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Insulin Sensitization and Resistance Interrelationship in a Prediabetic Rat: A Quantitative Molecular Model

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Abstract

Aims: To establish the insulin sensitization and resistance interrelationship through the quantitative modeling of number of insulin receptor molecules per molecule of insulin as response to plasma glucose levels.

Methods: Fenugreek seeds (FGS) powder and its soluble dietary fiber (SDF) were fed through diet to 50 day old prediabetic obese rats (WNIN/GR-Ob) for nine weeks. Fasting plasma glucose (FPG), fasting plasma insulin (FPI) levels and number of erythrocyte insulin receptors (NO-EIR) were determined. Data were analyzed for a) homeostatic model assessment for insulin resistance (HOMA-IRS), b) total number of insulin molecules in the plasma, c) total NO-EIR in one ml of blood and d) ratio of NO-EIR per molecule of FPI.

Results: The results showed a significant ($p<0.05$) reduction in FPG and the number of insulin molecules in plasma and a significant ($p<0.05$) increase in NO-EIR in one ml of blood of experimental rats compared to control rats. The NO-EIR per molecule of FPI was significantly ($p<0.05$) higher in all experimental rats ranging from 9 to 71 fold in males and 17 to 199 fold in females as comparison with control rats. Feeding of the SDF/FGS showed improved efficacy of insulin receptor molecules per molecule of insulin. Among them the NO-EIR per molecule of insulin was significantly ($p<0.05$) greater in rats fed with SDF in both the genders when compared to rats fed with FGS. This effect was seen in the HOMA–IRS levels.

Conclusions: It appears that insulin sensitivity could be defined as molar ratio of available NO-EIR molecules per molecule of FPI. When the molar ratio of receptor per molecule of insulin was higher, the sensitization was greatest. If the number of insulin receptors per molecule of insulin is least, it is resistance. Obese rats fed with FGS/SDF showed improvement in plasma glucose levels by increasing the insulin sensitivity.

Keywords: Fenugreek seeds; Soluble dietary fiber; Hypoglycemic effect; Obese rat; Insulin; Insulin receptors; Insulin sensitization; Insulin resistance

Abbreviations: EIR: Erythrocyte Insulin Receptor; FGS: Fenugreek Seeds; FPG: Fasting Plasma Glucose; FPI: Fasting Plasma Insulin; HOMA-IRS: Homeostatic Model Assessment for Insulin Resistance; NCLAS: National Centre for Laboratory Animal Sciences; NIDDM: Non Insulin Dependent Diabetes Mellitus; NIN: National Institute of Nutrition; NO-EIR: Number of Erythrocyte Insulin Receptors; PCV: Packed Cell Volume; RBC: Red Blood Cells; RIA: Radioimmunoassay; RRA: Radio Receptor Assay; SDF: Soluble Dietary Fiber

Introduction

Insulin and its receptor play important role in the homeostasis of plasma glucose [1-5]. Based on its mechanism of action, various drugs have been developed, called insulin secretagogues, which stimulate beta cells of pancreas for a) secretion of additional insulin e.g. sulphonylureas and b) insulin sensitizers e.g. metformin. The sensitizers increases action of the existing insulin and facilitate greater uptake of glucose from plasma [6-8]. Hence they are called insulin sensitizers.

Insulin sensitization is commonly understood as glucose clearance from plasma without additional inputs of insulin. In contrast, insulin resistance is thought to be poor glucose clearance despite presence of high amounts of insulin [9]. Several laboratories have elucidated the molecular mechanism of insulin action. However, the interrelationship between insulin sensitization and resistance at the quantitative molecular level has not been reported so far [10]. The simplistic assumption is that, one molecule of insulin binds with one molecule of its receptor and this binding was linear with concentration to insulin. It could be conceived that when the insulin levels are higher, its binding with the receptors will be greater leading to enhanced glucose transport. However, in type II non-insulin dependent diabetic mellitus (NIDDM), despite of presence of adequate insulin, there is poor clearance of plasma glucose, leading to hyperglycemia [11].

For clear understanding of insulin sensitization and resistance the following four possibilities can be considered: 1) Insulin molecules might undergo structural change to facilitate greater binding with its receptors and allow more glucose entry into tissue. 2) The receptor molecule might undergo a structural change. 3) The number of insulin molecules might increase. 4) There could be a quantitative change in the number of insulin receptors per molecule of insulin.

On examination of the possibilities it is observed that there is no so far reported evidence of any change in the structure of insulin or insulin receptors in literature during sensitization. The third possibility of increase in the number of molecules of insulin does not arise as per definition. Thus, the fourth possibility may be an increase in the

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molar ratio of 'insulin binding sites for molecule of insulin'. Hence, we examined this possibility and focused our present study on it.

