Mediterranean Journal of Nutrition and Metabolism 7 (2014) 119–132
DOI:10.3233/MNM-140012
IOS Press

Dietary proteins differentially influence adipokines and insulin sensitivity in high fructose–fed rats

S Sreeja[a], Baskaran Yogalakshmi[a], Rajagopalan Geetha[a], Mutlur Krishnamoorthy Radika[a], Krishnamoorthy Bhavani[b] and Carani Venkatraman Anuradha[a,*]

[a]*Department of Biochemistry and Biotechnology, Annamalai University, Tamil Nadu, India*
[b]*Department of Pathology, Mahatma Gandhi Medical College and Research Institute, Puducherry, India*

Abstract.
BACKGROUND: The type of dietary protein plays an important role in lipid and lipoprotein metabolism. This study tests the hypothesis that different dietary proteins may alter insulin sensitivity in rats after fructose ingestion.
OBJECTIVE: The aim is to investigate the effects of different dietary proteins in a rat model of metabolic syndrome created by feeding a high fructose diet.
METHODS: Rats were fed with starch or high fructose diet containing casein, egg albumin or soy protein for 8 weeks. Insulin sensitivity, oxidative stress markers, lipid profile and adipokines were measured.
RESULTS: F-CAS group registered insulin resistance and oxidative stress. Compared to the F-CAS group, F-EGG and F-SOY animals showed improved insulin sensitivity and lower fasting levels of glucose, insulin and lipids and increased antioxidant protection. In addition, F-EGG and F-SOY animals showed improved glucose metabolism compared to F-CAS group. Protein variation also affects the levels of adipokines and adipokine receptor mRNA expression in liver.
CONCLUSIONS: These findings demonstrate that soy protein had a better effect than egg protein with respect to insulin sensitivity, glucose and lipid homeostasis, antioxidant status and adipokine expression, while egg protein was more effective than soy in reducing oxidative damage despite continued intake of fructose.

Keywords: High fructose diet, adipokines, casein, egg protein, soy protein, insulin sensitivity

1. Introduction

The prevalence of metabolic syndrome (MS), a cluster of disorders like dyslipidemia, glucose intolerance and hypertension is increasing worldwide and is known to originate from insulin resistance and compensatory hyperinsulinemia [1]. The development of the MS involves a complex interaction between genetic, metabolic and environmental factors including diet. Studies have focused on the macronutrient content of diet in relation to insulin resistance and have firmly established that consumption of diet containing high quantities of refined sugars (sucrose/fructose), protein and saturated fats with low fibre content can increase the risk for insulin resistance [2]. However, data concerning the effects of various dietary protein types on the pathogenesis of insulin resistance and MS are sparse in the literature.

Dietary proteins are of high biological importance and are consumed in one or the other form in daily life. Caseins, the milk proteins are well known for their good nutritive value and excellent functional properties. Egg protein is a low-cost, high-quality protein and is considered to have one of the best amino acid profile for human nutrition

*Corresponding author: Dr. Carani Venkatraman Anuradha, Professor, Department of Biochemistry and Biotechnology, Annamalai University, Annamalai Nagar 608 002, Tamil Nadu, India. Tel.: +91 4144 239141; Fax: +91 4144 238080; E-mail: cvaradha@yahoo.com.

ISSN 1973-798X/14/$27.50

[3]. Soy protein is the most widely used vegetable protein which is shown to have several health benefits due to its hypolipidemic and hypotensive actions [4, 5]. In addition, soy protein and its components but not casein act to improve insulin sensitivity and promote weight loss in experimental animals [6] and humans [7]. On the other hand, the effects of egg protein on insulin resistance are less documented.

Adipose tissue secretes numerous cytokines like adiponectin, leptin, tumor necrosis factor (TNF)-α and interleukin (IL) 6 which regulate insulin action/sensitivity, glucose metabolism and lipid homeostasis. Studies have shown that the production and action of these adipokines are affected in obesity, and the MS. For example, plasma adiponectin levels are lower while leptin, TNF-α and IL6 levels were higher in obese subjects [8] and animals [9] compared to the control group. Literature search revealed that the type of dietary protein appears to be a crucial factor since parameters like glucose, insulin, cholesterol and blood pressure are altered differentially in experimental animals [10] and humans [11] fed various dietary proteins. Dietary protein source may also affect the levels of adipokines and their receptors in obesity which needs to be studied.

Diet containing fructose (60%) as the sole source of carbohydrate and casein as the protein type is generally used to induce insulin resistance in experimental rodents [12]. These animals exhibit nearly all the clinical manifestations associated with human MS. This study was designed to investigate whether insulin sensitivity, glucose-metabolizing enzymes, plasma biochemistry, antioxidant status, adipokines and the gene expression of adipokine receptors in liver could be changed in rats fed with a high fructose diet containing proteins other than casein namely soy protein or egg albumin.

2. Materials and methods

2.1. Fine chemicals, kits and solvents

Defatted soy protein, egg albumin powder and fat-free casein were purchased from Sakthi Sugars Limited, Coimbatore, India, SKM Egg Products Export Limited, Erode, India and Sisco Research Laboratory, Mumbai, India respectively.

Insulin and glucose assay kits were obtained from Monobind Microwells Inc, CA, USA and Agappe Diagnostics Pvt. Ltd, Kerala, India respectively. Assay kits for TNF-α and IL6 were obtained from Koma Biotech, Seoul, South Korea and kits for adiponectin and leptin were from Invitrogen, CA, USA. Supersensitive polymer - horseradish peroxidase immunohistochemistry detection kit was purchased from Biogenex laboratories, San Ramon, CA, USA. Primers were purchased from Sigma-Aldrich, MO, USA and the SYBR Green-qPCR master mix was purchased from Thermo Scientific, MA, USA. Anti-8 hydroxy guanosine (8OHG) goat pAb was purchased from Merck (Calbiochem), Darmstadt, Germany.

2.2. Experimental design and diet

The experiments were performed in accordance with the guidelines of the Institutional Animal Ethics Committee, Rajah Muthiah Medical College and Hospital, Annamalai Nagar and all the experimental procedures were approved by the committee (No. 160/1999/CPCSEA/770). Adult male albino rats of Wistar strain weighing 140–160 g were individually housed under hygienic conditions in polypropylene cages under 12 hr light/ 12 hr dark cycle (at 22–24°C). After acclimatization for a period of 1 week, rats were distributed randomly into six experimental groups containing 6 animals each.

The animals were allowed free access to water and to any one of the six semi-synthetic diets varying carbohydrate or protein type. The composition of the diet for each group is given in Table 1. The diets were prepared fresh every day. The diet provided a metabolizable energy of 3.84 Kcal/g of which 65.75% was obtained from starch in the control diet and from fructose in the fructose diet. The body weight of the animals was recorded every fourth day and food and water intake were measured daily. Blood was collected just prior to sacrifice and plasma was separated by centrifugation of the blood samples at $371 \times g$ for 10 min. Portions of the liver were either frozen immediately in liquid nitrogen or fixed in 10% formalin for histology.

