Dietary ginger improves glucose dysregulation in a long-term high-fat high-fructose fed prediabetic rat model

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The rapid increase in global diabetes burden with its associated morbidity and mortality is a major health concern for humans. Prediabetes is a condition which predispose a person not only to diabetes but also to the associated complications including morbidity even in the absence of an apparent hyperglycemia. However, appropriate dietary intervention may not only prevent but also improve one’s condition as diet is the major contributor to such metabolic disorders. Here, we investigated the effect of dietary ginger (Zingiber officinale Roscoe) on the markers of insulin resistance and pathophysiology in a diet-induced prediabetic rat model. Male Sprague-Dawley (SD) rats were fed the following diets: control (5% groundnut oil + 65% corn starch), high fat high fructose (HFHF; 25% beef tallow + 35% fructose) and HFHF with 3% ginger (HFHFG) for eight months. Plasma markers of insulin resistance, lipid profile, oral glucose tolerance (OGTT; 2nd and 5th month), intraperitoneal insulin tolerance (ITT), plasma total antioxidant capacity (TAC), liver histology and pancreatic immunohistochemistry (IHC) were examined. The impaired OGTT, ITT and insulin sensitivity indices with observed hyperinsulinemia and hypertriglyceridemia suggest that HFHF feeding resulted in prediabetes in rats. HFHF feeding also decreased insulin secretion in the pancreas, increased lipid accumulation in liver and total oxidants in plasma. The effects of HFHF feeding on glucose regulation, pathophysiology of pancreas and liver; total oxidative stress were improved by ginger feeding. The present study demonstrated that long-term HFHF feeding induces prediabetes in experimental rats while dietary ginger neutralizes the HFHF induced impairment in glucose regulation, dyslipidemia, and oxidative stress.

Keywords: Diet, Glucose dysregulation, Hyperinsulinemia, Hyperglycemia, Hypertriglyceridemia, Insulin resistance, metabolic syndrome, Type 2 diabetes, Zingiber officinale

The rapid increase in the prevalence of metabolic syndrome (MS) worldwide is a serious concern. The prevalence of MS is 34% in US adults population while 50% in the people who are above 60 years while in India it varies from 11 to 41 per cent as there is diversity in the geography and extent of urbanization. As per the International Diabetic Federation, MS is defined as a cluster of metabolic abnormalities such as type 2 diabetes (T2D), hyperglycemia, obesity, dyslipidemia, insulin resistance (IR) and hypertension. Suggested etiological factors for the rapid increase in the prevalence of MS are malnutrition, lifestyle changes, socioeconomic transitions, increasing affluence, urbanization, and mechanization.

Among the risk factors, IR is important contributor to the onset and progression of other components of MS, and the prevalence of IR is also higher among Asian Indians. During IR a subnormal amount of insulin is required to elicit a quantitatively normal response, and subjects with IR, exhibit close to normal or normal glycemic state, with increased levels of circulating insulin. The IR is not only a risk factor for MS but also a predisposing factor to type 2 diabetes (T2D). Diabetes, one of the most threatening life-style disorders, 90% T2D, affects 415 million people worldwide (about 8.8% of the total population) which may reach up to 642 million by 2040. India ranks second, next to China, with 69.2 million which may rise to 115 million in mid of this century.

Before leading to T2D, IR is shown to predispose an individual to a state of prediabetes/glucose dysregulation (GD) which is all the more deleterious as it goes undiagnosed for many years. Clinically GD is defined as an intermediate state between normoglycemia and T2D, during GD a person exhibit near normal or slightly higher fasting glucose (IFG) with impaired glucose tolerance (IGT). People with
IGT have a higher risk to develop diabetes and other complications when it clusters with the components of MS\(^{10}\). Among the strategies to target the progression of prediabetes to diabetes, diet & lifestyle intervention are promising\(^{12}\).

Since diet is the major contributor of metabolic disorders associated with GD, one can effectively target the same by improving the quality of the diet\(^{13}\). Moreover, dietary intervention is advantageous over lifestyle modification where the required commitments of both subjects with GD and healthcare professionals make it hard to practice. This encouraged researchers to explore the protective effects of dietary components on metabolic complications.

