Establishment of Mouse Model for Obesity and Type 2 Diabetes and Expression of Secreted Frizzled Related Protein 4 (SFRP-4) As A Biomarker

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Abstract

Type 2 diabetes is a metabolic, progressive and chronic disease associated with β-cell dysfunction and insulin resistance that leads to a decrease in insulin production followed by a decrease in insulin secretion. The main objective of the study was to develop a mouse model for type 2 diabetes that resembles most of the features of human T2D along with SFRP4 expression. Initially, all parameters to be studied in the research were measured and recorded prior to the start of the experiment. In order to accomplish this model, two groups of mice were fed high fat diet (HFD) and two groups were fed low fat diet (LFD) and tap water for a period of twelve weeks. Mice exposure to HFD has shown diet-induced obesity. At the end of the feeding period, the serum lipid profile including triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and total cholesterol (TC) of all experimental animals was evaluated. Significant increase in serum levels of TG, TC and LDL while low serum HDL levels were detected in HFD-fed mice serum. Similarly, the serum-SFRP4 level of all animals was measured using the ELISA kit. The serum level of SFRP4 was shown to be elevated (P < 0.01) in mice fed with HFD compared to control objects (P < 0.01). SFRP4 was associated with a high risk of developing type 2 diabetes. In conclusion, HFD dietary manipulation induces obesity, hyperglycemia, abnormal lipid profile and significant SFRP4 levels that ultimately lead to type 2 diabetes. SFRP4 may be a potential biomarker for early detection of type 2 diabetes.

Keywords: Type 2 diabetes; High and low-fat diet; Obesity; SFRP4; Lipid profile; ELISA.

INTRODUCTION

There is a massive increase in the number of patients with diabetes and a great challenge for the world's population and global health societies. Type 2 Diabetes (T2D) is caused by inadequate secretion of the pancreatic β-cell receptor or insulin resistance. The key features of T2D are the insulin asset in target tissues and the comparative glucose secretion defect in pancreatic β-neurons. In the preclinical age of illness, β-cell hyper-insulinemia and hyperplasia occur in response to insulin resistance. Due to the inability of the β-cell to pay for insulin resistance, relative glucose impairment develops into an overt T2D [1].

Obesity is a major cause of insulin resistance. Adiposity can reduce insulin resistance to developmental variables and lead to heterogeneity of type 2 diabetes [2]. Impaired insulin secretion is one of the main symbols of type 2 diabetes (T2D) due to a complicated relationship between disabled insulin secretion and decreased insulin sensitivity [3]. A turning point occurs when the secretion of genetically susceptible b-cells does not meet the requirements of peripheral insulin resistance. While it is present in T2D, damaged insulin reactions may be due to a reduced, feasible secretive function in earlier phases and a long-term decrease in B-cell mass [4].

SFRP4 has been shown to have a molecular link between defective insulin secretion and inflammation of the islet. SFRP4 demonstrates its impact on a wide range of genes is affected by Wnt signaling, expressed in the clampdown of two separate Ca2 + voltage channels, and causes a decrease in insulin exocytosis. SFRP4 levels above normal generate
less insulin, resulting in decreased carbohydrate metabolism [5]. As a result, SFRP4 has been shown to increase cellular concentrations of reactive oxygen species in endothelial bodies [6].

SFRP4 was found to be related to the Wnt signaling pathway and inflammatory markers. Interleukin-1β has been reported to have caused the secretion of SFRP4 from islands [7]. Thus, by inhibiting SFRP4 at a certain stage, we can increase the amount of insulin in the human body and reduce the activity of Ca2+ pumps [5]. It is important to note that SFRP4 was seen as a biomarker for the identification of T2D from disease people [8]. Further studies have shown that SFRP4 is an effective phosphatural agent that causes tumors. Since SFRP4 is a binding protein, SFRP4 may be identified several years before the onset of the disorder. SFRP4 is a protein that circulates, has phosphatonin-like characteristics, and promotes hypophosphatemia and phosphaturia [9].