Table 1
Diet composition

Ingredients (g/100 g)	Control Diet			Fructose Diet		
	Casein protein (C-CAS)	Egg Albumin (C-EGG)	Soy protein (C-SOY)	Casein protein (F-CAS)	Egg albumin (F-EGG)	Soy protein (F-SOY)
Corn starch	60.0	60.0	60.0	–	–	–
Fructose	–	–	–	60.0	60.0	60.0
Casein (fat free)	20.0	–	–	20.0	–	–
Egg Albumin	–	20.0	–	–	20.0	–
Soy protein	–	–	20.0	–	–	20.0
Groundnut oil	5.0	5.0	5.0	5.0	5.0	5.0
Wheat bran	11.0	11.0	11.0	11.0	11.0	11.0
Salt mixture♣	3.5	3.5	3.5	3.5	3.5	3.5
Vitamin mixture$^{\psi}$	0.5	0.5	0.5	0.5	0.5	0.5

♣The composition of mineral mix (g/kg)-M $gSO_4 \cdot 7H_2O$−30.5; NaCl 65.2; KCl−105.7; KH_2PO_4−200.2; $MgCO_3$−3.65$Mg(OH)_{2.}3H_2O$−38.8; $FeC_6H_5O_7 \cdot 5H_2O$−40.0; $CaCO_3$−512.4; KI−0.8; NaF−0.9; $CuSO_4 \cdot 5H_2O$−1.4; $MnSO_4$−0.4 and $CONH_3$−0.05. $^{\Psi}$The composition of vitamin mix (g/kg)- thiamine mono nitrate-3; riboflavin-3; pyridoxine HCl-3.5; nicotinamide-15; D-calcium pantothenate-8; folic acid-1; D-biotin-0.1; cyanocobalamin-0.005; vitamin A acetate-0.6; α-tocopherol acetate-25 and choline chloride-10.

2.3. Biochemical parameters - Glucose, insulin and insulin sensitivity indices

Plasma glucose and insulin were assayed using kits and the degree of insulin sensitivity was assessed by computing homeostatic model assessment (HOMA) [13], quantitative insulin sensitivity check index (QUICKI) [14], and fasting insulin resistance index (FIRI) [15]. The formulae used are given below:

HOMA = [Insulin (μU/ml) × glucose (mM)]/22.5

QUICKI = 1/[log(insulin μU/ml) × log (glucose mg/dl)]

FIRI = [Fasting insulin (μU/mL) × fasting glucose (mg/dL)]/25

2.4. Measurement of glycolytic and gluconeogenic enzymes and lipid profile

The activities of hexokinase, pyruvate kinase, glucose-6-phosphatase, and fructose 1, 6-bisphosphatase were assayed by standard procedures reported elsewhere [16].

Lipids were extracted from the plasma and liver according to the method of Folch et al. [17]. The content of triglycerides (TG), cholesterol and free fatty acids (FFA) were measured in plasma and liver by standard methods reported elsewhere [18].

2.5. Oxidative stress markers and levels of adipokines

Oxidative stress markers such as thiobarbituric acid reactive substance (TBARS), lipid hydroperoxides (LHP) and protein carbonyl (PC) content were quantified in plasma and liver by standard methods reported elsewhere [19]. Leptin, adiponectin, TNF-α and IL6 were assayed in plasma using sandwich enzyme linked immunosorbent assay kits according to manufacturers' instructions.

2.6. Enzymatic and non-enzymatic antioxidants

The activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidise (GPx) were assayed in the hemolysate and liver homogenate and the levels of reduced glutathione (GSH), ascorbic acid (vitamin C), α-tocopherol (vitamin E) were assayed in the plasma and liver homogenate by methods outlined elsewhere [18].

2.7. Histology and immunohistochemistry

At the end of 8th week, histological analysis of liver was performed. Liver samples were fixed at room temperature with 10% formalin and embedded in paraffin. Sections of 4–5 μm thickness were mounted on glass slides, deparaffinised and dehydrated in graded alcohol. Sections were stained with haematoxylin and eosin.

For immunohistochemistry, 4 μm paraffin-embedded liver sections were deparaffinized with xylene and rehydrated with graded concentrations of isopropyl alcohol. Slides were incubated overnight with anti-8OHG antibody (1:200 dilution). The slides were rinsed well with phosphate buffer and incubated with super enhancer reagent for 30 min. After rinsing with phosphate buffer, antibody binding was detected using supersensitive polymer - horseradish peroxidase immunohistochemistry detection system. Sections were counterstained with haematoxylin and observed under the light microscope.

2.8. RNA preparation and real time-polymerase chain reaction (RT-PCR) analysis

Total cellular RNA was extracted from rat liver using TriZol reagent. RNA concentration was determined spectrophotometrically at 260 nm using Biophotometer plus (Eppendorf, Hamburg, Germany) and the purity of RNA preparation was checked by calculating the absorbance ratio at 260/280 nm. RT-PCR was conducted as a two step PCR procedure. Total cellular RNA (2.0 μg) was reverse transcribed to cDNA in a reaction mixture containing Oligo $(dT)_{18}$ primer (0.5 μg/μl), RNase inhibitor (20 U/μl), 0.1 M DTT, RT Buffer (5X), dNTP mix (2.5 mM each) and M-MuLV Reverse Transcriptase (50 U/μl). Reaction mixture were incubated at 25°C for 10 min, then at 37°C for 1 hour followed by 85°C for 10 min, to prevent secondary structures and the transcribed cDNA was quantified (Biophotometer Plus, Eppendorf, Hamburg, Germany).

RT-PCR amplification was performed in a 20 μl reaction mixture containing cDNA (100 ng), 1 μl each of 0.3 μM of reverse and forward primers, 10 μl Maxima SYBR green qPCR master mix and sterile water. The nucleotide sequences of primers used are given in the Appendix. PCR program was conducted using Real-time PCR system Mastercycler ep realplex (Eppendorf, Hamburg, Germany) in universal cycling conditions (10 min at 95°C, 40 cycles of 2 min at 95°C, 30 sec at 60°C (or the optimal Tm) and 20 sec at 72°C). A melting curve analysis was made after each run to ensure a single amplified product for every reaction. The amount of target gene, normalized to an endogenous control glyceraldehyde 3 phosphate dehydrogenase (GAPDH) by $2^{-\Delta\Delta CT}$ method. The relative quantity was expressed in bar graphs as fold change with respect to control.

2.9. Statistical analysis

The values obtained are expressed as means ± SD of 6 rats for biochemical analysis, and those for RT-PCR analysis are means ± SD of 4 rats from each group. Statistical evaluation was done by one-way analysis of variance (ANOVA) followed by two-way ANOVA using Prism version 6.0 software (GraphPad, USA) with carbohydrate and protein type as variables. *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. Body weight, liver weight and liver index, glucose, insulin and insulin sensitivity measures (FIRI, HOMA, and QUICKI)

The initial and final body weights, liver weight and liver index (liver weight/body weight × 100) of the experimental rats are given in Table 2. Fructose feeding caused significant rise in final body weight in all three groups (F-CAS, F-EGG and F-SOY) irrespective of protein source and the final body weights were higher than the respective starch-fed controls (C-CAS, C-EGG and C-SOY). Rats of F-CAS group gained more body weight than those of F-EGG or F-SOY groups. There was no noticeable change in liver index for all fructose-fed and starch-fed groups at the end of experimental period. The values did not differ significantly between C-CAS, C-EGG and C-SOY groups. The carbohydrate and protein type seem to have no interactive effects on the final body weight and liver index.