For insulin sensitization, metformin is a commonly used drug for treating type 2 diabetes. As against these sensitization drugs, diet plays an important role in controlling glucose levels. Fiber foods and gums were found to bring glycemic control in diabetic subjects. Fenugreek seed is one such rich source of soluble fiber and regularly used in culinary dishes in Asian countries. Extensive work done in our National Institute of Nutrition on humans by Sharma and others had shown the usefulness of inclusion of FGS and its components in the diet, on reducing blood sugar. Further, the isolated soluble fiber is shown to be the principle component for lowering the glucose levels. Apart from reduction in glucose, it is reported that fiber reduces circulating insulin levels [12,13]. All these findings are attributed due to viscosity generated by the fiber and which in turn slows down digestion and absorption of glucose from intestine by Madar and Shomer, 1990 [14]. Further studies by Raghuram et al, 2004 on the effect of FGS on intravenous glucose disposition in NIDDM subjects showed that there is a significant reduction in plasma glucose and increase in red blood cell insulin receptors [15]. These findings were interpreted as sensitization of insulin.

Hence, feeding of FGS powder and isolated fiber were chosen for conducting careful controlled study in a prediabetic obese rat model established at our center. These are models for type II diabetic rats (showing hyperglycemia, hyperinsulinemia, hypercholesterolemia and hypertriglyceridemia) [16,17], and are used in the present study for finding the quantitative interrelationship between insulin and insulin receptor to explain insulin sensitization and resistance.

Udayasekhararao et al., had used two different levels of FGS, 10% and 20% in the diet fed to rats for their study [18]. Hence in the present study, in tune with the equivalent levels of two doses of FGS (10 and 20%) and SDF (2.5 and 5%) were used separately as dietary method of controlling plasma glucose levels.

Materials and Methods

Isotope and plant material

Radioisotope Na ^{125}I and A-14 tyrosyl ^{125}I monoiodoinsulin (2000 Ci/millimole) were obtained from Board of Radiation and Isotope Technology (BRIT), Mumbai, India and Amersham, UK, respectively. Twenty five kilograms of FGS were purchased from local super market, cleaned, powdered in cyclone mill and used for isolation of SDF fraction.

Preparation of experimental diets

The experimental diets were casein based and prepared according to Reeves et al. [19]. Control diet consisted vitamin mixture, salt mixture, cellulose, casein, oil, choline chloride and starch. FGS was incorporated at 10% and 20% levels along with the other constituents. Similarly in case of SDF at 2.5% and 5% incorporated in the diet. The composition of the five diets is given in Table 1. Based on the earlier study at our institute the above levels of FGS/SDF were incorporated in the diet [18]. The diets were made isonitrogenous by adjusting 20% protein level.

Experimental animals

Prediabetic obese rats (WNIN/GR-Ob) were obtained from National Centre for Laboratory Animal Sciences (NCLAS) at NIN, Hyderabad, India, after due approval from the Institute Animal Ethics Committee (IAEC). Before the start of the experiment, animals were screened for their fasting plasma glucose (FPG) and fasting plasma insulin (FPI) levels. Animals with FPG 6mmol/L or more were taken for the experiment.

Experimental design

The study was conducted in 50-day-old WNIN/GR-Ob rats (males 319g & females 278g) and the feeding period was for 9 weeks. Fifty-five (thirty males and twenty five females) obese rats were divided into following five groups; Control (Group I), 10% FGS (Group II), 2.5% isolated SDF fraction (Group III), 20% FGS (Group IV), and 5% isolated SDF fraction (Group V). Each group comprised of six males and five females. The rats were housed individually in plastic cages with grilled bottoms and diet and water were provided ad-libitum for 9 weeks and the following parameters were monitored. Daily food intake, weekly body weights, FPG levels at initial, three, six and nine weeks and FPI levels at initial and nine weeks of feeding. Erythrocyte insulin receptor (EIR) (A-14 ^{125}I-insulin binding) binding assay was performed after nine weeks.

Methodology

The SDF fraction was isolated from FGS according to Dea and Morrison 1975 [20] and analyzed for insoluble, soluble and total dietary fiber contents by enzymatic and gravimetric method of AOAC [21]. The isolated SDF fraction showed moisture 8.85%, protein 6.51%, fat 0.98%, ash 1.17%, total dietary fiber 82.49%, insoluble dietary fiber 15.58% and SDF 66.91% and this was used in preparation of experimental diets.

FPG levels were determined by glucoseoxidase method [22]. Radioimmunoassay (RIA) of FPI was performed according to Yalow and Berson 1960 [23]. Homeostatic model assessment for insulin resistance (HOMA-IRS) was calculated using FPG and FPI values [24,25]. Radio receptor assay (RRA) of insulin binding of erythrocytes was determined according to Gambhir et al. and Robinson et al. [26,27]. The methodology involves two steps: 1) number of insulin receptors per RBC 2) calculation of total number of erythrocyte insulin receptors (NO-EIR) in one ml of blood.