MATERIALS AND METHOD

Animal handling
Six to eight weeks old mice have been used in the study. Male and female Albion mice were obtained from the Department of Pharmacy and Physiology, Government College University, Faisalabad. Mice have been randomly divided into four groups. All animals were kept in usual cages (six mice per cage) under measured room temperature (22°C) with 55±5% humidity in 12 hours of light and 12 hours of dark cycle. Procedures for supervision of mice have been followed and permission to conduct research has been sought.

Experimental design
Mice were divided into four groups, two male and two females. Male groups were labeled G1, G2, while female groups were labeled G3, G4. Group 1 and 3 were fed a normal diet (ND) that is used as a control, while G2 and G4 were fed a high fat diet (HFD). There were six mice in each group.

The mice were assigned to two dietary regimens. Group 1 and 3 are fed with a normal diet (control) and pure drinking water, while Group 2 and Group 4 are fed a high fat diet along with 15% fructose water [10]. The normal chow diet was purchased from the Annie food shop of the Chinese bazaar of Faisalabad. HFD was prepared in our lab by adding oil to the pellet diet. The composition of HFD, LFD and fructose water are shown in Table 1.

| Ingredients     | LFD       | HFD       |
|-----------------|-----------|-----------|
| Fats (g%)       | 12.77     | 43        |
| Carbohydrates (g%) | 45    | 27        |
| Proteins (g%)   | 31.21     | 23        |
| Crude fiber (g%) | 9.52    | 7         |
| Energetic values (k/cal%) | 350 | 563       |
| Drinking water  | No fructose | 15% fructose |

Weight of the mice was assessed and documented 3 to 4 times a week using weight balance throughout the entire study. Glucose level was initially determined as well as after starting dietary treatment and was checked 2-3 times a week by glucometer. Blood was taken intravenously from the tail to determine the glucose level.

Blood was obtained from the veins of the face. Blood was obtained from facial veins. The collected sample was placed on ice for 3 hours. Centrifuged at 3000 rpm for 15mint followed by serum separation [11]. Serum was stored at -40 ° C until further analysis was carried out. SFRP4 levels were measured using the ELISA kit. Micro lab 300, the ELI Tech Group's semi-automated clinical chemical analyzer, was used for lipid profile and biological techniques.

STATISTICAL ANALYSIS
Final data was evaluated statistically by using two-way ANOVA. All data are shown as mean ± SEM and P<0.05 was considered to be significant. In addition, t-tests were performed to assess statistical significance between the HFD and LFD groups. A two-way analysis of the variance table was also carried out to analyze the comparison between two experimental groups [12].

RESULTS
In order to evaluate the effect of diet on both experimental groups, the mice were treated with HFD and LFD. The mouse model was developed by feeding mice for 12 weeks with chow and HFD containing more fat. Mice fed with HFD developed a diet that induced obesity and, ultimately, type 2 diabetes. The results obtained from this model can help us to understand the pathogenesis of type 2 diabetes and the significance of T2D therapy.
BGR Value

The result shows the effect of dietary treatment on all groups that overall HFD-fed mice groups have a higher BGR value compared to ND-fed mice groups as shown in Figure 1. HFD-fed animals are severely hyperglycemic, whereas chow-fed animals are mildly glycemic. Data on serum glucose levels among all experimental groups, first at the beginning of the week and second after 12 weeks of dietary treatment. According to our results, the blood glucose level was low at an early stage before the start of dietary treatment compared to the BGR of 12 weeks of diet, which is very significant (P<0.01). At the initial week, BGR values were not significant (P>0.05), but BGR values became high after dietary treatment (G1: 194.00±11.02b; G2: 209.17±8.21b; G3: 216.33±6.07ab; G4: 246.00±10.75a). Due to consumption of fat content, HFD-mice has a higher BGR value (G1: 209.17±8.21b; G4: 246.00±10.75a) compared to ND-fed mice (G1: 194.00±11.02b; G3: 216.33±6.07ab). Total mean is 216,38±5.84A.