Table 2
Body weight, liver weight, liver index, plasma levels of glucose and insulin and insulin sensitivity indices at the end of 8 weeks

Parameters	C-CAS	C-EGG	C-SOY	F-CAS	F-EGG	F-SOY	Two-Way ANOVA		
							CARB	PROT	INTER
Body weight									
Initial (g)	142.8 ± 10.2	144.2 ± 11.3	145.4 ± 9.5	146.8 ± 10.6	144.4 ± 8.9	143.6 ± 12.8	NS	NS	NS
Final (g)	164.3 ± 14.3	162.5 ± 12.2	163.2 ± 10.3	182.3 ± 14.9	177.5 ± 13.6	165.2 ± 14.6	$P < 0.05$	NS	NS
Liver weight (g)	5.39 ± 0.2^{a}	5.17 ± 0.2^{a}	5.18 ± 0.2^{a}	7.33 ± 0.6^{b}	6.87 ± 0.4^{c}	6.32 ± 0.3^{c}	$P < 0.0001$	$P < 0.05$	NS
Liver index (%)	3.28 ± 0.1^{a}	3.18 ± 0.2^{a}	3.17 ± 0.2^{a}	4.02 ± 0.28^{b}	3.87 ± 0.1^{b}	3.82 ± 0.2^{b}	$P < 0.0001$	NS	NS
Glucose (mg/dl)	92.4 ± 5.8^{a}	90.1 ± 5.3^{a}	86.4 ± 4.1^{a}	202.5 ± 16.9^{b}	158.4 ± 12.4^{c}	112.8 ± 9.5^{d}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Insulin (μIU/ml)	17.2 ± 0.7^{a}	17.1 ± 0.6^{a}	16.9 ± 0.8^{a}	30.5 ± 2.4^{b}	24.3 ± 1.9^{c}	19.3 ± 1.5^{d}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
HOMA	3.94 ± 0.4^{a}	3.81 ± 0.3^{a}	3.61 ± 0.4^{a}	16.3 ± 2.6^{b}	9.56 ± 1.5^{c}	5.41 ± 0.9^{a}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
QUICKI	0.41 ± 0.01^{a}	0.41 ± 0.01^{a}	0.42 ± 0.01^{a}	0.28 ± 0.01^{b}	0.32 ± 0.01^{c}	0.37 ± 0.01^{d}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
FIRI	63.8 ± 6.83^{a}	61.7 ± 5.79^{a}	58.59 ± 5.83^{a}	264.7 ± 24.2^{b}	154.9 ± 12.4^{c}	87.78 ± 7.4^{d}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

Values are means ± SD of 6 rats from each group. C-CAS- starch+casein diet-fed rats; C-EGG- starch+egg albumin diet-fed rats; C-SOY- starch+soy protein diet-fed rats; F-CAS-fructose+casein diet-fed rats; F-EGG- fructose+egg albumin diet-fed rats; F-SOY- fructose+soy protein diet-fed rats. NS- not significant; CARB-Carbohydrate; PROT-Protein; INTER-Interaction. Values that bear different superscripts are significantly different with each other [one way ANOVA followed by Tukey's test ($p < 0.05$)].

At the end of 8 weeks, significant increase in blood glucose and insulin levels were seen in all fructose-fed groups as compared to the respective starch-fed groups irrespective of the protein source (Table 1). Among the groups fed fructose diet, glucose and insulin levels of rats fed casein were significantly higher than those that were fed any of the other two proteins. The C-SOY, C-EGG and C-CAS groups did not show any change in glucose and insulin levels between one another. The type of carbohydrate and protein showed both independent and interactive effects on glucose and insulin.

All fructose-fed groups showed lower level of insulin sensitivity than the respective starch-fed control groups (Table 2). Within the fructose-fed groups, the HOMA and FIRI values were significantly increased in F-CAS group as compared to F-EGG or F-SOY groups. Although the values were reduced in F-EGG group compared to F-CAS group, the decrease was not as high as that seen in F-SOY group. QUICKI, another index of insulin sensitivity was lower in F-CAS rats than F-EGG and F-SOY. Thus soy protein showed better improvement in insulin sensitivity than egg protein when given along with fructose. Among the starch diet-fed animals (C-SOY, C-EGG and C-CAS groups), no variation in insulin sensitivity indices were observed suggesting that protein source did not have any impact on insulin sensitivity in control animals (Table 2).

3.2. Glycolytic and gluconeogenic enzymes and lipid profile

Table 3 gives the activities of hepatic carbohydrate-metabolizing enzymes of rats at the end of experimental period. The activities of hexokinase and pyruvate kinase were significantly decreased and those of glucose 6 phosphatase and fructose 1, 6-bis phosphatase were significantly increased in F-CAS group when compared with F-EGG or F-SOY groups. The C-SOY, C-EGG and C-CAS groups did not show any significant variation between one another in the activities of these enzymes. Altered activities were seen in all fructose-fed rats as compared to their starch-fed counterparts. The type of carbohydrate and protein showed both independent and interactive effects on glucose 6 phosphatase and fructose 1, 6-bis phosphatase while there was no interactive effect on hexokinase and pyruvate kinase.

The levels of cholesterol, TG and FFA in fructose-fed groups were significantly higher than the respective starch-fed groups (Table 3). Among fructose fed groups, casein inclusion caused significant elevation of lipids followed by egg albumin and soy protein. In starch diet-fed animals, the type of protein did not affect the lipid levels in plasma and liver and all three groups showed normal lipid levels. Statistically, the protein and carbohydrate source showed both independent and interactive effects on lipid profile.

Table 3
Liver glycolytic and gluconeogenic enzymes and plasma and liver lipid profile of experimental animals at the end of the experimental period

Parameters	C-CAS	C-EGG	C-SOY	F-CAS	F-EGG	F-SOY	Two-Way ANOVA		
							CARB	PROT	INTER
Hexokinase (μmoles of glucose phosphorylated.h^{-1}.mg protein^{-1})	9.06 ± 0.5^{a}	9.44 ± 0.6^{a}	9.80 ± 0.3^{a}	6.74 ± 0.2^{b}	7.49 ± 0.3^{c}	7.80 ± 0.5^{c}	$P < 0.0001$	$P < 0.001$	NS
Pyruvate kinase (μmoles of pyruvate formed.min^{-1}.mg protein^{-1})	5.96 ± 0.2^{a}	6.12 ± 0.4^{a}	6.35 ± 0.5^{a}	3.69 ± 0.2^{b}	7.13 ± 0.6^{c}	7.95 ± 0.4^{d}	NS	$P < 0.0001$	$P < 0.0001$
Glucose-6-phosphatase (μg of Pi liberated.min^{-1}.mg protein^{-1})	28.1 ± 1.2^{a}	27.2 ± 1.4^{a}	27.2 ± 1.1^{a}	37.8 ± 1.8^{b}	31.8 ± 1.7^{c}	29.8 ± 1.8^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Fructose 1,6 bis phosphatase (μg of Pi liberated.min^{-1}.mg protein^{-1})	4.83 ± 0.3^{a}	4.72 ± 0.2^{a}	4.54 ± 0.2^{a}	14.2 ± 0.9^{b}	12.7 ± 0.9^{c}	8.13 ± 0.5^{d}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Lipid profile									
Cholesterol									
Plasma (mmol/l)	2.44 ± 0.09^{a}	2.43 ± 0.07^{a}	2.45 ± 0.05^{a}	3.5 ± 0.4^{b}	2.9 ± 0.2^{c}	2.8 ± 0.1^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Liver (mg/g tissue)	3.37 ± 0.14^{a}	3.52 ± 0.21^{a}	3.29 ± 0.17^{a}	5.91 ± 0.32^{b}	5.23 ± 0.2^{c}	4.28 ± 0.2^{d}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Triglycerides									
Plasma (mmol/l)	1.09 ± 0.03^{a}	0.99 ± 0.05^{a}	0.89 ± 0.09^{a}	1.95 ± 0.2^{b}	1.32 ± 0.2^{c}	1.2 ± 0.1^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Liver (mg/g tissue)	2.9 ± 0.08^{a}	2.8 ± 0.09^{a}	2.7 ± 0.07^{a}	4.9 ± 0.54^{b}	4.4 ± 0.4^{c}	4.31 ± 0.17^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Free fatty acid									
Plasma (mmol/l)	2.99 ± 0.07^{a}	2.89 ± 0.06^{a}	2.87 ± 0.09^{a}	5.85 ± 0.4^{b}	5.03 ± 0.2^{c}	3.86 ± 0.1^{d}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Liver (mg/g tissue)	3.90 ± 0.15^{a}	3.98 ± 0.19^{a}	3.8 ± 0.13^{a}	9.08 ± 0.57^{b}	7.8 ± 0.35^{c}	5.1 ± 0.29^{d}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

Values are means ± SD of 6 rats from each group. C-CAS- starch+casein diet-fed rats; C-EGG- starch+egg albumin diet-fed rats; C-SOY- starch+soy protein diet-fed rats; F-CAS-fructose+casein diet-fed rats; F-EGG- fructose+egg albumin diet-fed rats; F-SOY- fructose+soy protein diet-fed rats. NS- not significant; CARB-Carbohydrate; PROT-Protein; INTER-Interaction. Values that bear different superscripts are significantly different with each other [one way ANOVA followed by Tukey's test ($p < 0.05$)].

