Flaxseed and quercetin improves anti-inflammatory cytokines level and insulin sensitivity in animal model of metabolic syndrome fructose-fed rats

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The purpose of this study was to assess the beneficial effect of quercetin, flaxseed and/or in combination as synergetic, in an animal model of metabolic syndrome (MetS), high fructose (HF)-fed rats. Fifty male Sprague-Dawley rats, 3 months old, weighing between 110 to 120 g were randomly divided into 5 groups. Rats were given drinking water (negative (-ve) control rats) or 10% fructose in drinking water (HF; fructose-fed rats) with standard chow for 8 weeks. After 4 weeks of HF feeding, rats were further divided into matched 4 subgroups. Different groups of animals (n = 10, each group) were administered 10% HF (5 mg/kg, +ve control), flaxseed (F; 50 mg/kg), quercetin (Q; 50 mg/kg), flaxseed + quercetin, (FQ; 25 mg/kg each), respectively. All ingredients were given orally, once daily and subsequently for 4 weeks. Serum glucose, insulin, lipids profile, leptin, and adiponectin were estimated. After 4 weeks of feeding, a significant increase in blood glucose level was observed in HF fed rats compared to normal rats, but this was significantly decreased after administration of F, Q and FQ. The serum insulin level in HF fed rats was significantly decreased after administration of F and FQ groups. Significantly, higher concentrations of triacylglycerols (TG), total cholesterol and low density lipoprotein cholesterol (LDL- C) were observed in HF fed rats, and these increases were lower after administration of F, Q and FQ. There was a significant increase in serum high density lipoprotein cholesterol (HDL-C) in FQ group. The increase of serum leptin level was decreased significantly in F, Q and FQ groups. Whereas, the reduction of serum adiponectin level in HF fed rats was increased in F, Q and FQ groups. These data suggests that protective effect of flaxseed and quercetin consumption as functional foods could be less risky for people with decreased insulin sensitivity and increased oxidative stress, such as those with the metabolic syndrome or type 2 diabetes.

Key words: Flaxseed, protective effect, quercetin, insulin sensitivity, metabolic syndrome, type 2 diabetes.

INTRODUCTION

The metabolic syndrome (MetS) is a constellation of risk factors, including impaired fasting glucose, hypertension, central adiposity, predisposing to higher risks of oxidative stress, type 2 diabetes and atherosclerotic cardiovascular disease (CVD) (Park et al., 2007; Grattagliano et al., 2008; Chen et al., 2008; Ishizaka et al., 2009). The etiopathology of the metabolic syndrome has not yet been fully elucidated. Recent studies have highlighted
the involvement of a pro-inflammatory state that induces insulin resistance and leads to clinical and biochemical manifestations of the metabolic syndrome (Horiuchi and Mogi, 2011).

Obesity/insulin resistance is associated with metabolic syndrome, which plays a pivotal role in cardiovascular risk. The mechanisms that link obesity, insulin resistance, and endothelial dysfunction are numerous and complex (Steinberg et al., 1996). Increase in visceral fat, usually involved in obesity, leads to an imbalanced production of metabolic products, hormones, and adipocytokines including tumor necrosis factor-α (TNF-α), free fatty acids (FFAs) or adiponectin which causes decreased insulin sensitivity in skeletal muscle and liver, and impairs endothelial function through direct or indirect mechanisms.

Insulin itself acts as cytokine at sufficiently high concentrations, and this may underlie vascular damage and dysfunction in human and animal studies (Absher et al., 1997, 1999). There are a number of recognized cytokines that are related to obesity, metabolic syndrome and cardiovascular disease, including adipocyte-related peptide adiponectin and inflammatory marker, interleukin-1b (IL-1b) (Dinarello, 1998, 2005; Huyppens, 2007). Researchers observed that obesity is inversely correlated with adiponectin, a marker of anti-inflammation (Brooks et al., 2007). Similarly, IL-1b is a mediator of systemic pro-inflammatory pathways and may provide an index of the inflammatory processes that are known to accompany atherosclerosis.