Weight

The weight of all experimental animals was measured at the beginning of the week and after 12 weeks of two experimental dietary treatments to assess body weight gain. Figure 2 shows the body weight of all groups. The weight was found to be highly significant (P<0.01) in HFD animals compared to LFD control animals. Higher total caloric intake in the HFD group resulted in high body weight gain (g) compared to other experimental groups over a 12-week feed period. Compared to all groups, HFD-fed mice showed higher body weight gain than other groups (G1:32.17±1.40bc; G2:35.17±1.01b; G3:29.83±0.70c; G4:43.50±1.78a). Of all the G4 (HFD-F) weight gain (43.50±1.78a) is higher than the other groups which are very significant. Second, the male G2 (HFD-M) had a weight gain (35.17±1.01b) compared to the other two ND-fed groups. Whereas ND-fed mice also show a slight increase in weight with a 12-week diet experiment.
Expression of SFRP4 protein

SFRP4 circulating is expressed more in obese objects (G2: 41.14±2.36a; G4: 44.50±2.39a) than in lean objects (G1: 28.60±3.14b; G3: 30.57±2.89b). The results indicate the difference between the initial and final SFRP4 values as shown in Figure 3. It can be seen that SFRP4 levels have been highly expressed following dietary treatment. The data show a very significant value (P<0.01). SFRP4 expression in the HFD-fed group of mice was more than the ND-fed group of mice. Serum SFRP4 is always correlated with body fat percentage and BMI (P<0.01). Group 4 (HFD-F) shows a higher expression of SFRP4 (41.14±2.36a; P<0.01) than all groups of HFD-fed male G3 mice (30.57±2.89b; P<0.01). Similarly, ND-fed mice also raise the level of SFRP4 but are less compared to HFD animals.

Cholesterol Level

Significantly higher serum cholesterol levels were observed in animals after 12 weeks of dietary treatment compared to normal values. Figure 4 shows a significant difference between ND-fed objects and HFD-fed objects (P<0.01). There was a large difference between the initial data (G1: 144.50±5.13e; G2: 171.67±3.54d; G3: 171.17±3.76d; G4: 175.67±3.26d) and the 12-week evaluation data (G1: 227.83±3.46c; G2: 262.17±3.55b; G3: 268.00±4.62ab; G4: 282.17±3.73a). In our results, all groups, whether on ND or HFD, show elevation in cholesterol but overall HF-fed mice are more elevated than on normal diet-fed mice. Total mean is as 260.04±4.54A.
HDL Cholesterol Level

Serum circulating HDL was evaluated from the serum of all experimental animals. Highly significant data (P<0.01) was observed. As low HDL is associated with type 2 diabetes, the present results are almost in line with this hypothesis, but G2 (HFD-M) shows high elevation (131.00±4.18a) in HDL level. There is no difference in the initial and final value of HDL even to a small extent in G1 (70.83±6.76c; 70.50±3.75c). Group 2 (HFD-M) showed a significant increase in HDL levels (70.17±4.23c; 131.00±4.18a) with HFD diet. In all groups except G2, the HDL level is normal or close to normal as shown in Figure 5.

LDL Cholesterol Level

The LDL level was found to be highly significant (P<0.01) in diabetic objects. Results show a high difference between initial and final dietary value with the exception of G1 (95.33±7.64c; 95.33±7.64c) which shows no difference in both before and after dietary treatment as shown in Figure 6. Animals fed with HFD show a further increase in LDL levels (G2: 219.83±11.90a; G4: 198.00±12.83a). Group 3 of the ND experiment also showed an increase in the LDL level (178.50±3.91b). The data represent an overall mean of 172.92±10.83A after 12 weeks of dietary treatment.
Triglycerides level

![Comparison of triglycerides level](image)

The results of Figure 7 indicate a clear increase in triglyceride levels when the initial and final data were compared. The level of triglycerides was highly significant (P<0.01) after 12 weeks of dietary treatment in experimental mice. HFD-fed groups showed an increase in triglycerides (G2: 273.17±14.22b; G4: 317.67±13.11b) while ND-fed mice (G1: 267.50±18.70b; G3: 340.50±13.22a). G3 showed abnormal results with a maximum increase in triglyceride levels.

DISCUSSION

The main objective of this research was to develop a type 2 diabetes mouse model that would explain the metabolism and natural history of human T2D disease and help determine protein expression. Here, a mice model has been established that resembles some of the main features of human T2D. The study was divided into two groups: the first focuses on the development of the mouse model for type 2 diabetes and obesity, and the second focuses on the evaluation of the secreted frizzled protein 4 expressions in both diet-induced and normal mouse models using ELISA.

