food intake and energy expenditure[32].

Other features of the metabolic syndrome detected in rats fed high fructose diet included a significant elevation in the serum levels of total cholesterol, LDL-C, triglycerides and significant decrease in serum HDL-C.

Similar to our results, Taghibiglou *et al.*[33] concluded that fructose feeding in hamsters causes insulin resistance, hypertriglyceridemia, hepatic very-low-density lipoprotein over-production. In another study, consumption of moderate amounts of fructose significantly and dose dependently increased plasma triglyceride levels only in carbohydrate sensitive men[34].Moreover, Rader[35]reported that a low HDL cholesterol level is even more common in patients with insulin resistance than is hypertriglyceridemia.

In insulin-resistant states, two mechanisms lower HDL cholesterol: cholesterol ester transfer protein mediating the transfer of cholesterol from HDL to the apo-B- containing lipoproteins; and upregulation of enzymes, such as hepatic lipase and endothelial lipase, thus promoting hypercatabolism of HDL.

In contrast to our results Bantle *et al.*[36]demonstrated that fructose diet produced significantly higher fasting, postprandial, and daylong plasma triacylglycerol values in older men, although this effect of fructose was not seen in younger (< 40 y of age) men or in the older (≥ 40 y of age) women included in the study. The fructose diet had no significant effects on fasting plasma cholesterol, HDL cholesterol, or LDL cholesterol in either men or women

Rutledge and Adeli[37] suggested that dietary fructose has a direct impact on hepatic lipid metabolism by bypassing the enzyme phosphofructokinase, the regulatory step imposed on glucose. Allowing unregulated flow of fructose-derived carbons into lipogenesis . In addition to increased lipid production, fructose has been found to decrease lipid oxidation in humans[38].

In our study, rats fed high fructose diets showed highly significant elevation in their systolic blood pressure at the end of the study in comparison with the control rats. Even there was elevation reported in systolic blood pressure from the random samples taken after the first month.

Similar to insulin resistance and hyperlipidemia, many published experiments have shown that high-fructose diets induce hypertension in animals[39&40]. Our results clearly showed that SBP is highly correlated with insulin resistance (r; 0.652); this is in agreement with the fact that insulin resistance is one of the important mechanisms underling the development of the metabolic syndrome [41].

Hypertension in rats with the metabolic syndrome, due to chronic consumption of a high refined sugar has been reported to be associated with oxidative stress[42], the increase in sympathetic neural outflow and plasma catecholamine concentrations associated with increased plasma insulin concentrations[43], the anti-natriuretic effect of insulin to increase fluid reabsorption & lastly, the activated renin-angiotensin system found in obese individuals. [44]

Laboratory and experimental evidences indicate that atherosclerosis, in addition to being a disease of lipid accumulation, also represents a chronic inflammatory process[45]. Based on these data, hs-CRP has been used as a marker of cardiovascular risk in the present study.

The present results showed a significant rise in the levels of inflammatory markers, hs-CRP and TNF-α in FG when compared with the CG. Moreover hs-CRP was positively correlated with systolic blood pressure (r = 0.733).

These results are in accordance with results of Women's Health Study where levels of hs-CRP were shown to correlate with the major components of the metabolic syndrome[46]. Numerous studies have revealed that persons who have the most or all features of the metabolic syndrome have increased levels of CRP[47&48].

Moreover, Andrea *et al.*[49] found increased TNF-α mRNA expression (5-fold), plasma concentration of TNF- α (8-fold), and protein expression of TNF- α (more than 3-fold in small coronary arteries) in Zucker obese fatty rats. Also Hotamisligil [50] found high TNF-α levels in metabolic syndrome patients.

The increases in proinflammatory cytokines including IL-6, TNF- α and CRP reflect overproduction by the expanded adipose tissue mass[51]. Studies done by Weisberg *et al.*[52], suggested that monocyte-derived macrophages reside in adipose tissue and may be at least in part the source of the generation of pro-inflammatory cytokines locally and in the plasma.

Our results showed a significant increase in the levels of nitric oxide (NO) in FG when compared with the CG. This is in agreement with results obtained by **Zahedi *et al.*** [53]**,** who found higher NO metabolites concentrations in subjects with metabolic syndrome and type 2 diabetes. Also an experiment done by Blouet *et al.* [54]on rats fed a high-sucrose diet for six weeks inducing insulin resistance,& showed that high-sucrose diet was accompanied with higher production of superoxide anion that account for the increase in NO scavenging and the resulting

production of peroxynitrite (a stable footprint of NO oxidation) which indicate a decrease in NO bioavailability in the studied rats.

These results and ours apparently contrasts with what had previously been reported under conditions of diet-induced oxidative stress in rodents associated with a reduction in NO production[55]. In rats fed a high-refined sugar and/or high-fat diet, an impairment of endothelial-dependant vasodilation was associated with decrease in endothelial nitric-oxide synthase eNOS expression, NO production and bioavailability, and reduced insulin-induced eNOS activation[56].