Procedure for step 1: Erythrocytes were purified using Ficoll-Histopaque solution and RBC pellet was diluted with buffer containing 50mM HEPES, 50mM Tris, 10mM $MgCl_2$, 2mM EDTA, 10mM $CaCl_2$, 50mM NaCl, 5mM KCl, 10mM Dextrose and 0.1% BSA. RBC pellet was diluted with buffer pH 7.0. RBC was counted in a neurocytometer,

Ingredient	Dietary treatments (g/kg diet)				
	Group-I (Control)	Group-II (10% FGS)	Group-III (2.5% SDF)	Group-IV (20% FGS)	Group-V (5% SDF)
Vitamin mix	10	10	10	10	10
Mineral mix	40	40	40	40	40
Groundnut oil	50	50	50	50	50
Cellulose	50	50	50	50	50
Casein	290	255	290	220	290
SDF	-	-	25	-	50
FGS	-	100	-	200	-
Corn starch	558	493	533	428	508
Choline chloride	2	2	2	2	2
Protein (%energy)	20	20	20	20	20

FGS: Fenugreek seeds; SDF: Soluble dietary fiber fraction isolated from fenugreek seeds

Table 1: Composition of diets fed to obese rats for 9 weeks.

Group	Males		Females	
	Initial	Final	Initial	Final
Group I (Control)	17.27±1.16	27.26±0.66	57.08± 1.96	114.32±6.58
Group II (10% FGS)	19.92±2.53[a]	7.28±0.39[b]	62.07±11.49[a]	20.17±0.9[b]
Group III (2.5% SDF)	20.36±2.53[a]	7.06±0.30[b]	62.87±10.09[a]	8.36±0.33[b]
Group IV (20% FGS)	18.00±0.86[a]	4.83±0.17[b]	63.87±14.25[a]	7.61±0.19[b]
Group V (5% SDF)	19.97±1.19[a]	4.28±0.12[b]	63.87± 5.93[a]	4.72±0.23[b]

Values are mean ± SD (males 6 and females 5) in each group. Values bearing different superscripts i.e. 'a' and 'b' are statistically different by Duncan's multiple range test at p<0.05 for males and females respectively. Incorporation of FGS/SDF (Group II-V) in the diet to obese rats significantly (p<0.05) decreased HOMA-IRS levels compared to their initial HOMA-IRS levels. Where as in control rat's HOMA-IRS levels were significantly (p<0.05) increased. FGS: Fenugreek seeds; SDF: Soluble dietary fiber

Table 2: Effect of SDF/ Seeds of fenugreek on HOMA-IRS levels in obese rats.

Treatment	Males	Females
Control	29±16.0[a]	23±14.3[a]
10%FGS	121±35.0[b]	152±58.2[b]
2.5% SDF	306±96.5[b]	216±115.5[b]
20%FGS	216±115.5[b]	234±109.6[b]
5%SDF	573±287.1[b]	388±42.2[b]

Number of red blood cells has been taken as 7 million per Cubic milli meter. Values are mean ± SD. No. of males 6 and female 5 in each group. Values bearing different superscripts i.e. 'a' and 'b' are statistically different by Duncan's multiple range test at p<0.05 for males and females respectively. Supplementation of both levels of SDF or FGS in the diet for nine weeks to obese rats significantly (p<0.05) increased the insulin receptors in erythrocytes of experimental rats compared to control rats. FGS: Fenugreek seeds; SDF: Soluble dietary fiber

Table 3: Number of ($x10^{17}$) of insulin receptors in erythrocytes per ml of blood at the end of 9 weeks.

Group	Males		Females	
Treatment	Initial	9th week	Initial	9th week
Control	104±51.7	188±63.3	241±112.1	727±425.3
10%FGS	176±143.9[a]	88±51.2[b]	627±647.8[a]	279±479.8[b]
2.5% SDF	176±134.6[a]	88±65.9[b]	794±680.8[a]	108±74.4[b]
20%FGS	78±37.5[a]	38±23.2[b]	463±610.0[a]	49±24.5[b]
5%SDF	162±132.5[a]	53±23.1[b]	588±655.4[a]	63±54.8[b]

Packed cell volume has been taken as 45% and plasma volume 550 micro liters in blood of all animals. Values are mean ± SD. No. of males 6 and female 5 in each group. Values bearing different superscripts i.e. 'a' and 'b' are statistically different by Duncan's multiple range test at p<0.05 for males and females respectively FGS: Fenugreek seeds; SDF: Soluble dietary fiber

Table 4: Number ($x10^9$) of insulin molecules in the plasma obtained from one ml blood of obese rats.