3.3. Oxidative stress markers and adipokines

Table 4 lists the levels of TBARS, LHP and PC in plasma and liver of experimental animals at the end of 8 weeks. All the fructose-fed groups showed higher levels of oxidation products when compared to their starch-fed counterparts. F-CAS rats showed significantly higher levels of these compounds as compared to F-SOY and F-EGG groups. F-SOY group showed values closer to those observed for C-SOY. There were no significant differences in the levels of these markers among C-SOY, C-EGG and C-CAS groups. The type of carbohydrate and protein showed both independent and interactive effects on lipid peroxidation indices.

Adipokine levels did not differ significantly between the three groups fed starch diet (Table 4). However, rats of F-CAS showed hyperleptinemia and increased levels of TNF-α and IL6 and decreased levels of adiponectin compared to F-SOY and F-EGG. The concentrations of adipokines were within normal limits in F-SOY and F-EGG groups. Thus, replacement with either soy or egg protein prevented the alterations in adipokine levels induced by consumption

Table 4
Oxidative stress markers and plasma adipokines in experimental rats at the end of the experimental period

Parameters	C-CAS	C-EGG	C-SOY	F-CAS	F-EGG	F-SOY	Two-Way ANOVA		
							CARB	PROT	INTER
Lipid peroxidation indices									
TBARS									
Plasma (μmol/dl)	1.02 ± 0.06^{a}	0.97 ± 0.02^{a}	0.88 ± 0.04^{a}	4.97 ± 0.1^{b}	3.07 ± 0.1^{c}	2.12 ± 0.2^{d}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Liver (μmol/mg protein)	2.53 ± 0.1^{a}	2.27 ± 0.2^{a}	2.37 ± 0.1^{a}	7.14 ± 0.2^{b}	3.19 ± 0.1^{c}	3.5 ± 0.1^{d}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
LHP									
Plasma (nmol/dl)	1.89 ± 0.06^{a}	1.23 ± 0.03^{b}	1.26 ± 0.05^{b}	4.64 ± 0.1^{c}	3.07 ± 0.2^{d}	3.06 ± 0.2^{d}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Liver (μmol/mg protein)	5.55 ± 0.5^{a}	5.47 ± 0.4^{a}	5.22 ± 0.4^{a}	12.2 ± 0.9^{b}	7.96 ± 0.6^{c}	8.7 ± 0.5^{d}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
PC									
Plasma (μmol/dl)	3.34 ± 0.2^{a}	2.34 ± 0.2^{b}	2.24 ± 0.2^{b}	8.78 ± 0.6^{c}	6.87 ± 0.4^{d}	4.56 ± 0.3^{e}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Liver (μmol/mg protein)	4.92 ± 0.2^{a}	4.77 ± 0.2^{a}	4.40 ± 0.1^{a}	9.06 ± 0.6^{b}	7.09 ± 0.4^{c}	6.91 ± 0.4^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Adipokines									
Leptin (ng/ml)	0.42 ± 0.01^{a}	0.41 ± 0.01^{a}	0.40 ± 0.01^{a}	1.22 ± 0.04^{b}	0.68 ± 0.02^{c}	0.81 ± 0.02^{d}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Adiponectin (μg/ml)	4.4 ± 0.2^{a}	4.6 ± 0.2^{a}	4.8 ± 0.3^{a}	2.2 ± 0.08^{b}	5.6 ± 0.4^{c}	5.8 ± 0.4^{c}	NS	$P < 0.0001$	$P < 0.0001$
TNF-α (pg/ml)	78.2 ± 3.9^{a}	76.2 ± 2.5^{a}	74.2 ± 3.2^{a}	141.9 ± 12.0^{b}	129.2 ± 11.7^{c}	122.3 ± 9.7^{c}	$P < 0.0001$	$P < 0.05$	NS
IL-6 (pg/ml)	151.9 ± 11.2^{a}	150.2 ± 8.5^{b}	149.8 ± 12.4^{a}	472.9 ± 21.6^{b}	255.1 ± 17.0^{c}	295.1 ± 17.0^{d}	*$P < 0.0001$*	*$P < 0.0001$*	*$P < 0.0001$*

Values are means ± SD of 6 rats from each group. C-CAS- starch+casein diet-fed rats; C-EGG- starch+egg albumin diet-fed rats; C-SOY- starch+soy protein diet-fed rats; F-CAS-fructose+casein diet-fed rats; F-EGG- fructose+egg albumin diet-fed rats; F-SOY- fructose+soy protein diet-fed rats. NS- not significant; CARB-Carbohydrate; PROT-Protein; INTER-Interaction. Values that bear different superscripts are significantly different with each other [one way ANOVA followed by Tukey's test ($p < 0.05$)].

of high fructose diet. The protein and carbohydrate sources showed independent and interactive effects on both leptin and IL6 and while carbohydrate and protein types did not have significant effect on adiponectin and TNF-α.

3.4. Levels of enzymatic and non-enzymatic antioxidants

The activities of enzymatic antioxidants (SOD, CAT and GPx) and the levels of non-enzymatic antioxidants (vitamins C and E and GSH) measured at the end of the experimental period are presented in Table 5. F-EGG or F-SOY groups showed increased activities of enzymatic and non-enzymatic antioxidants than F-CAS. There was no significant difference within the starch-fed groups but the starch diet fed animals showed increased antioxidant levels as compared to the respective fructose-fed animals. The protein and carbohydrate sources showed independent and interactive effects on both enzymatic antioxidants and non-enzymatic antioxidants.

3.5. Histopathological and immunohistochemical analysis

Figs. 1 (A–F) show the histological sections of liver stained with haematoxylin and eosin stain. Staining reveals necrosis and inflammatory cell infiltration in liver of F-CAS group (Fig. 1D). Sections from F-EGG (Fig. 1E) and F-SOY (Fig. 1F) groups show reduction in inflammatory cell infiltration and necrosis. The abnormalities associated with high fructose feeding were markedly reduced. Egg protein also reduced liver damage but not to that extent shown by soy protein. Sections from C-SOY, C-EGG and C-CAS groups show normal architecture of the liver (Figs. 1A, B and C).