The Mediterranean diet which include a high intake of plant food content, such as vegetables, legumes, and fruits, have been directly associated with the prevention of obesity, type 2 diabetes, and other cardiovascular risk factors (Estruch et al., 2006). The protective effect of plant foods that contain flavonoids, polyphenolic compounds against chronic pathologies such as, obesity, diabetes and cardiovascular disease mortality, is reported by many workers (Knekt et al., 2002; Mink et al., 2007).

Quercetin (3,3’,4’,5,7-pentahydroxyflavone) is one of the most widely used flavonol in human dietary sources (Hertog et al., 1993). The intervention trials with quercetin in human subjects is an effort in the development of dietary supplements with a higher dose, which might prove useful for the prevention or treatment of functional alterations clustered in the metabolic syndrome (Middleton et al., 2000; Duarte et al., 2002; Comalada et al., 2006; Rivera et al., 2008). Other human studies, however, failed to confirm these effects (Williamson and Manac, 2005).

Flaxseed is a complex food containing high amounts of polyunsaturated fatty acids (PUFA), mainly α-linolenic acid (ALA), an (n-3) fatty acid, as well as soluble fiber, lignan precursors, and other substances that may have health benefits (Hall et al., 2006; Basset et al., 2009). A number of studies have shown that flax oil supplementation can reduce serum triacylglycerols and cholesterol concentrations, thus leading to reduced CVD risk (Cunnane et al., 1993; Craig et al., 1999). Furthermore, n-3 PUFA of flaxseed oil has anti-inflammatory properties that are mediated by the production of anti-inflammatory cytokines (Cohen et al., 2005). However, the effects of these foods on MetS remain unclear.

In this study, we determined the beneficial effects of quercetin, flaxseed and/or in combination as synergistic in an animal model of MetS HF fed rats. In particular, we measured various parameters related to MetS, such as hyperlipidaemia, hyperglycaemia, hyperinsulinaemia and on formation of anti-inflammatory cytokines such as leptin and adiponectin.

**MATERIAL AND METHODS**

**Animals**

Male Sprague-Dawley rats were purchased from the Laboratory Animal Center (Science section and medical studies, Malaz, Riyadh, King Saud University, Saudi Arabia) and housed in plastic cages with a 12:12 h light-dark cycle, at a constant temperature of 22 to 24°C. They were given standard chow *ad libitum* for the duration of the study and allowed 1 week to adapt to the laboratory environment before experiments.

**Animal model and drug administration**

All the animals used in the present study were treated in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by Committee of King Saud University, College of Pharmacy (Riyadh, Saudi Arabia). Fifty male rats, 3 month old, weighing between 110 and 120 g were randomly divided into 5 groups. The initial body weights of the rats were recorded with no significant difference between control and HF-fed groups. Rats were given drinking water (negative (-ve) control rats) or 10% fructose in drinking water (HF-fed rats), with standard chow for 8 weeks (Hu et al., 2009). Fresh drinking water was replaced every 2 days. After 4 weeks fructose feeding, HF-fed rats were further divided into matched 4 subgroups. Different groups of animals (*n* = 10; each group) were administered; 10% fructose in drinking water (5 mg/kg, as a +ve control group), F (50 mg/kg), Q (50 mg/kg), FQ (25 mg/kg each), respectively. All ingredients were given orally once daily, subsequently for 4 weeks.

**Biochemical assays**

After an overnight fasting (food deprivation), rats were anesthetized and blood was withdrawn by heart puncture, in tubes protected from light, then centrifuged at 3,000 g for 10 min at 4°C. Plasma was immediately isolated, aliquoted and stored at -80°C until analyzed. Serum glucose, total cholesterol, HDL-C, and TG levels were estimated calorimetrically using kits according to United Diagnostics Industry. LDL-C was calculated using the Friedwald equation \[
L = C - H - 0.167T; \] where \( H \) is HDL-C, \( I \) is LDL-C, \( C \) is total cholesterol. \( T \) is TG, and \( k \) is 0.20 (mg/dl). Serum insulin, leptin and adiponectin were measured using enzyme linked immunosorbent assay (ELISA) kits, insulin (American Laboratory Products Company, Windham, NJ), leptin and adiponectin (AniBiotech Oy, Orgenium Laboratories Division, Vantaa, Finland).
The reading was taken using ELISA microplate reader (VERSA Max, Molecular Devices Corporation, MN, USA).