diluted to a concentration 4.0 $x10^9$ cells/ml and used for erythrocyte receptor assay. One hundred micro liters of purified erythrocyte suspension (0.75-1.5 $x10^9$ cells/ml) were incubated at 15^0c for 30 minutes in the presence of 16.7 p moles/L of A-14 ^{125}I Insulin (specific activity 2000Ci/mmol) (30000cpm) and increasing concentrations of cold insulin in the physiological range of 16.7-1670 p moles/L. The incubation was terminated by the addition of 2.7 ml of normal saline. The tubes were centrifuged at 1500g for 15 minutes at 4°C and the supernatant removed by aspiration. The pellet was washed once again with cold saline as mentioned above. To the pellet hundred micro liters of 50% formaldehyde was added to harden RBC. The pellet was washed with normal saline, centrifuged, supernatant aspirated and tubes were inverted on a filter paper to drain and the internal surface wiped to dryness with tissue paper wicks completely and the radioactivity of the pellet was counted in the Gama counter. The total binding was calculated the percentage of radioactive insulin bound by the RBC in the absence of non radioactive insulin. Non specific binding was assessed by the radioactive insulin bound in the presence of thousand fold excess of non radioactive insulin. Difference between the total and non specific binding was considered. Competitive binding curves were obtained for each red blood cell suspension. From these curves the number of insulin receptor sites in femato moles per cell was calculated.

$$\text{Erythrocyte insulin receptor sites / cell } \left(\text{f moles / RBC}\right) = \frac{\text{Total binding} - \text{NSB / 2200 x mole}}{\text{Number of erythrocytes}}$$

Mole=$6.023x10^{23}$molecules; 2200dpm=1fmole; NSB=Non specific binding; Erythrocytes concentration= (0.75-1.5 $x10^9$ cells/ml).

Step 2: Calculation of total number of erythrocyte insulin receptors (NO-EIR) in one ml of blood. This involves the following sequence: a) the number of red blood cells (RBC) in one micro liter blood was obtained by counting the RBC in the field under the microscope (i.e., 7 x 10^6). b) Multiplication of the RBC in one micro liter with 1000, this will give the RBC in one ml. (7 $x10^6x10^3$=7 $x10^9$). c) Insulin receptors per erythrocyte obtained in step 1 (as femato moles) multiplied with total RBC count per ml of blood. One femato mole corresponds to $6.023x10^8$ molecules of insulin receptor sites (one femato mole is equal to 1 x 10^{-15} x 6.023x 10^{23} (Avogadro number) = 6.023 $x10^8$ molecules). d) Total number of EIR is equal to number of femato moles of receptors per erythrocyte multiplied by total number of erythrocytes. For examples if receptors 5femato moles/RBC=5femato moles x $6.023x10^8$ x $7x10^9$ RBC in one ml of blood = 210.805 x 10^{17}. The total number of ($x10^{17}$) of insulin receptors in erythrocytes per ml of blood at the end of 9 weeks in each treatment is given in Table 3.

For understanding of the fundamental interrelationship of insulin and its receptor, the simplistic assumption of one molecule of insulin binds with one molecule of its receptor was made and the data obtained was re-analyzed. This was done in the following three steps: I) determination of the total number of insulin receptor molecules on erythrocytes in one ml blood arrived mentioned above and II) calculating total number of insulin molecules in the plasma obtained from one ml of blood. III) Calculation of insulin sensitivity and resistance index.

Treatment	Total receptor molecules / total insulin molecules	No. receptor molecules per molecule of insulin
Males		
Control	29 $x10^{17}$/ 0.188 $x10^{12}$	153 $x10^{5a}$
10%FGS	121$x10^{17}$/ 0.088 $x10^{12}$	1373 $x10^{5b}$
2.5% SDF	306 $x10^{17}$/ 0.088 $x10^{12}$	3491$x10^{5b}$
20%FGS	216 $x10^{17}$/ 0.038 $x10^{12}$	5765 $x10^{5b}$
5%SDF	573 $x10^{17}$/ 0.053 $x10^{12}$	10839 $x10^{5b}$
Females		
Control	23 $x10^{17}$/ 0.727 $x10^{12}$	31 $x10^{5a}$
10%FGS	152 $x10^{17}$/ 0.279 $x10^{12}$	544 $x10^{5b}$
2.5% SDF	454 $x10^{17}$/ 0.109 $x10^{12}$	4189 $x10^{5b}$
20%FGS	234 $x10^{17}$ / 0.049 $x10^{12}$	4803 $x10^{5b}$
5%SDF	388 $x10^{17}$ / 0.063 $x10^{12}$	6159 $x10^{5b}$

Each group consists of 6 males and 5 females. Values bearing different superscripts i.e. 'a' and 'b' are statistically different by Duncan's multiple range test at p<0.05 for males and females respectively. Treatment with both the levels of SDF or FGS in the diet for nine weeks to obese rats showed a significant (p<0.05) increase in insulin receptors per molecule of insulin in all the experimental rats compared to control rats. FGS: Fenugreek seeds; SDF: Soluble dietary fiber

Table 5: Insulin sensitivity and resistance index: expressed as a ratio of number of receptors on erythrocytes per molecule of insulin in plasma of obese rats at the end of 9 weeks.