*Zingiber officinale* Roscoe, commonly known as Ginger, is one such dietary ingredients that possess several beneficial effects\(^{14-16}\) including analgesic\(^{14}\), anticancer\(^{17}\), antimicrobial, antioxidant\(^{18}\), larvicidal\(^{14}\), neuro-\(^{19}\) and hepatoprotective\(^{19}\) and anti-Alzheimer effects\(^{20}\). Despite its promising effects on hyperlipidemia and obesity, the hypoglycemic and antidiabetic properties of ginger are reported to be mild to moderate in normal and experimental diabetic animal models\(^{21}\). The observations in T2D patients suggest that supplementation of ginger improved the insulin sensitivity indices and plasma triglycerides but not plasma glucose and HbA1C\(^{22}\). Here, we explored the effects of dietary ginger *per se* on GD and the components of MS in long-term high-fat and high-fructose-fed rats which phenotypically mimic the conditions of prediabetes.

**Materials and Methods**

**Experimental design**

The procedures involved in animal experimentation were approved by the Institutional Animal Ethics Committee, National Institute of Nutrition. Weanling male Sprague-Dawley (SD) rats were divided into three groups (n=6) and housed individually in a temperature (22±2°C) and light-controlled (12 h cycle) animal facility. The animals were fed a diet containing 5% groundnut oil (GNO) + 65 % corn starch (control diet-CON) or a blend of 5% GNO and 25% beef tallow as a source of dietary fat and 35 % fructose (high fat high fructose diet- HFHF) or HFHF diet with 3% ginger powder (HFHFHG) for a period of eight months. The ginger powder was prepared as mentioned previously\(^{23}\) by lyophilizing finely chopped fresh *Z. officinale* purchased from the local market. The percentage (g/100 g) of other ingredients was as follows: casein 25, cellulose 5, mineral mix 4, vitamin mix 1, L-cysteine 0.3, choline chloride 0.2\(^{24}\).

**Food intake and metabolic efficiency**

The animals had free access to water and food. The food intakes and body weights were recorded daily and weekly respectively. From the food intake data, monthly average food intake was calculated while average weight gain was derived out of weekly weight gain data. The metabolic efficiency was calculated as a ratio between the energy intake and the body weight gain for every month\(^{25}\).

**Oral glucose tolerance test (OGTT), insulin sensitivity indices and plasma lipid profile**

OGTT was carried out at 2\(^{nd}\) and 5\(^{th}\) months of feeding to estimate the glucose tolerance. Briefly, the animals were fasted overnight (16 h) and given a single oral dose of glucose (3 g/kg body wt.) and blood samples were collected in EDTA-coated tubes at 0, 30, 60, and 120 min from the orbital plexus. Additional time point (90 min) was introduced after 5 months of feeding to improve the visibility of GD. Plasma was separated, the levels of glucose and insulin were estimated; various insulin sensitivity related indices such as homeostasis model assessment of insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), glucose to insulin ratio (G/I)\(^{26}\), homeostasis model assessment for pancreatic β cell function (HOMA–β)\(^{27}\) and the area under the curve (AUC) for glucose & insulin\(^{28}\) were derived. The plasma collected after eight months of feeding was also estimated for the levels of glucose and insulin and the aforementioned indices were derived. The plasma collected after completion of 2\(^{nd}\), 5\(^{th}\) and 8\(^{th}\) months of feeding was analyzed for lipid profile.

**Intraperitoneal insulin tolerance test (ITT)**

ITT was performed after 8 months in (16 h fasted) rats by administering recombinant human insulin (0.75 U/kg body wt.; Human mixtard; Novo Nordisk India Pvt. Ltd., India) intraperitoneally\(^{29}\). Glucose levels were estimated in tail vein blood using a handheld blood glucose monitor (OneTouch; Johnson & Johnson, Milpitas, CA) at five time points: 0, 15, 30, 45, and 60 min after insulin injection. Percentage glucose disappearance was calculated considering the glucose levels at 0 min as 100 per cent.
Body mass analysis using dual energy X-ray absorptiometry (DEXA)
Rats were scanned for their total body mass before the initiation and after eight months of feeding using Discovery-A Hologic body mass analyzer (QDR-series- ASN 82382; version-12.5). Each scan was performed by placing the anesthetized animals (isoflurane inhalation) with their abdomen down over the scanner platform. After scanning, the percentage of lean, fat was calculated using the manufacturer’s protocol.