Table 5
Enzymatic and non-enzymatic antioxidants in experimental animals at the end of the experimental period

Parameters	C-CAS	C-EGG	C-SOY	F-CAS	F-EGG	F-SOY	TWO-WAY ANOVA		
							CARB	PROT	INTER
Enzymatic antioxidants									
SOD									
Hemolysate (U/mg Hb)	3.59 ± 0.2^{a}	3.96 ± 0.1^{a}	3.57 ± 0.1^{a}	1.19 ± 0.1^{b}	2.9 ± 0.2^{c}	3.2 ± 0.2^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Liver (U/mg protein)	5.2 ± 0.25^{a}	4.7 ± 0.20^{a}	4.76 ± 0.22^{a}	3.53 ± 0.21^{b}	4.2 ± 0.26^{c}	4.3 ± 0.28^{c}	$P < 0.0001$	NS	$P < 0.0001$
CAT									
Hemolysate (μmoles of H_2O_2 consumed/min/mg Hb)	40.2 ± 2.8^{a}	39.9 ± 1.2^{a}	38.8 ± 2.5^{a}	21.45 ± 1.7^{b}	34.12 ± 3^{c}	36.2 ± 2.9^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Liver (μmoles of H_2O_2 consumed/min/mg protein)	46.5 ± 4^{a}	45.12 ± 3.8^{a}	45 ± 3.5^{a}	26.7 ± 2.2^{b}	36.2 ± 2.5^{c}	38.7 ± 2.8^{c}	$P < 0.0001$	$P < 0.005$	$P < 0.005$
GPX									
Hemolysate (μmoles of GSH consumed/min/mg Hb)	8.6 ± 0.3^{a}	8.2 ± 0.2^{a}	7.9 ± 0.5^{a}	5.45 ± 0.4^{b}	6.89 ± 0.5^{c}	7.09 ± 0.6^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Liver (μmoles of GSH consumed/min/mg protein)	7.9 ± 0.3^{a}	7.8 ± 0.3^{a}	7.5 ± 0.2^{a}	2.9 ± 0.1^{b}	6.11 ± 0.4^{c}	6.8 ± 0.6^{c}	$P < 0.0001$	$P < 0.005$	$P < 0.0001$
Non-enzymatic antioxidants									
Vitamin C									
Plasma (mg/dl)	2.4 ± 0.1^{a}	2.39 ± 0.09^{a}	2.36 ± 0.13^{a}	0.98 ± 0.09^{b}	1.7 ± 0.07^{c}	1.8 ± 0.05^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Liver (μg/mg protein)	4.6 ± 0.15^{a}	4.5 ± 0.13^{a}	4.4 ± 0.11^{a}	1.89 ± 0.08^{b}	3.65 ± 0.08^{c}	3.99 ± 0.06^{d}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Vitamin E									
Plasma (mg/dl)	1.76 ± 0.08^{a}	1.71 ± 0.08^{a}	1.6 ± 0.11^{a}	0.65 ± 0.10^{b}	1.2 ± 0.06^{c}	1.4 ± 0.07^{c}	$P < 0.005$	$P < 0.05$	$P < 0.0001$
Liver (μg/mg protein)	4.83 ± 0.20^{a}	4.63 ± 0.21^{a}	4.49 ± 0.25^{a}	2.27 ± 0.28^{b}	3.5 ± 0.19^{c}	3.7 ± 0.14^{c}	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
GSH									
Plasma (mg/dl)	18.01 ± 1.2^{a}	17.8 ± 0.9^{a}	17.4 ± 0.8^{a}	10.9 ± 0.5^{b}	14.3 ± 0.8^{c}	15.1 ± 0.9^{c}	$P < 0.0001$	$P < 0.0005$	$P < 0.0001$
Liver (μg/mg protein)	72.2 ± 3.2^{a}	70.9 ± 5.4^{a}	69.9 ± 2.9^{a}	52.08 ± 4.2^{b}	65.2 ± 5.1^{c}	63.9 ± 3^{c}	$P < 0.0005$	$P < 0.05$	$P < 0.0001$

Values are means ± SD of 6 rats from each group. C-CAS- starch+casein diet-fed rats; C-EGG- starch+egg albumin diet-fed rats; C-SOY- starch+soy protein diet-fed rats; F-CAS-fructose+casein diet-fed rats; F-EGG- fructose+egg albumin diet-fed rats; F-SOY- fructose+soy protein diet-fed rats. NS- not significant; CARB-Carbohydrate; PROT-Protein; INTER-Interaction. Values that bear different superscripts are significantly different with each other [one way ANOVA followed by Tukey's test ($p < 0.05$)].

The photomicrographs showing the immunochemical localization of 8OHG in liver are given in Fig. 2. The intensity of 8OHG staining was more distinct in F-CAS group than in control diet-fed groups. There was a marked reduction in 8OHG immunoreactivity in the liver of fructose-fed rats given either egg or soy protein. The C-SOY, C-EGG and C-CAS groups showed no difference in the immunoreactivity as compared to one another (Figs. 2A, B and C).

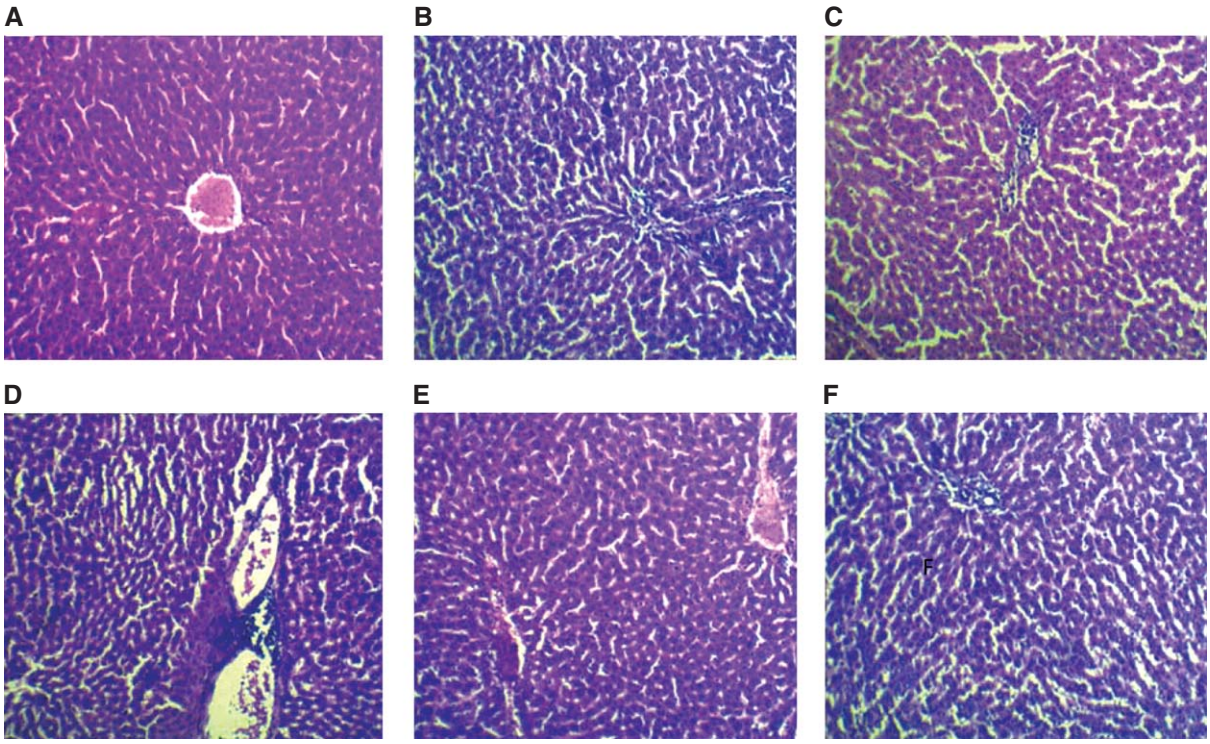

Fig. 1. Histopathological analysis of liver by haematoxylin and eosin (H and E) staining (10x). Liver sections from C-CAS (Fig. 1A), C-EGG (Fig. 1B) and C-SOY (Fig. 1C) appear normal; hepatocytes arranged around the central vein in a trabecular pattern. Section from F-CAS group shows central vein composed of perivascular arrangement of dense inflammatory cells (Fig. 1D). On the other hand, liver sections from rats supplemented with egg (Fig. 1E) and soy protein (Fig. 1F) show reduction in infiammatory cell infiltration and congestion.