Treatment	Ratio	Value/ Control	Value/10%FGS	Value/2.5%SDF	Value/20%FGS	Value/5%SDF
Males						
Control	153 x10^5	-1-	-	-	-	0.014
10%FGS	1373 x10^5	8.97	-1-	-	-	0.130
2.5% SDF	3491 x10^5	22.8	2.54	-1-	-	0.320
20%FGS	5765 x10^5	37.7	2.19	1.65	-1-	0.530
5%SDF	10839 x10^5	70.8	7.89	3.10	1.88	1-
Females						
Control	31 x10^5	-1-	-	-	-	0.005
10%FGS	544 x10^5	17.5	-1-	-	-	0.088
2.5% SDF	4181x10^5	134.9	7.68	-1-	-	0.680
20%FGS	4803 x10^5	154.9	8.82	1.15	-1-	0.780
5%SDF	6159 x10^5	198.7	11.32	1.47	1.28	-1-

Each group consists of 6 males and 5 females. Feeding of either two levels of FGS or SDF in the diet to obese rats showed an increase in the insulin receptors per molecule of insulin in all the experimental animals compared to control animals. It is also observed that the prevalence of insulin resistance in control rats. FGS: Fenugreek seeds; SDF: Soluble dietary fiber

Table 6: Efficacy of insulin receptors per molecule of insulin in different treatments.

Calculating the number of insulin molecules in one ml of blood: a) for this purpose first the packed cell volume (PCV) was determined. The PCV volume was subtracted from one ml blood and this gave plasma volume in one ml of blood. b) The number of insulin molecules in the plasma volume was calculated by multiplying with the number of micro units of insulin obtained by RIA with a factor 41.83x10^8; (since one micro unit of insulin corresponds to 6.945 x 10^{-15} x 6.023 x 10^{23} (Avogadro number)=41.83x10^8 molecules).This was shown in Table 4.

Calculation of insulin sensitivity and resistance index: The total number of (NO-EIR) receptor molecules in one ml of blood obtained in step-I, was divided by the total number of insulin (FPI) molecules in the plasma of one ml of blood arrived in step-II. This has been expressed as a ratio of number of receptors on erythrocytes per molecule of insulin in plasma of obese rats at the end of 9 weeks was shown in Table 5.

Efficacy of insulin receptors per molecule of insulin in different treatments: The ratio of number of receptors on erythrocytes per molecule of insulin in plasma of each experimental group was divided by the ratio of number of receptors on erythrocytes per molecule of insulin in plasma of control group which gave the efficacy value for each experimental group (as expressed insulin receptors per molecule of insulin) shown in Table 6.

Statistical analyses

The data was analyzed appropriately by one way and/or two way analysis of variance (ANOVA), paired 't' test, Duncan's multiple range test and/or non-parametric test of Wilcoxon signed ranks using SPSS/PC+ package for statistical significance [28].

Results

Fasting plasma glucose

FPG (m moles/L) levels in male and female obese rats shown in Figure 1. After three weeks of feeding, a significant ($p<0.05$) decrease in FPG levels was observed in group V fed females only. However, after six weeks of feeding, a significant ($p<0.05$) decrease in FPG levels was observed in all experimental groups irrespective of gender. However significant ($p<0.05$) increase in FPG was seen in control (Group I) animals compared to their initial FPG values. At the end of 9 weeks, the FPG levels were significantly ($p<0.01$) decreased in comparison with initial levels by 26 and 35% in group II, 30 and 42% in group III, 36 and 39% in group IV, 35 and 44% in group V males and females respectively, while the levels rose significantly ($p<0.05$) in control (25 and 23%) in males and females, respectively.

Fasting plasma insulin

The FPI (micro units/ml) levels in obese rats shown in Figure 2. After 9 weeks of feeding the respective diets, there was a significant ($p<0.05$) decrease in FPI levels in all experimental groups compared to initial levels in both the genders, more drastic in females (50-89%), than in males (51-67%) while significant ($p<0.05$) increase in the control (group I) animals.

Homeostatic model assessment for insulin resistance

Female obese rats had significantly high HOMA-IRS values initially than males in experimental rats and to the control rats of both genders, the values increased significantly ($p<0.05$) by 9 weeks of feeding. On the other hand there was a significant ($p<0.05$) decrease in HOMA-IRS values of all experimental rats of both genders by 9 weeks of feeding and the decrease was highly marked in females than males (Table 2).

Erythrocyte insulin receptor

The EIR (f moles/cell) of male and female rats obtained in the present study have shown in Figure 3. After 9 weeks of feeding experimental diets to obese rats, the EIR numbers per erythrocyte significantly ($p<0.05$) increased in experimental rats (7-15 fold increase) whereas no change was observed in control rats.

Total NO-EIR and FPI molecules in one ml of blood and their interrelationship

Results on total NO-EIR molecules per ml of blood at the end of ninth week for males and females have shown in Table-3. There was a drastic increase in NO-EIR in all the treatments ranging from 121x10^{17} to 573 x10^{17} in males and 152 x10^{17} to 388x10^{17} in females compared to control males and females respectively, which are 29 x10^{17} and 23 x10^{17}. This corresponds to 4-20 folds increase in males and 7-17 folds increase in females compared to control groups.