However, in the latter studies, these observations were made after more than 4 months of studying which is long duration in contrast to our study.

Another explanation for the unexpected increase in the levels of nitric oxide in our study could be the fact that inflammatory cytokines like TNF-α are known to trigger the transcription of inducible nitric-oxide synthase (iNOS), a proinflammatory mediator in chronic inflammatory states including obesity-linked diabetes[57].

Therefore, we suggested that a decrease in NO bioavailability is the first impairment that affects NO metabolism in the course of insulin resistance, and that subsequent impairment in NO metabolism lags behind.

One of the main goals of our study was to assess skin microcirculation under baseline conditions and maximum skin hyperaemia in response to heating (vasodilatory capacity) by Laser Doppler Flowmeter (LDF). Our results showed that the maximum skin hyperaemia induced by heating of the skin to ≥44°C (vasodilatory capacity) is impaired in FG. These results are in agreement with many of the intervention studies that investigated the effect of metabolic syndrome parameters; insulin resistance, hypertension, obesity and dyslipidemia on microcirculation.

It was revealed that the increases in blood flow, in response to body heating was markedly less in hypertensives than normal rats[58&59]. It was suggested that this difference indicate structural change in the skin vasculature in hypertension caused by rarefaction, vascular hypertrophy, or both[59].Moreover, there is evidence that experimental elevation of blood pressure causes an increase in generation of reactive oxygen species (ROS) in endothelial cells, which may trigger adverse functional and structural changes in microvessels [60].

Štulc *et al.*[61], noticed blunted skin vasodilator response to heating in hypercholesterolemic patients. Moreover in obese women, it was shown that postocclusive capillary recruitment , microvascular endothelium–dependent vasodilatation & insulin-induced increase of microvascular endothelium–dependent vasodilatation are decreased[62]. Studies done by Caballero *et al.* [66] revealed a significant inverse correlation between microvascular reactivity and systolic blood pressure, body mass index and index of insulin resistance HOMA. The previous mentioned studies are on line with our correlation studies as we found that % change between blood flow at 30^0Cand 44^0C after local heating of the skin was negatively correlated with body mass index(r = - 0.663), systolic blood pressure (r = - 0.807) and HOMA (r = - 0.589).

The pathophysiological mechanism behind the relationship between obesity and microvascular dysfunction is probably multi factorial. Adipose tissue secretes substances, such as FFAs, TNF-α, and adiponectin, that can influence microvascular function. An increase in FFAs impairs vascular function in resistance vessels in humans and in microvasculature in rats [63]. In addition, acute TNF-α elevation impairs insulin-induced capillary recruitment and glucose uptake in rats[64].Adiponectin levels are reduced in obesity and adiponectin has a vasoprotective effect, as demonstrated by associations between hypoadiponectinemia and impaired endothelial function in resistance vessels [65].

Regarding skin vasomotion, our results showed a significant lower frequency values in frequency-2 i.e sympathetic dependent) at 30^0 C and in the frequency-2 &frequency- 3 i.e sympathetic and myogenic dependent, at 44^0C & a significant decrease in power of vasomotion at all frequency ranges after local heating of the skin to 44^0C in FG as compared to the CG

Our results match those obtained by Rossi *et al.*[67] who reported that, the newly diagnosed essential hypertensive patients showed a reduced post-ischemic increase in sympathetic- and myogenic-dependent vasomotion, together with a normal post-ischemic response of the endothelial-dependent vasomotion. Moreover, De Jongh *et al.* [68] suggested that there is a decreased endothelial- and sympathetic-dependent skin vasomotion in obese women under basal conditions. More recently, John *et al.* [69]revealed that in acute insulin resistance induced by peripheral vasoconstrictor α-methyl serotonin (αMT), there is reduction in the myogenic component of vasomotion by 27% compared to baseline. They suggested that insulin directly interacts with insulin receptors on the vascular smooth muscle of the terminal arterioles that control capillary recruitment. The findings, however, do not rule out indirect effects of insulin for example via endothelial mechanisms to cause rhythmic contractions and relaxations of vascular smooth

muscle. The vasoconstrictor α MT that induces an acute state of insulin resistance blocks these vascular actions of insulin suggesting that vascular dysfunction of insulin resistance may involve a specific loss of effect of insulin on the vascular smooth muscle contribution to vasomotion in skeletal muscle.

In contrast to our results, Gryglewska *et al.* [70] found that in patients with masked hypertension the skin flow motion was characterized by higher power spectral density values of sympathetic and myogenic origin than in truly normotensive subjects.

Conclusion, the microvascular dysfunction is a hallmark in our results that may be a potential factor explaining the clustering of several components of the metabolic syndrome such as hypertension, obesity, and insulin resistance. Our results strongly suggest that targeting micro vascular and endothelial dysfunctions in patients with metabolic syndrome might help to prevent cardiovascular morbidity in those patients.

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