3.6. mRNA expression of adipokine receptors in liver

Fig. 3 shows the mRNA levels of adipokine receptors in liver. TNFR1 and TNFR2 expression were similar in all fructose-fed groups (F-CAS, F-EGG and F-SOY), irrespective of protein type. AdipoR1 and adipoR2 expression were decreased to 40 and 31% respectively in F-CAS compared to C-CAS group (C-CAS, 100%) whereas the expression levels were 70 and 75% respectively in F-EGG and 80 and 90% during F-SOY substitution compared to their respective starch-fed groups (C-EGG and C-SOY, 100%). Elevation in mRNA levels of LepR and IL6R were observed in F-CAS (6.1 and 5.9 folds respectively), F-EGG (1.9 and 2.1 folds respectively) and F-SOY (3.9 folds each) group of rats compared to their respective starch administered rats. Egg and soy protein administration suppressed the expression of LepR and IL6R when compared to casein. No significant changes in the mRNA expression of cytokine receptors were observed in starch-fed groups.

4. Discussion

Dietary models of insulin resistance and obesity are created by manipulating the macronutrient content of the diet. The milk protein, casein is commonly the dietary protein used in the preparation of such diets. The present experimental study in rats investigated whether varying the type of protein, for example, egg albumin or soy protein for casein, would influence the adverse effects of feeding high quantities of fructose. Our findings suggest that proteins from both soy and egg improve insulin sensitivity, maintain glucose and lipid homeostasis, potentiate antioxidant defense, reduce oxidative stress and also have beneficial effects on adipokine levels and the gene expression of adipokine receptors when substituted for casein. However, soy protein improved lipid homeostasis and insulin sensitivity to a

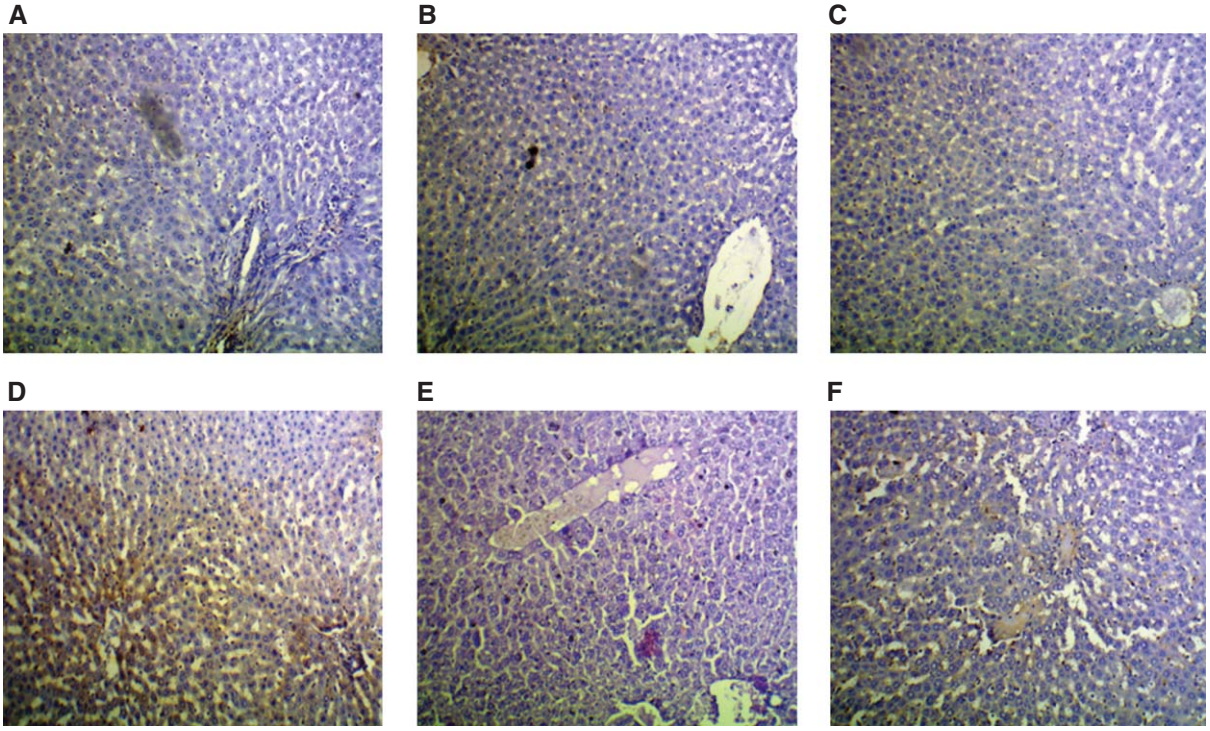

Fig. 2. Liver sections for 8OHG immunoreactivity. The photomicrographs depict the immunochemical localization of 8 hydroxy guanosine (8OHG) in liver (10x). The intensity of 8OHG staining was more distinct in F-CAS group than in starch diet-fed groups (Fig. 2D). There was a marked reduction in 8OHG immunoreactivity in the liver of fructose fed rats given either egg or soy protein (Fig. 2E and F). C-SOY, C-EGG and C-CAS groups (Fig. 2A, B and C respectively) show no apparent differences in immunoreactivity with one another.

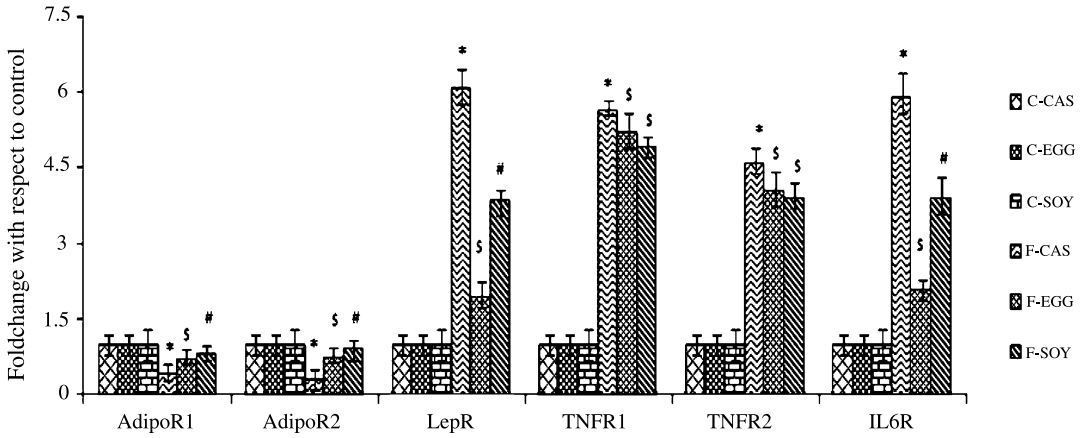

Fig. 3. Effects of dietary proteins on the mRNA expression of adipokine receptors. Significant reductions in adipoR1 and adipoR2 expression were observed in F-CAS group, compared to F-SOY and F-EGG groups. TNFR1 and TNFR2 expression were similar in all fructose fed groups (F-CAS, F-EGG and F-SOY). The gene expression of IL6R and LepR were higher in F-CAS group and the expression was reduced in F-EGG or F-SOY groups. Egg protein reduced LepR and IL6R expression to a greater extent than soy protein. C-SOY, C-EGG and C-CAS groups show no apparent differences in the mRNA levels of adipokine receptors. Values are means $\pm$ SD, ($n = 4$). Statistical analysis was performed using one way ANOVA followed by Tukey's Multiple Range Test. Values without a common superscript symbol differ by $p < 0.05$.

greater extent than egg albumin while egg albumin was more effective in reducing oxidative stress and inflammation. However, the protein type did not have an impact on glucose, insulin or insulin sensitivity indices when the diet contained only starch as the carbohydrate source.