Results of the total number of insulin molecules in plasma obtained from one ml blood are shown in Table 4. At the end of ninth week treatment with different doses of FGS/SDF indicated that the total number insulin molecules dropped drastically ranging from 88x10^9 to 53x10^9 in males and 279x10^9 to 63x10^9 in females compared to 188x10^9 and 727x10^9 in the respective control males and females. This reduction in number of insulin molecules in experimental group rats corresponds to 53-80% in males and 62-93% in females compared to control males and females at the end of experiment.

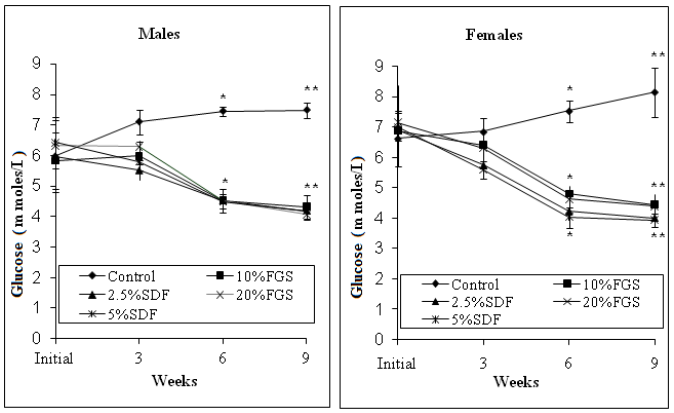

Figure 1: Effect of SDF fraction/seeds of fenugreek on fasting plasma glucose levels in obese rats. Values are mean ± SD (males 6, females 5 in each group). Feeding of two levels of FGS or SDF fraction in the diet to experimental group rats respectively after nine weeks showed significant (p<0.01) decrease in fasting plasma glucose levels when compared to control rats. *p <0.05; **p<0.01 paired't' test. FGS: Fenugreek seeds; SDF: Soluble dietary fiber.

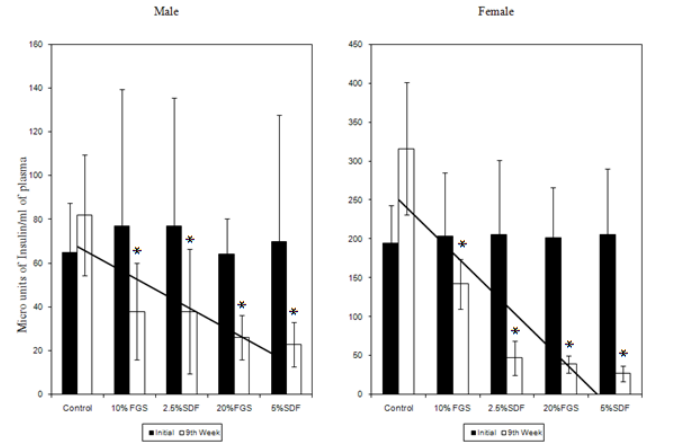

Figure 2: Effect of SDF/Seeds of fenugreek on fasting plasma insulin levels in obese rats. Values are mean ± SD (males 6, females 5 in each group). Feeding of both levels of SDF or FGS in the diet for nine weeks to obese rats resulted significant (p<0.05) reduction in fasting plasma insulin levels in experimental group rats when compared to control group rats. FGS: Fenugreek seeds; SDF: Soluble dietary fiber.

Results of insulin sensitivity and resistance index expressed as a ratio of the total NO-EIR per molecule of insulin in plasma of obese rats at the end of ninth week are shown in Table 5. The NO-EIR per molecule of insulin significantly (p<0.05) increased in all the experimental groups and ranged from 1,373 x10^5 to 10,840 x10^5 in males and 544 x10^5 to 6,160 x10^5 in females compared to control males and females which was 153 x10^5 and 31 x10^5.

Results of the efficacy of insulin receptors per molecule of insulin in different treatment are shown in Table 6. Treatment with FGS/SDF to obese rats resulted increase in efficacy 9 to 71 fold in males and 17 to 199 fold in females. The highest increase being in rats fed with 5%SDF in both the genders. Difference in the effect of treatment between males and females could conceivably due to their hormonal differences.

Discussion

The results of present study showed that on feeding of FGS or SDF mixed with diet for a period of nine weeks to type II prediabetic model rats (WNIN/GR-Ob), resulted in a significant (p<0.01) decrease in FPG (Figure 1) and the number of insulin molecules (Figure 2) compared to control rats. Maximum decrease occurred in animals fed with 5% SDF. These results are in conformity with studies on humans and animals [13,29-31].

The mechanism of the glucose reduction can be due to the fiber content in the FGS and particularly the SDF rich in galactomannans. The viscosity generated by the SDF containing galactomannan slows down the digestion process of starch [32,33,14]. Further, it also contributes to slow diffusion of glucose from intestine to blood. As a result, the levels of glucose in the blood are low and this lowered glucose levels causes less secretion of insulin. Secretion of insulin normally depends on the levels of glucose. Insulin secretion is proportional to plasma glucose itself. This is because glucose itself is an insulinogogue [10].