Plasma total antioxidant capacity
The total antioxidant capacity (TAC) of the plasma was estimated using a commercial kit (Total antioxidant power kit, Oxford biomedical research, Oxford, MI, USA) as per the manufacturer’s protocol. Briefly, the cupric reducing antioxidant capacity of plasma was compared and expressed as mM Trolox equivalents.

Histopathology and immunohistochemistry
After eight months of feeding, the animals were sacrificed, liver and pancreas tissues were collected. The histological changes in the liver were examined as described. Briefly, the liver tissues were fixed in 10% buffered formalin. Microsections (5 μM thickness) were prepared from paraffin blocks, stained with Hematoxylin and Eosin (H&E) and examined microscopically. Pancreatic insulin-positive area and strength of insulin positivity were examined in 4% paraformaldehyde fixed pancreas tissues immunohistochemically. The insulin-positive cells were determined in a unit area of pancreatic tissue by incubating the sections with primary insulin antibody (mouse monoclonal IgG; sc-56418; 1:100 dilution; Santa Cruz Biotechnology, Inc.) and insulin positivity was identified using immunocruz mouse staining system (sc-2017; Santa Cruz Biotechnology, Inc).

Statistical analysis
The data were analyzed using IBM SPSS 19 statistical program. The results were expressed as mean ± SD. One-way analysis of variance was used to test the significance of difference among dietary groups. Post hoc comparisons were performed using LSD test. P value <0.05 was considered statistically significant.

Results
The food intake and the metabolic efficiency of the feed were decreased significantly in HFHF animals when compared to control animals. Feeding 3% ginger in the diet did not improve food intake or metabolic efficiency (Fig. 1A & B). Irrespective of the ginger in diet high-fat feeding increased the fat mass and decreased the lean mass significantly after eight months (Fig. 1C & D), despite there being a significant decrease in food intake and metabolic efficiency.

HFHF diet significantly impaired the glucose tolerance at two months (Fig. 2A); the impairment was at a greater magnitude after 5 months of feeding (Fig. 2B) as evidenced by the AUC pattern of glucose and insulin (Fig 2C & D). Dietary ginger improved the impairment significantly during both the time points.
The data on ITT showed that there was a significant decrease in the percentage of glucose disappearance in HFHF fed animals at 30 min. However, during other time points (0, 15, 45 and 60 min) there was only a trend of decrease observed. The impairment in glucose disappearance was significantly improved to normal levels by the dietary ginger (Fig. 3).

The plasma markers of insulin sensitivity and lipid profile were not altered among experimental groups during 2nd and 5th month of feeding (data not shown). However, there was a significant increase in the levels of triglycerides and insulin in the plasma of HFHF fed animals after 8 months of feeding. Dietary ginger ameliorated the observed changes in triglycerides and insulin. Circulating free fatty acid (FFA) levels were significantly decreased by dietary ginger when compared to both control and HFHF fed animals (Fig. 4).

Fig. 2—Oral glucose tolerance test curve at (A) two, and (B) five months; Area under the curve (AUC) for (C) glucose, (D) insulin after five months of experimental feeding. [CON, control diet; HFHF, diet provided high fat and high fructose; and HFHFG, the diet provided 3% ginger in addition to the HFHF. Values are mean ± SD of 6 rats in each group. Statistical significance is shown by letters and values with different letters are significantly different (P <0.05)]

Fig. 3—Plasma glucose disappearance after an intravenous injection of insulin. [The glucose values were recorded and the value at 0 min was considered 100% and the consecutive clearance of glucose from the circulation was calculated accordingly. CON, control diet; HFHF, diet provided high fat and high fructose; and HFHFG, the diet provided 3% ginger in addition to the HFHF. Values are mean ± SD of 6 rats in each group. Statistical significance is shown by letters and values with different letters are significantly different (P <0.05)]

Fig. 4—The levels of (A) plasma triglycerides-TG & insulin-PI; and (B) free fatty acids–FFA after eight months of experimental feeding. CON, control diet; HFHF, diet provided high fat and high fructose; and HFHFG, the diet provided 3% ginger in addition to the HFHF. [Values are mean ± SD of six rats in each group. Statistical significance is shown by letters and values with different letters are significantly different (P <0.05)]
In the HFHF fed animals, HOMA-IR was significantly increased, while the QUICKI significantly decreased after 5th & 8th months of feeding and both the indices were improved by the dietary ginger. HOMA-β and the G/I were altered

Table 2: The histopathology of liver (n = 4) and immunohistochmistry of pancreas (n = 3)