Impaired glucose tolerance, hyperinsulinemia, dyslipidaemia and ectopic lipid deposition are associated with high fructose diet (60%) feeding in animal models [20, 21]. In such studies fructose and casein are the carbohydrate and protein sources respectively. Hepatic and peripheral insulin resistance in fructose-fed animals arise due to defects in the downstream events of insulin signaling. Oxidative stress, production of inflammatory cytokines and accumulation of intracellular lipid metabolites like FFA, diacylglycerol and ceramides that can produce lipotoxicity are responsible for activation of stress-related kinases resulting in decrease in insulin signaling during fructose feeding [22]. Increased oxidative stress in liver was evidenced in F-CAS group.

Signaling molecules secreted by adipocyte influence insulin sensitivity, glucose homeostasis, and lipid metabolism in different ways. Adiponectin reduces the expression of enzymes involved in gluconeogenesis like glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PEPCK) in the liver and directly stimulates glucose uptake in muscle and adipocytes [23]. Adiponectin also decreases fatty acid synthesis, promotes fatty acid oxidation, and reduces the accumulation of triglycerides in liver and skeletal muscle [24]. Adiponectin acts by activating two receptors, adipoR1 and adipoR2. AdipoR1 is widely expressed in tissues, including muscle, liver, and pancreas, whereas adipoR2 is found most abundantly in liver. Binding of adiponectin to its receptors activates peroxisome proliferator-activated receptor (PPAR)-α and AMP-activated protein kinase (AMPK). This causes insulin sensitivity and increased anti-inflammatory effects. Leptin regulates food intake, lipid metabolism and energy expenditure [25]. Leptin binds to its receptor (LepR) and initiates a phosphorylation cascade via Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3) pathway which in turn activates both AMPK and PPAR-γ coactivator (PGC)-1α in liver [26]. On the other hand, TNF-α and IL6 impair the PI3K-AKT pathway of insulin signaling pathway by inducing insulin receptor substrate (IRS) kinases that cause serine phosphorylation of IRS and partly by increasing the expression of suppressor of cytokine signaling (SOCS) [4]. These events block tyrosine phosphorylation of the IRS resulting in decreased flux through downstream signaling. TNF-α exerts its inhibitory effects on insulin signalling predominantly through stimulation of TNFR1 and TNFR2 [27] whereas the IL6 binds to the class I family of cytokine receptors, that uses Janus kinases (JAKs) as intracellular signaling pathway [28].

We observed decreased levels of adiponectin and increased levels of leptin, TNF-α and IL6 in F-CAS group. The levels of leptin and IL6 were reduced significantly by egg protein than soy protein. Soy protein diet may promote the conversion of large adipocytes to smaller ones this may be responsible for the enhanced levels of adiponectin and reduced levels of leptin. Nagasawa et al. [29] reported that the mRNA expression level in adipose tissue and the plasma concentration of the anti-atherogenic protein, adiponectin were higher whereas leptin mRNA levels were lower in rats given soy protein isolate than those fed casein. Studies suggest that the gene expression of adipokines is influenced by the total calorie content of diet and also by its composition [30]. Elevated expression of IL6R and LepR (approximately 6 fold each) and reduced expression of adipoR1 and adipoR2 in F-CAS (reduced by 60% and 69% respectively) were observed. Reports show that a decreased expression of adiponectin receptors could participate in the development of insulin resistance [31]. Egg and soy protein substitution prevented this drastic raise in the gene expression of IL6 and LepR indicating that they play a better role in preventing inflammation than casein even under frutose administration. No significant changes were observed in the expression of TNFR1 and TNFR2 between three protein-fed fructose groups.

Increased levels of lipids, decreased adiponectin, elevated TNF-α, IL6 and leptin may all contribute to insulin resistance observed in fructose+casein fed rats. Egg protein and soy protein maintained glucose and lipid homeostasis, reduced oxidative stress and inflammatory cytokine production and increased adiponectin. These effects may contribute to the improved insulin sensitivity. The observation on glucose-metabolizing enzymes also suggests that soy and egg protein-fed rats display glucose homeostasis and greater insulin sensitivity than casein-fed rats.

Proteins of animal origin, such as casein, are generally hypercholesterolemic and atherogenic, when compared with vegetable proteins, like soybean protein. Phytoestrogens of soy favorably affect glucose homeostasis and metabolism by inhibiting glucose uptake into intestinal brush border membrane vesicles and by enhancing glucose-stimulated insulin secretion [32]. Soy protein reduces the magnitude of postprandial insulin resistance, improves insulin sensitivity [33] and exerts antidiabetic effect in type 2 diabetes (T2D) subjects [34]. Compared with casein, soy protein improved fasting and postprandial plasma insulin responses, glucose tolerance and insulin sensitivity in rats [35].

Although casein, egg and soy proteins upon digestion generate bioactive peptides which have several properties including antioxidative and antihypertensive activities, differences in their amino acid composition have been proposed to mediate protein-dependent changes in glucose and insulin dynamics [36]. The presence of higher amounts of arginine and lower amounts of branched chain aminoacids and low lysine:arginine ratio in soy and egg protein might impart beneficial effects compared to casein. Studies have found that short and long-term oral L-arginine supplementation improved insulin sensitivity by increasing insulin sensitivity index and adiponectin levels, and exerted anti-inflammatory and antioxidant actions in T2D patients [37]. Low lysine:arginine ratio in soy protein has been shown to be related to its antiatherogenic effect [38] and supplementation of branched chain aminoacids has been to shown to increase insulin resistance in Wistar rats [39].

The treatment strategy for MS includes weight loss, increase in physical activity, and close attention toward dietary modification. Dietary modifications such as calorie restricted diet, moderate restriction in saturated fat and cholesterol intake and inclusion of dietary fibre are suggested to reduce the risk of MS and T2D. Awareness on the close relation that exists between type of protein and various life style-related diseases has begun to emerge recently. From our findings, we conclude that replacement of casein with egg albumin or soy protein reduces the manifestations of MS and improves insulin sensitivity by favourably modifying the adipokines. Protein-dependent variation in the regulation of insulin action through adipokines deserves further investigations in future at the level of adipocytes.

Acknowledgments

This work was supported and funded by a research grant from the Indian Council of Medical Research (ICMR), New Delhi, India.

Conflict of interest

None.

References

[1] Sadikot S, Hermans M. Here we go again . . . The metabolic syndrome revisited! Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2010;4:111-20.

[2] van Dam RM, Willett WC, Rimm EB, Stampfer MJ, Hu FB. Dietary fat and meat intake in relation to risk of type 2 diabetes in men. Diabetes Care. 2002;25(3):417-24.

[3] USDA National Nutrient Database for Standard Reference.

[4] Friedman M. Nutritional value of proteins from different food sources: A Review. J Agric Food Chem. 1996;44:6-29.

[5] Hermansen K, Hansen B, Jacobsen R, Clausen P, Dalgaard M, Dinesen B, et al. Effects of soy supplementation on blood lipids and arterial function in hypercholesterolaemic subjects. Eur J Clin Nutr. 2005;59(7):843-50.

[6] Liao FH, Shieh MJ, Yang SC, Lin SH, Chien YW. Effectiveness of a soy-based compared with a traditional low-calorie diet on weight loss and lipid levels in overweight adults. Nutrition. 2007;23(7-8):551-6.

[7] Lukaszuk JM, Luebbers P, Gordon BA. Preliminary study: Soy milk as effective as skim milk in promoting weight loss. J Am Diet Assoc. 2007;107(10):1811-4.