The studies on erythrocytes and monocytes have been widely used as tools in clinical studies of insulin receptors and receptor data of blood cells have been extrapolated to target cells for insulin by earlier workers. The validity of extrapolation has been set to be supported by several studies like, Soll et al. and Olefsky et al. [34,35]. So, we have used EIR as represented to the target tissues and for assessing insulin sensitivity as shown by several workers using metformin [5,8,36-38]. The second finding was a significant (p<0.05) increase in total number of EIR molecules per RBC and the raise was as high 7-15 fold in experimental animals compared to control animals (Figure 3).

The key molecules involved, in glucose uptake by the target tissue, are insulin, insulin receptor and their interaction is mole to mole. We analyzed the data obtained in each treatment for insulin and its receptor in one ml of blood to arrive at a quantitative interrelationship between them and relate it to glucose levels. A computational finding was that the total number EIR molecules per molecule of insulin showed a marked increase in the experimental groups, which were treated with the FGS/SDF mixed diet compared to control group. The ratio of EIR to insulin was high for all the experimental rats compared to control rats.

The order of increase of receptors to insulin was approximately 9

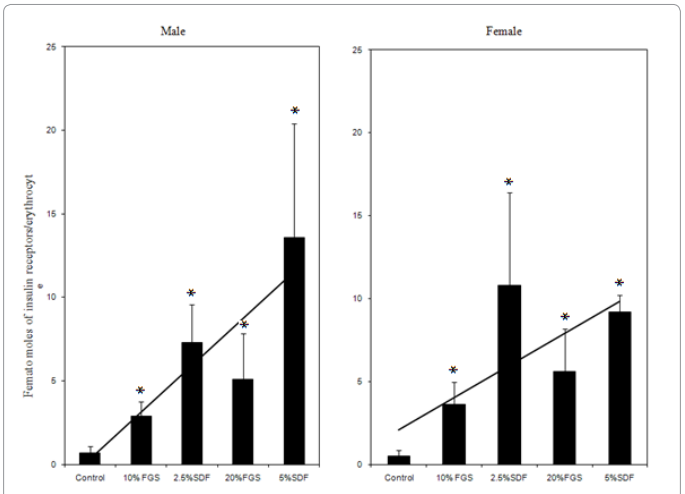

Figure 3: SDF/Seeds of FGS effect on erythrocyte insulin receptor binding in obese rats. Values are mean ± SD (males 6, females 5 in each group). Feeding of both levels of SDF or FGS in the diet to obese rats had significantly (p<0.05) increased the insulin receptors per erythrocyte. FGS: Fenugreek seeds; SDF: Soluble dietary fiber.

to 71 fold in males and 17 to 199 fold in females depending on the type of treatment compared to controls (Table 5 & Table 6). This significant increase in the ratio can be due to two reasons. There was a significant ($p<0.05$) increase in the number of insulin receptors and also a substantial decrease in the amount of insulin. Thus, for lower number of molecules of insulin there were an increased number of receptors available. This increased number of receptors with decreased number of insulin molecules had better interaction leading to higher transport of glucose into the cell. This effect can be seen in the HOMA–IRS levels (Table 2; Figure 1). This can be called sensitization. Conversely in the control group the ratio of EIR/FPI to was low and glucose levels were high. Low amount of EIR and an increased amount of FPI molecules contribute this low ratio. This can viewed as insulin resistance. Lowered EIR despite higher amounts of FPI presence may not lead to effective clearance of glucose.

Given the reduced number of receptors availability for interaction to the increased amount of circulating insulin, the chance of the interaction of receptor with ligand becomes less. This is primarily because of the internalization of the receptor and ligand complex leading to a lower availability of surface receptors. The effect is fewer interactions (despite high levels of circulating insulin molecules) leading to hyperglycemia and hyperinsulinemia [39-40].

Thus, insulin resistance is but non-availability of enough surface receptors for the action of insulin. The combined effect of such interaction can alone influence the plasma glucose levels. This type of mechanism of insulin sensitivity and resistance is in tune with the modern concept of endocrinology where the action is inversely proportional to the ligand and receptor concentrations [39]. This phenomenon stated means, higher is ligand less is the number of receptors and converse is true. The action is not directly proportional as originally thought. Thus, hyperglycemia in type II diabetes can be viewed as non-availability of enough EIR and not lack of insulin per se probably this is the reason why clinicians prefer advising metformin, a known sensitizer to newly diagnosed type II diabetic patients. In terms of molecular physiology there is an up-regulation or increase of receptors/binding sites when the ligand (insulin) is very less. This leads to more glucose clearance from plasma as even for single molecule of insulin more receptors are available for binding to enable the glucose transport into the cells. This can be interpreted as sensitization. In contrast in case of down regulation of receptors, the availability of binding sites on the cell surface decreases per molecule of insulin (ligand) and hence the reaction reduces, leading to less transport of glucose into the cells. This can be viewed as resistance where there is too much ligand (insulin). This type of up regulation and down regulation relation to the ligand concentration is well documented in cell culture experiments [40,41].

