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Research Article

High carbohydrate diets enhances lipogenesis and reduces Paraoxonase-1 activity in Isoproterenol induced Myocardial Infarction

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Abstract

Improving diet is a critical component in the management of cardiovascular diseases such as myocardial infarction (MI). Most of the staple diets used worldwide, especially by Asians are high in carbohydrates. The key aim of this study was to investigate how different high carbohydrate diets (HCD) affect paraoxonase-1 (PON1) and lipogenic enzymes activity along with the lipid levels. Rats were fed with different carbohydrate (75%) diets (rice, cassava or corn starch) and standard diet (60% carbohydrates) for 60 days followed by the subcutaneous injection of isoproterenol for the induction of MI. The activity of creatine kinase-MB and the activity of xanthine oxidase showed an increased activity after the consumption of HCD with a maximum shown in rice fed group. Consumption of HCD enhanced lipogenesis as indicated by the high levels of total cholesterol, LDL+ VLDL cholesterol and triglycerides and decreased level of HDL. HCD fed groups showed a decreased activity of PON1 with a maximum decrease in activity in rice fed group. Thus the study showed that consumption of HCD's especially rice may provide a favorable condition for the onset of MI. It is mediated by enhanced oxidative stress, elevated lipogenesis and decreased PON1 activity.

Key words: High carbohydrate diet, paraoxonase 1, myocardial infarction, isoproterenol

INTRODUCTION

Cardiovascular diseases (CVD) are the major contributor to the global burden of disease among the noncommunicable diseases (NCDs)[1].The role of diet and nutrition as determinants of chronic NCDs is well established and they therefore occupy a prominent position in prevention activities WHO report-2002[2]. Many researches show that certain dietary patterns can influence cardiovascular health by modifying risk factors such as obesity, dyslipidemia, and hypertension, as well as factors involved in systemic inflammation, insulin sensitivity, oxidative stress, endothelial function, thrombosis, and cardiac rhythm[3,4].Studies have shown that high-carbohydrate diet (HCD) consumption produces a rat model for obesity and the increased carbohydrate availability in the immediate postnatal life of rats induces adaptations predisposing to adult-onset obesity[5,6].

The majority of the carbohydrate derived from a normal diet reaches the body's peripheral tissues as glucose.Glucose is utilized by all cells and serves as metabolic fuel for muscle, liver, heart, kidneys and gut. It is the obligate energy source for brain, renal medulla and erythrocytes.Reports show that when glucose is administered in excess of the amount which can be directly oxidized for energy and glycogen production. It is directed to lipogenesis thus promoting fat deposition[7,8].

Paraoxonase1 (PON1) is an esterase synthesized by the liver, closely associated to HDL.PON1 secretion is stimulated by HDL, which is also an important factor in the stabilization of the enzyme activity[9].

PON1 is also responsible for HDL's antioxidant function[10].The primary protective effect of HDL is its pivotal role in reverse-cholesterol transport. PON1 component of HDL has an ability to metabolize lipid peroxidesand protect against their accumulation on LDL. Studies by Ferretti reports that *in vitro*glycation of HDL partially inhibit PON1[11].There are reports that point out thatPON1 activity can be modulated by diet[12,13]. PON1 plays an important role in lipid metabolism and the onset of cardiovascular disease[14]. PON1 has been proposed to play an important role in protecting LDL and HDL from oxidation in vitro, thus lowering the risk of developing atherosclerosis[15,16].

Myocardial infarction (MI) is one of the main causes of death from cardiovascular disease. MI is defined as an acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand. Isoproterenol (ISP) has been used as a model compound to induce infarct-like lesions in the rat and various other animal species[17]. The lesions are most prevalent in areas of the heart that are most susceptible to ischemia[18]. Reports showed that ISP-induced myocardial metabolic alterations are similar to those occurring in human beings following a myocardial infarction[19,20].

Rice, cassava and corn starch are some of the importantHCD's used worldwide. Rice is grown worldwide and provides food for more than half of the world's population, especially those living in China, India and Japan[21]. Therefore rice is considered as a traditional staple food in India and other Asian countries[22]. Role of various HCD's that are important staple diets in many parts of the world in influencing CVD is not well studied. Understanding the association of HCD's with CVD may provide a strategy for early intervention in the natural progression of CVD's. Therefore the purpose of the present study was to investigate different commonly used HCD's such as rice, cassava and corn starch in providing a favourable environment for the onset of MI.