<table>
<thead>
<tr>
<th>Histopathology (liver)</th>
<th>CON</th>
<th>HFHF</th>
<th>HFHFG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal vacuolation</td>
<td>0/4</td>
<td>4/4</td>
<td>1/4</td>
</tr>
<tr>
<td>Immunohistochemistry (pancreas)</td>
<td>S</td>
<td>M</td>
<td>S</td>
</tr>
</tbody>
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[Strength of insulin positivity was classified as S-Strong, M-Moderate and Mi-mild. CON, control diet; HFHF, diet provided high fat and high fructose; HFHFG, the diet provided 3% ginger in addition to the HFHF]

in HFHF fed animals after eight months of feeding. Although the dietary ginger does not significantly improve these indices, there was a trend of improvement observed in HFHFG fed animals (Table 1). Feeding HFHF significantly decreased the TAC as evidenced by decreased levels of Trolox equivalence while feeding 3% ginger significantly increased the TAC in plasma (Fig. 5)

The histological examination showed scattered vacuole formation in liver tissues of HFHF fed animals (4/4; 100%) while ginger fed group showed vacuolation only in one out of four animals (25%). Immunohistochemistry of pancreas tissue showed that there is a decrease in the % β cell area as evidenced by the presence of insulin (average 70% in CON Vs 60% in HFHF). However increased insulin positivity was seen in the pancreas of HFHFG animals (90%). The strength of the stain was also comparable in CON and HFHFG fed animals (strong positivity) while it decreased in HFHF fed animals (moderate) (Fig. 6 & Table 2).
Discussion

The Western dietary habit, such as consumption of high fat and high fructose (HFHF), in combination, reaches almost all part of the urban and some part of the rural India; the result is higher incidence of the metabolic disorders countrywide. Diet-related approaches are always preferable to counteract metabolic disorders. Hence, the present study explored the beneficial effects of long-term feeding of 3% ginger in HFHF fed experimental rat model of prediabetes.

The significant decrease in food intake and metabolic efficiency of the diet in HFHF fed animals could be due to the amount of beef tallow (25%) and fructose (35%) which might have made the diet less palatable and significantly energy inefficient (Fig. 1 A & B). Interestingly despite the lesser intake and efficiency, the HFHF feeding increased the adiposity significantly irrespective of the ginger in the diet as evidenced by increased percentage of body fat and decreased the percentage of lean mass (Fig. 1 C & D). Studies on chemical induced and high-fat fed obese rodent models showed that dietary ginger significantly reduced the total body weight. In both the studies, the protective effects of ginger were examined with an alcoholic extract. In the present study, the effect of ginger was evaluated against the combined effect of high-fat as well as high-fructose which could have doubled the deleterious effects on adiposity. Therefore, feeding 3% ginger may not be adequate to neutralize the combined effects of high fat and high fructose in the diet. Furthermore, the effects may vary according to the preparation of formulation of ginger used for the study.

The insulin sensitization effect of ginger was explored in diabetic and experimental IR rat models, and the results are in contrast as there are differences in the induction of disease phenotype and treatment. In the present study dietary ginger significantly improved the glucose tolerance as evidenced by improvement in OGTT pattern at second and fifth months (Fig. 2 A & B). The effects are more prominent after the fifth month as evidenced by the significantly improved AUC pattern of glucose and insulin (Fig. 2 C & D). Interestingly, feeding ginger for a longer duration significantly improved insulin sensitivity as evidenced by the improvement in indices such as HOMA-IR & QUICKI along with the observed trend of improvement in the G/I & HOMA-β after eight months of experimental feeding.

The significant decrease in glucose disposal after an insulin dose observed in HFHF fed animals suggested the existence of decreased insulin sensitivity in peripheral target tissues. We further determined the fasting plasma levels of glucose and insulin during various durations (2, 5 & 8 months) to understand whether the decreased glucose clearance results in frank hyperglycemia. Interestingly, throughout the study, we have not observed frank hyperglycemia (data not shown) which is in contrast to a similar study where the observations are in agreement with our study except for the fasting hyperglycemia which might be due to a higher level of fat (40%) and fructose (60%) in the diet. However, after eight months of feeding, there was a significant increase in the levels of insulin in HFHF fed animals and feeding 3 % ginger could improve the hyperinsulinemia without affecting glucose levels in the animals. These observations on glucose clearance and plasma insulin suggest that HFHF could deleteriously predispose the animals to a state of prediabetes which may be neutralized by dietary ginger. It is supported by the observation on plasma triglycerides which was significantly increased in HFHF fed animals, suggesting decreased clearance of lipids and or increased release of lipids into the circulation, which is a hallmark of IR. It is well established that in a state of prediabetes, the glucose levels are near normal to slightly higher with hyperinsulinemia and hypertriglyceridemia. With the above observations, our experimental animals typically represent a prediabetic animal model and feeding 3 % ginger could efficiently improve the effects of HFHF by improving the insulin sensitivity.