Statistical analysis

All values were expressed as mean ± Standard error (SE). Data were statistically analyzed using one way Analysis of variance (ANOVA) for multiple group comparison, followed by Student’s unpaired t-test for group comparison. Significance was set at p ≤ 0.05. Data were computed for statistical analysis by using GraphPad Prism Software.

RESULTS

After 4 weeks of feeding, a significant increase in serum glucose and insulin levels were observed in HF fed rats compared to negative (-ve) control group. However, this increase significantly decreased after administration of F (p ≤ 0.001), Q (p ≤ 0.05) and F+Q (p ≤ 0.001) in serum glucose (Figure 1A). Serum insulin significantly decreased after administration of F (p ≤ 0.001) and F+Q (p ≤ 0.001) groups, whereas no significant difference could be observed in Q group (Figure 1B). Serum lipid profiles were measured after 4 weeks of HF fed rats. Significantly higher concentrations of TG (p ≤ 0.05), total cholesterol (p ≤ 0.001) and LDL-C (p ≤ 0.001) was observed in HF fed rats compared to negative (-ve) control group, but these increases were significantly lowered after administration of F (p ≤ 0.001), Q (p ≤ 0.01) and F+Q (p ≤ 0.001) (Figure 2A, B and C). A significant increase in serum HDL-C was observed in FQ group (p ≤ 0.001) compared to HF fed rats (Figure 2D). Serum leptin level decreased in F (p ≤ 0.001), Q (p ≤ 0.01) and F+Q (p ≤ 0.001) groups (Figure 3A) whereas, serum adiponectin level increased in F (p ≤ 0.001), Q (p ≤ 0.01) and F+Q (p ≤ 0.001) groups (Figure 3B).

DISCUSSION

Fructose rich diet was used for the induction of diabetes, which is characterized by insulin resistance and metabolic syndrome, very much close to type 2 diabetes in human. Several studies reported that fructose feeding for long term induces diabetes associated with insulin resistance and metabolic syndrome in experimental animals (Veerapur et al., 2010; Reungjui et al., 2007). In the present study, in rats receiving HF, plasma insulin, glucose, TG, total cholesterol and LDL-C were increased significantly, whereas HDL-C was significantly decreased, compared to negative (-ve) control group. These results are consistent with several earlier reports (Thresher et al., 2000; Busserolles et al., 2002, 2003) which support that consumption of HF diet leads to the development of insulin resistance which plays a pivotal role in the pathogenic mechanism of human type 2 diabetes, and is the cause of all metabolic complications (Veerapur et al., 2010). High dietary fructose have been associated with enhanced oxidative damage in rats (Busserolles et al., 2002) and development of insulin resistance; beta-cell dysfunction, and impaired glucose tolerance (Paolisso and Giugliano, 1996; Bloch-Damti and Bashan, 2005).

In the present results of rats fed HF diet, the cluster of metabolic syndrome were reversed by administration of F, Q and/or a combination (Figure 1A and B). Some flavonoids may affect fasting glucose, insulin, and lipid profile by insulin-enhancing activity in vitro and may regulate the expression of genes involved in glucose uptake and insulin signaling in rats fed HF (Rivera et al., 2008). Epidemiological evidence indicated that flaxseed seemed to decrease fasting glucose, prevent the increase of HbA1c and delays the development of diabetes (Prasad, 2001). Castilla et al. (2006) found that consumption of grape juice, rich source of quercetin have improving effect on HDL-C level which was paralleled by an increase in apo A1 concentrations. This indicates that flavonoids may affect hepatic apo A1 secretion in vivo (Hotamisligil et al., 1995). Flaxseed contains both n-3 fatty acids and lignans; flaxseed lignans alone had produced serum lipids in hyperlipidaemic rats (Felmllee et al., 2009). Thus, the presence of lignans could have contributed to the lipid-lowering properties of flaxseed supplementation by inhibiting fatty acid and cholesterol synthesis in the liver and hence reducing the hepatic lipid levels (Fukumitsu et al., 2008). It may be attributable; in particular, since ALA-rich flax oil can act as a better substrate for mitochondrial and peroxisomal β-oxidation, thus stimulating increased oxidation of lipids in the liver (Ide et al., 2000). Thus flaxseed consumption could be predictive of benefits for people at risk of developing cardiovascular disease.