MATERIALS AND METHODS

Animals

Female Swiss albino rats weighing 100-120g was used for the study. Rats were housed at standard laboratory conditions (25 ± 2^{o}C, relative humidity 50 ± 10%, 12 h light/12 h dark photoperiod). They were fed with a high carbohydrate diets (HCD) [corn starch, white polished rice or cassava] (Table1), standard pellet diet (VRK's scientific choice laboratory animal feed[@]) and tap water ad libitum for a period of 60 days. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee of the department [IAEC- KU 16/2010-11 BC MI (27)] and animals were handled using the laboratory animal welfare guidelines[23].

Experimental Protocol

Animals were divided into following groups, each group containing 6 animals and the treatment period for the study was 60 days.

Group I: Standard pellet diet (CON)
Group II: Corn starch diet (CS)
Group III: Cassava diet (C)
Group IV: Rice diet (R)
Group V:Standard pellet diet + isoproterenol (ISP)
Group VI:Corn starch diet + isoproterenol (CS+I)
Group VII: Rice + isoproterenol (R+I)
Group VIII: Cassava + isoproterenol (C+I).

MI was induced by a subcutaneous injection of isoproterenol (ISP, Sigma Aldrich) at a dose of 100 mg/kg body weight in normal saline on the 59th day, followed by a booster dose after 24 hours[24,25]. On the 61st day the animals were sacrificed, blood samples and heart tissues were collected and transferred to ice cold containers for further biochemical estimations.

Biochemical Analysis

The activity of creatine kinase-MB (CK-MB) was estimated by using commercialkit from Vital Diagnostics (P) Ltd, India.Xanthine Oxidase was estimated for by the method of Hashimoto[26].

Extraction and estimation of heart tissue lipids

The lipid was extracted from the heart tissue by the method of Folch et al.,[27]. Briefly the tissue were homogenized and extracted with chloroform: methanol (2:1). The mixture was incubated at 50°C for 15 min. it was filtered and the residue washed with chloroform: methanol (2: 1) at least three times. The filtrate was combined. To the filtrate 0.7% KCl was added and mixed. The aqueous upper phase was removed and the lower layer was washed three times with chloroform: methanol: KCl (2:48:47) solution. The washed lower layer of chloroform was evaporated to dryness and the residue was dissolved in a known volume of chloroform. Aliquots of the extract were used for the estimation of various lipids.

Estimation of tissue cholesterol, triglycerides and free fatty acids level

The level of cholesterol was estimated by the method of Zaket al.,[28] Triglycerides by the method of Van Handel and Zilversmith[29], and free fatty acid by the method of Falholt et al.,[30].

Estimation of activity of HMG CoA reductase, malic enzyme and Glucose-6-phosphate dehydrogenase in liver tissue

HMG CoA reductase activity was estimated by the method of Rao and Ramakrishnan[31], malic enzyme by the method of Ochoa[32], and Glucose 6 phosphate dehydrogenase by the method of Kornberg and Horecker[33].

Estimation of serum lipid profile

The levels of serum lipids such as total cholesterol (TC), high density lipoprotein (HDL) cholesterol, low density lipoprotein- very low density lipoprotein (LDL-VLDL) cholesterol and triglycerides (TG) was estimated by commercially available kit as per instructions (Agappe Diagnostics, India).

Analysis of Paraoxonase1 (PON1) activity in serum

PON1 activity was measured by the method of Mackness[34]. Briefly, serum was added to Tris buffer(100 mmol/L, pH 8.0) containing 2 mmol/L CaCl2 and 5.5 mmol/L paraoxon(Sigma AldrichCo). The rate of generation of p-nitrophenol was determined at 405 nm and 25°C with a UV-Vis Spectrophotometer. PON1 activitywas expressed as mmoles of p-nitrophenol liberated per minute per mg protein.

Statistical Analysis

The results were analyzed using a statistical programme SPSS/PC+, Version 11.5 (SPSS Inc., Chicago, IL, USA).A one-way ANOVA was employed for comparison among the six groups. Post-hoc multiple comparison tests of significant differences among groups was determined. Pair fed comparisons between the groups was made by Duncan's multiple range tests (Steel RGD, 1960). $p<0.05$ was considered to be significant.