The lipid-lowering effects of aqueous extract of ginger have been reported in normal and in experimental models which could be attributed to its inhibition of the expression of lipogenic genes in the liver. Lipid accumulation in non-adipose tissues is suggested to increase the lipotoxicity and alter the metabolic homeostasis. Moreover, high fructose consumption may cause lipid accumulation in the liver. In our study, we have used HFHF to induce metabolic syndrome and hence we have examined the liver histology. The observation showed that feeding HFHF diet resulted in the visible accumulation of fat vacuoles in the liver tissues while animals
co-administered with ginger reversed the fatty accumulation caused by the HFHF feeding which could be attributed to its inhibitory effect over lipogenic gene expression\(^4\). The data on the circulating FFA is also in support of the lipid lowering effect of ginger as evidenced by the significantly decreased levels of FFA in HFHFG animals despite there being high levels of fructose and fat in the diet. The observation could also be ascribed to the effect of ginger on improved lipid utilization in peripheral tissues. The histological evidence, as well as circulating levels of triglycerides, suggest that the increased lipotoxicity in HFHF fed animals might have played an important role in increasing insulin resistance, and thereby could have caused the GD in the experimental animals.

Studies suggest that the mechanism of insulin sensitization effect of ginger could be due to its stimulatory effect over antioxidants, improving the insulin signal transduction mechanisms and glucose uptake\(^4,45\). It has also been postulated that dietary ginger may prevent pancreatic \(\beta\) cell damage through its antioxidant potential and thereby increase the insulin sensitivity\(^46\). In the present study, the TAC was examined to understand how the antioxidant status played a role in improving insulin sensitivity. The significant improvement of TAC observed in HFHFG animals when compared to HFHF animals suggests a decreased oxidative damage in ginger fed animals which could be partly attributed to its protective effects on tissues associated with insulin sensitivity, which is in agreement with the observations in other disease models\(^47\).

We further explored whether the oxidative stress had any direct impact on pancreases, which resulted in a prediabetic state in the experimental animals. Hence, we carried out IHC to identify insulin positive areas, the strength of insulin positivity. The average insulin-positive area and the strength of the insulin positivity in the pancreas were decreased in HFHF fed animals while feeding ginger improved both the parameters. It is noteworthy that insulin resistance leads to the highest demand for insulin which results in the atrophy of pancreatic islets eventually diminishing its ability to secrete insulin, and this is clinically defined as the establishment of T2D\(^48\). Interestingly, microphotograph of IHC in HFHF fed rats showed that the stain intensity is indistinguishable in HFHFG fed animals (Fig. 6) which represent damage in the insulin-secreting cells. The IHC pattern of HFHF (Table 2) showed that one out of three pancreases showed negligible insulin positivity (~20% of \(\beta\)-cell area) suggesting that HFHF feeding adequately predisposed the animals into a prediabetic state. It has been previously reported that dietary ginger exhibits protective effect over pancreatic \(\beta\) cells by decreasing the cytotoxicity\(^46\). Together these results suggest that dietary ginger suppresses dyslipidemia, hepatic lipotoxicity, and oxidative stress and thereby improves insulin sensitivity in HFHF fed animals.

In conclusion, feeding a diet with high fat and high fructose for eight months can induce a prediabetic state in the experimental animals as evidenced by GD, dyslipidemia, and increased oxidative stress. The findings are supported by increased lipid accumulation in the liver, decreased insulin secreting cells as well as insulin secretion in pancreases. Co-administering ginger powder for 8 months (~500 mg/animal/day) has decreased the deleterious effects of HFHF feeding, and the protective effects can be attributed to the insulin-sensitizing, antioxidant, lipid lowering properties of ginger.

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Author disclosure statement

None of the authors have any conflicts of interest.

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