The results of the present study revealed a significant decrease in serum leptin and increase in adiponectin levels after administration of Q (p ≤ 0.001), F (p ≤ 0.05) and F+Q (p ≤ 0.001) in HF fed rats (Figure 3A and B). Quercetin has been shown to exert anti-inflammatory effects. In peripheral blood mononuclear cells, quercetin dose-dependently inhibited the gene expression and production of the pro-inflammatory cytokine TNF-α (Nair et al., 2006). The mechanism of this effect was the modulation of the NF-kB signal transduction cascade. Therefore, a high dose of quercetin might have improved the inflammatory status by decreasing the production of TNF-α in visceral adipose tissue, and increasing plasma levels of adiponectin (Rivera et al., 2008).

The serum leptin level was lowered but serum adiponectin level was increased significantly in F and F+Q treatments (Figure 3A and B). Flaxseed has recently gained popularity as a functional food. Consumption of flaxseed has been shown to lessen insulin resistance, hyperlipidemia, atherosclerosis and hypertension and...
Figure 1. Serum Glucose and Insulin levels after administration of flaxseed, quercetin and in combination in fructose fed rats for 4 weeks. Values are expressed as mean ± SD. ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05, a: compared to (–ve) control group, b: compared to (+ve) control group.

Figure 2. Serum lipid profile level after administration of flaxseed, quercetin and in combination, in fructose fed rats for 4 weeks. Values are expressed as mean ± SD. ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05, a: compared to (–ve) control group, b: Compared to (+ve) control group.
Figure 3. The leptin and adiponectin levels after administration of flaxseed, quercetin and in combination, in fructose fed rats for 4-weeks. Values are expressed as mean ± SD. ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05, a: compared to (-ve) control group, b: compared to (+ve) control group.

decrease the incidence of cardiac arrhythmias. These effects of dietary flaxseed have been attributed, in part, to the rich ALA content of flaxseed (Ander et al., 2004; Dupasquier et al., 2007). However, the mechanism to explain the induction of these effects by ALA remains elusive. ALA in adipose tissue is strongly associated with increased leptin expression and subsequent reduction of atherosclerosis. Therefore, it is suggested that flaxseed may induce its anti-atherogenic effects in part via an ALA-mediated modulation of leptin expression (McGuillough et al., 2011).

Adiponectin is the most highly expressed and secreted adipokine, with beneficial effects on metabolism, inflammation, and vascular function. It plays a role in insulin sensitivity, LDL oxidation, endothelial nitric-oxide synthase (eNOS) activation, inflammation suppression and fatty acid catabolism (Halleux et al., 2001; Haluzik et al., 2004; Iacobellis et al., 2005). Thus, hypo adiponectinemia is of interest as a biomarker of both cardiovascular disease and metabolic syndrome. A study conducted on mice showed that replacing 15% of lipids with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (specifically, the replacement lipid consisted of 6% EPA and 51% DHA) improved insulin sensitivity as well as raised plasma adiponectin level, independent of food intake or adiposity. It also showed that the adiponectin gene expression was up-regulated in mature adipocytes after this intervention (Albert et al., 2005).

Conclusion

These data are encouraging and suggest that anti-inflammatory and protective effect of flaxseed and quercetin consumption as functional foods could be an important mechanism contributing to the reduced risk for people with decreased insulin sensitivity and increased oxidative stress, such as those with the metabolic syndrome or type 2 diabetes.

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