When the reaction is mole to mole, it can be asked, why more receptors are necessary? This can be understood for example, from animal breeding experiments. For getting more litters of pups in a limited period of time the breeder keeps higher ratios of females to males. The reason being that once the female is mated and conceived, the females have to undergo periods like gestation, delivery, lactation and get ready for the next cycle. Thus, there is lag period. To increase productivity, if females are more, then the litter obtained is more for fixed duration of time. Similarly on interaction of insulin molecule with its receptor, the complex gets internalized, forms coated pits for degradation of hormone, then gets recycled to membrane surface and gets integrated for next interaction with the ligand. Hence there is lag in the interactions.

The other possible reason would be due to rheological properties. While the receptor is membrane bound and fixed, the ligand is in constant flow/circulation in association with several big and small molecules. Hence the probable chance of binding would increase if the number of receptors is more and reduced if the receptors are less. In this current study it was observed that among the various treatments the isolated SDF is more effective as the available number of surface receptors is high. Consequently, the reduction in glucose was higher in all the groups are fed with FGS/SDF.

Thus, fundamental difference in type II diabetes lies with the non-availability of enough surface receptors and not lack of ligand (insulin). In contrast, in type I diabetes there is inadequacy of insulin (ligand) [11]. Basically, the cell of the body increases the number of receptors to a hormone when it chemically detects a low concentration of those molecules. The up-regulation/increase in binding sites is what makes cells sensitive to the ligands.

Sensitization mechanism of metformin could be due to the inhibition of gluconeogenisis leading to lowered levels of plasma glucose and consequently lowered insulin secretion. In conclusion the mechanism of insulin sensitization may have a practical clinical implication, probably reducing the indiscriminate use of insulin and insulin secretagogues, which down-regulates the receptors while encouraging dietary method of sensitization for effective glucose clearance from blood.

Most interestingly, the epidemic-like nature of the type II diabetes among large population of highly polished rice eaters in southern states of India could possibly be due to lack of fiber and lack of up-regulation of insulin receptors [42,43]. This can viewed as a consequence of fast entry of glucose and constant demand on the beta cells, thus causing "insult to the insulin secreting cells" and release of insulin, leading to insulin resistance. This constant higher glucose increase due to starch digestion leads to higher secretion insulin leading to "pancreatic exhaustion" eventually leading diminished function over period time. This may result may show as initially impaired glucose tolerance (IGT) and then to type II diabetes. Thus, there is paradigm shift in the understanding of entire scenario from more insulin (type II) to no insulin in type I diabetes.

With these observations, it is tempting to speculate on the pathogenesis of type II diabetes. It is observed that in type II diabetic patients, the gluconeogenisis goes up by three fold [8]. The glucose released into blood is itself an insulinogogue. Thus, leads to higher circulating insulin. The circulating higher insulin down regulates the insulin receptors with poor clearance of plasma glucose leading to hyperglycemia. This can be corrected by using metformin, which is a gluconeogenisis inhibitor [38].

The ultimate question is what causes the three-fold increase in the gluconeogenisis (which is inhibited by metformin) in the type II diabetes? We propose that there is endogenous metformin-like molecule. The expression of these molecules gets truncate/reduce slowly with age leading to increased gluconeogenisis. The identification of endogenous metformin-like molecules (as in case of detection of endorphins, enkaphelins) would pave way for understanding the genesis of type II diabetes. We further hypothesize that this molecule is genetically controlled as well as by diet. Experiments are being conducted for the identification of endogenous metformin-like molecules *in vivo*.

The above results showed that it is SDF (galactomannan) that exerted highly significant effect than FGS powder. It means that

insulin sensitivity is high in animals given 5% SDF. We found that for every molecule of insulin there are a large number of insulin receptor molecules. Since in many patients the fasting glucose is estimated along with insulin, apart from these parameters, if EIR is also estimated in RBC, the sensitization can be arrived by calculating the EIR molecules per molecule of FPI. This can form a good diagnostic tool for effective treatment of diabetic patient and arriving at insulin resistance.

Thus, it appears that insulin sensitivity can be defined as molar ratio of available EIR molecules per molecule of FPI. Higher is the molar ratio of receptor per molecule of insulin, greater is the sensitivity and lesser is the number of receptors per molecules of insulin it is resistance. Thus, the fenugreek seed powder or soluble fiber could be used from early stages of dietary plan for sensitization of insulin to control plasma glucose levels instead of using drugs. Since fenugreek seed is regularly used as condiment in the culinary practice, it would be safe to advocate in the management of type 2 diabetes as a non pharmacological method right from the early stage of detection of diabetes instead of banking on drugs. Lack of soluble dietary fiber in the diet could eventually lead to insulin resistance and thus aggravate the disorder.

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Duality of Interest

The authors declare that there is no duality of interest associated with this manuscript.

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