Our diet shows that the fat content was almost 4 times higher in HFD than in LFD, while the carbohydrate content in LFD was almost three times higher than in HFD. Obviously, because of the high fat content, the moisture content of HFD was lower than that of LFD. Energy values for high fat diets and low-fat diets were 360 and 553 kcal per 100 g of food. However, the main contributor to obesity is excessive caloric intake in combination with energy-dense food availability [13].

Interestingly, because drinking water contains fructose, mice reduce food intake. Although the total caloric intake of the HFD mouse was high compared to LFD. The present study agreed with the results of the already existing study that HFD and fructose had increased their weight compared to control animals [14]. Hyperglycemia is generally induced within 4 weeks of the HFD diet [15].

In this study, the lipid profile test is performed both at the initial stage and after 12 weeks of dietary treatment. It is clear that HFD affects the serum concentration of high-density lipoprotein (HDL), low density lipoprotein (LDL) and triglyceride (TG). ND also increases their concentration but compares less to HFD. Increased fat intake leads to an increase in blood cholesterol levels. HFD-fed mice were found to have a high blood glucose level (p<0.05) after diet.

The present study shows that HDL cholesterol was almost normal or near normal except in one group (G2) where HDL was accidentally increased. However, overall data was very significant (P<0.01). Type 2 Diabetes characterized by dyslipidemia is associated with normal low HDL cholesterol, high triglyceride levels and LDL cholesterol [16].

In this study, the serum SFRP4 level was evaluated at the initial stage and after 12 weeks of duration using the enzyme-linked immunosorbent assay (ELISA) kit. Serum SFRP4 levels were found to be over-expressed and elevated to a high degree in HFD-fed animals compared to the initial data. Similarly, ND-fed mice also show elevated serum SFRP4 to some extent, but not to the same extent as HFD-fed animals that were our target. SFRP4 was over-expressed in type 2 diabetes.
In our research, we found that T2D mice showed statistically higher concentrations of SFRP4 serum than those of apparently good artifacts (P< 0.01). In addition, T2DM respondents were found to have increased blood levels of SFRP4 even prior to the development of open hyperglycemia [5]. These results show that the circulating concentration of SFRP4 was associated with a declining glucose metabolism.

In addition, we found that SFRP4 was positively associated with IL-1β (P < 0.001) and demonstrated that suppression of IL-1β releases of hormones may have an effect on SFRP4. It may be associated with acute, low-grade islet swelling and defective insulin secretion. In T2D, SFRP4 was recognized as an early mediator of pancreatic β-cell dysfunction by combining global gene expression assessment with network analysis of human islets in patients with T2D [5]. Results show that SFRP4 has a very significant (P<0.01) correlation with obesity and BGR (0.656; 0.316) respectively. In response to metabolic stress, chronic low-grade inflammation of the islet tends to cause T2D [17].

Over-expression of SFRP4 has been reported to pose a five-fold higher risk to the development of diabetes [18]. The main effect of SFRP4 in B-cells was to reduce glucose-stimulated insulin secretion [5]. The study showed that long-term HF dietary eating profoundly changed the development of WNT signaling proteins in pancreatic islets and significantly reduced the development of Wnt4 in β-neurones. WNT4 signaling performs a key position in the control of glucose-caused insulin secretion. Extensive knowledge of the signaling pathway for type 2 diabetes can encourage the development of new therapies [19].

CONCLUSIONS

Obesity and type 2 diabetes are closely related. Obesity results in insulin resistance, inadequate insulin secretion and decreased insulin sensitivity in patients and ultimately a cause of T2D. SFRP4 is found to be associated with defective insulin secretion and inflammation of the islet. In type 2 diabetic patients, an increased level of SFRP4 has been observed. Our research work concluded that high-fat diet (HFD) results in the induction of obesity, hyperglycaemia, abnormal lipid profile and significantly high levels of SFRP4, which ultimately lead to type 2 diabetes. It was also concluded that SFRP4 may be a potential biomarker for early detection of type 2 diabetes.

AUTHOR’S NOTE

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