RESULT

The activities of cardiac toxicity marker, CK-MB showedsignificant ($p<0.05$) increase in the serum of HCD group when compared to standard pellet diet group. The increase in activity was maximum in rice fed group when compared to other HCD group and in rice fed ISP- induced group when compared to cornstarch and cassava fed ISP-induced groups (Table 2).

The analysis of serum lipid profile showed an increased serum lipid levels in HCD fed rats. The level of total cholesterol, LDL+VLDL cholesterol and triglycerides were seen significantly ($p<0.05$) elevated in rice fed group when compared to standard pellet diet and rice fed ISP-induced group when compared to other ISP-induced groups (Table 3).

The evaluation of tissue lipids showed a maximum deposition of lipids in the HCD fed group when compared to standard pelleted diet and also in HCD fed ISP-induced group when compared to standard diet fed ISP-induced group. The level of total cholesterol in tissue was seen elevated significantly ($p<0.05$) along with freefatty acid and triglycerides in the rice fed group when compared to other HCD's and rice fed ISP-induced group when compared to other ISP-induced groups (Table 4).

The analysis of PON1 activity revealed that the consumption of HCD and also MI induction decreased the activity when compared to the standard pelleted diet. The activity was decreased significantly ($p<0.05$) in the rice fed among the HCD and rice fed ISP-induced when compared to other ISP-induced groups (Figure 1).

The activity of lipogenic enzymes was seen increased in all the HCD fed groups when compared to standard pelleted diet and HCD fed ISP-induced groups when compared to standard diet fed ISP-induced group. The activity of HMG CoA reductase, Malic enzymes and glucose-6-phosphate dehydrogenase was seen increased significantly ($p<0.05$) in rice fed group when compared to other HCD's and in rice fed ISP-induced groups when compared to other HCD fed ISP-induced groups (Table 5).

The activity of xanthine oxidase was elevatedsignificantly ($p<0.05$) in the serum of HCD fed groups. Rice fed group showed the maximum increased activity when compared to other HCD's and rice fed ISP-induced group when compared to other ISP-induced groups (Figure 2).

DISCUSSION

Diet and nutrition are important factors in the promotion and maintenance of good health. The dietary state of the body determines the substrate supply to the heart.The stability of body composition requires that intakes of protein, carbohydrate, and fat are balanced in terms of macronutrient oxidation over time [35, 36].

Carbohydrate consumption reduces the need to use fat as fuel, so carbohydrate is important in controlling the balance between fat intake and fat oxidation [37]. Excess carbohydrates get converted to lipids through lipogenesis pathway and get deposited as fat in the body. Thus the HCD may affect the level of adiposity. Our present study revealed that consumption of HCD the levels of lipids elevated, which might be due to the conversion of excess carbohydrate into fat. The levels of TC, LDL+VLDL and TG in serum were higher and the HDL level decreased in therice fed among the HCD's and rice fed ISP-induced group when compared to other ISP-induced groups. This is in agreement with previous

studies which states that increased carbohydrate intake may adversely affect blood lipids and glucose metabolism[38,39].

The analysis of lipogenic enzymes in our study showed anincreased activity after HCD consumption. This may be due to the activation of lipogenic enzymes by HCD. The highest activity of lipogenic enzymes and highest lipid level in the myocardium was shown by rats fed rice among the HCD's and also in rice fed ISP-induced group.This is in agreement with the studies by Aman et al., which states that ISP injected ratshave increased levels of serum and heart tissue lipids and decreased serum HDL cholesterol levels. Increased biosynthesis and decreased utilization of cholesterol after the administration of ISP might have led to elevated cholesterol levels[40]. David et al., also suggested that accumulation of lipid and glucose metabolites in the myocardium could contribute to the development and progression of heart failure[41].This is in agreement with our studies since the activities of HMG CoA activity and lipogenesis enzymes were enhanced in the ISP group indicating increased biosynthesis of lipids.