[8] Kern PA, Di Gregorio GB, Lu T, Rassouli N, Ranganathan G. Adiponectin expression from human adipose tissue: Relation to obesity, insulin resistance, and tumor necrosis factor-alpha expression. Diabetes. 2003;52(7):1779-85.

[9] Rong X, Li Y, Ebihara K, Zhao M, Kusakabe T, Tomita T, et al. Irbesartan treatment up-regulates hepatic expression of PPAR alpha and its target genes in obese Koletsky (fa(k)/fa(k)) rats: A link to amelioration of hypertriglyceridaemia. Br J Pharmacol. 2010;160(7):1796-807.

[10] Jacobucci HB, Sgarbieri VC, Dias NFGP, Borges P, Tanikawa C. Impact of different dietary protein on rat growth, blood serum lipids and protein and liver cholesterol. Nutr Res. 2001;21:905-15.

[11] Anderson JW, Fuller J, Patterson K, Blair R, Tabor A. Soy compared to casein meal replacement shakes with energy-restricted diets for obese women: Randomized controlled trial. Metabolism. 2007;56(2):280-8.

[12] Sil R, Ray D, Chakraborti AS. Glycyrrhizin ameliorates insulin resistance, hyperglycemia, dyslipidemia and oxidative stress in fructose-induced metabolic syndrome-X in rat model. Indian J Exp Biol. 2013;51(2):129-38.

[13] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia.1985;28:412-9.

[14] Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ. Quantitative insulin sensitivity check index: A simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab. 2000;85(7):2402-10.

[15] Harati M, Ani M, Messripour M. Effect of vanadyl sulphate on fructose induced insulin resistance rat. Iranian Biomedical Research. 2003;7(4):179-82.

[16] Anitha Nandhini AT, Thirunavukkarasu V, Anuradha CV. Taurine modifies insulin signaling enzymes in the fructose-fed insulin resistant rats. Diabetes Metab. 2005;31(4 Pt 1):337-44.

[17] Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 1957;226(1):497-509.

[18] AnithaNandhini AT, Balakrishnan SD, Anuradha CV. Taurine modulates antioxidant potential and controls lipid peroxidation in the aorta of high fructose-fed rats. J Biochem Mol Biol Biophys. 2002;6(2):129-33.

[19] Balasaraswathi K, Rajasekar P, Anuradha CV. Changes in redox ratio and protein glycation in precataractous lens from fructose-fed rats: Effects of exogenous L-Carnitine. Clin Exp Pharmacol Physiol. 2008;35(2):168-73.

[20] Suwannaphet W, Meeprom A, Yibchok-Anun S, Adisakwattana S. Preventive effect of grape seed extract against high-fructose diet-induced insulin resistance and oxidative stress in rats. Food Chem Toxicol. 2010;48(7):1853-7.

[21] Lê KA, Ith M, Kreis R, Faeh D, Bortolotti M, Tran C, Boesch C, Tappy L. Fructose overconsumption causes dyslipidemia and ectopic lipid deposition in healthy subjects with and without a family history of type 2 diabetes. Am J Clin Nutr. 2009;89(6):1760-5.

[22] Kelley GL, Allan G, Azhar S. High dietary fructose induces a hepatic stress response resulting in cholesterol and lipid dysregulation. Endocrinology. 2004;145(2):548-55.

[23] Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med. 2002;8(11):1288-95.

[24] Yamauchi T, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature. 2003;423(6941):762-9.

[25] Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. Science. 1995;269(5223):540-3.

[26] Asilmaz E, Cohen P, Miyazaki M, Dobrzyn P, Ueki K, Fayzikhodjaeva G, et al. Site and mechanism of leptin action in a rodent form of congenital lipodystrophy. J Clin Invest. 2004;113(3):414-24.

[27] Straczkowski M, Kowalska I, Stepien A, Dzienis-Straczkowska S, Szelachowska M, Kinalska I. Increased plasma-soluble tumor necrosis factor-alpha receptor 2 level in lean nondiabetic offspring of type 2 diabetic subjects. Diabetes Care. 2002;25(10):1824-8.

[28] Ihle JN, Witthuhn BA, Quelle FW, Yamamoto K, Silvennoinen O. Signaling through the hematopoietic cytokine receptors. Annu Rev Immunol. 1995;13:369-98.

[29] Nagasawa A, Fukui K, Kojima M, Kishida K, Maeda N, Nagaretani H, et al. Divergent effects of soy protein diet on the expression of adipocytokines. Biochem Biophys Res Commun. 2003;311(4):909-14.

[30] Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, et al. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. J Clin Endocrinol Metab. 2001;86(8):3815-9.

[31] Inukai K, Nakashima Y, Watanabe M, Takata N, Sawa T, et al. Regulation of adiponectin receptor gene expression in diabetic mice. Am J Physiol Endocrinol Metab. 2005;288(5):E876-82.

[32] Liu D, Zhen W, Yang Z, Carter JD, Si H, Reynolds KA. Genistein acutely stimulates insulin secretion in pancreatic beta-cells through a cAMP-dependent protein kinase pathway. Diabetes. 2006;55(4):1043–50.

[33] Ae Park S, Choi MS, Cho SY, Seo JS, Jung UJ, Kim MJ, et al. Genistein and daidzein modulate hepatic glucose and lipid regulating enzyme activities in C57BL/KsJ-db/db mice. Life Sci. 2006;79(12):1207-13.

[34] Lavigne C, Marette A, Jacques H. Cod and soy proteins compared with casein improve glucose tolerance and insulin sensitivity in rats. Am J Physiol Endocrinol Metab. 2000;278(3):E491-500.

[35] Sanchez A, Hubbard RW. Plasma amino acids and the insulin/glucagon ratio as an explanation for the dietary protein modulation of atherosclerosis. Med Hypotheses. 1991;36(1):27-32.

[36] Aoyama Y, Wada M. Effects of dietary protein type on the response of lipid metabolism to orotic acid in rats. Biosci Biotechnol Biochem. 1999;63(11):2005-8.

[37] Lucotti P, Setola E, Monti LD, Galluccio E, Costa S, Sandoli EP, et al. Beneficial effects of a long-term oral L-arginine treatment added to a hypocaloric diet and exercise training program in obese, insulin-resistant type 2 diabetic patients. Am J Physiol Endocrinol Metab. 2006;291(5):E906-12.

[38] Rajamohan T, Kurup PA. Antiatherogenic effect of a low lysine: Arginine ratio of protein involves alteration in the aortic glycosaminoglycans and glycoproteins. J Biosci. 1990;15(4):305-11.

[39] Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. Cell Metab. 2009;9(4):311-26.

Appendix

List of genes and primer sequences used for the study

Gene	ID	NCBI Reference Sequence	Official symbol	Forward primer sequence (5′- 3′)	Reverse primer sequence (5′- 3′)
Glyceraldehyde-3-phosphate dehydrogenase	24383	NC_005103.3	GAPDH	aaggggaacccttgatatgg	cggagatgatgacccttttg
Adiponectin receptor 1	289036	NC_005112.3	AdipoR1	ctgaaggcactgtgtgtcgt	aaggagggcataggtggtct
Adiponectin receptor 2	312670	NC_005103.3	AdipoR2	gcttgggtctgagtggaatc	tagagggcagctcctgtgat
Leptin receptor	24536	NC_005104.3	Lepr	cacgaggtattcgatgcaaa	aggctggactgctccaatta
TNF receptor I	25625	NC_005103.3	Tnfrsf1a	tgcctcacactgagcatctt	ccccaaagtccacactcact
TNF receptor II	156767	NC_005104.3	Tnfrsf1b	cgctgttccaaggacaatct	ggttgaacccaaggacacag
Interleukin 6 receptor	24499	NC_005101.3	IL6R	gcctattgaaaatctgctctgg	gctctgaatgactctggcttt