PON1 have protective role against cardiovascular diseases. Beside the potent antioxidant properties, PON1 also shows favorable effects on macrophage cholesterolmetabolism[42].Reports by Mackness et al.and Kumar et al. states that PON1 is inactivated under oxidative stress conditions[43,44]. This is in agreement with our studies since induction of MI by ISP induces oxidative stress and we have observed reduced PON1 activity in HCD groups and ISP group.Decreased PON1 activity might also be due to the decreased HDL level observed. The activity was decreased significantly in the rice fed among the HCD's and rice fed ISP-induced group when compared to other ISP-induced groups.

Gupte at al., showed that chronic exposure to excess circulating fuels can have a toxic effect on the heart due to formation of noxious derivatives of glucose and lipid metabolism, such as reactive oxygen species (ROS) from glucose[45,46].Report by Raghuvanshiet al., states that xanthine oxidase during myocardial infarction play an important role in contributingfree radical mediated damage[47].Xanthine oxidases convert the excess hypoxanthine formed to xanthine, uric acid and superoxides.In our present study the level of xanthine oxidase was seen to be elevated in the rice fed group among the HCD's and rice fed ISP-induced group when compared to other ISP-induced groups.The increased activity of xanthine oxidase indicates the increased production of free radicals leading to oxidative stress after a HCD diet. The decreased activity of PON1 might haveadded to oxidative stress of the myocardium, which in turn increased the activity of xanthine oxidase. This is in line with the reports that PON1 has got antioxidant effect.

CONCLUSION

The present study revealed that consumption of HCD's especially rice renders the myocardium more susceptible to CVD. This is mediated by enhanced oxidative stress, decreased PON1 activity and elevated lipogenesis.

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Table 1. Composition of high carbohydrate diet

Composition	% diet
Carbohydrate*	75
Protein	15
Fat	5
Salt mixture°	4
Vitamin mixture#	1

*Source of carbohydrate: White rice, cassava or corn starch. White rice and cassava are cooked, drained, dried in oven and powdered.

°Composition of salt mixture: NaCl-105g, KCl-120g, KH2PO4-310g, Ca3(PO4)-149g, CaCO3-210g, anhrdrous MnSO4-0.20g, K2Al2(SO4)3 H2O-0.09g, anhydrous MgSO4- 90g, FePO4. H2O-14.7g, CuSO4.5H2O- 0.39g, NaF- 0.57g, KI- 0.05g, ZnCl2-15g, COCl2. 6 H2O-0.15g.

#Composition of vitamin mixture: Thiamine- 160mg, Riboflavin- 160mg, Pyridoxine- 120mg, Niacin- 1g, calcium panthothenate- 800mg, Inositol- 4g, Folic acid 80 mg, Cyanocobalamine 0.4g, Biotin- 4mg, Vitamin A- 200,00IU, Ergocalciferol- 30,000 IU, Tocopherol- 2.4 g, Menadione-60 mg, Corn starch- 150 g, Choline chloride- 40g.

@Composition of standard pellets (%): Moisture- 10, Crude proteins -22, Crude fibre-0.3, Crude fat-3, Carbohydrate-60, colour-1, phosphorous-0.5, Moisture-11:23.

Table 2: Activity of Creatine kinase-MB (CK-MB) in serum

Group	CK-MB (U/L in serum)
CON	37.235±1.32[a]
CS	43.56±1.62[b]
R	51.44±1.91[b]
C	46.21±1.72[b]
ISP	108.15±4.02[c]
CS+I	117.65±4.38[d]
R+I	128.92±4.82[e]
C+I	120.42±4.48[d]

Values are expressed as Mean ±SEM.
[a,b,c,d,e,f] Mean values within a column with unlike superscript letters were significantly different (P<0·05).

Table 3: Concentration of Total cholesterol, HDL cholesterol and LDL+VLDL cholesterol in serum

Groups	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL+VLDL cholesterol (mg/dl)	Triglycerides (mg/dl)
CON	64.46±2.4[a]	39.75±1.48[a]	26.36±0.98[a]	65.44±2.4[a]
CS	77.70±2.8[b]	36.59±1.36[b]	31.28±1.16[b]	74.72±2.7[b]
R	84.09±3.1[c]	31.75±1.18[c]	36.18±1.34[c]	90.82±3.3[c]
C	80.10±2.9[b]	34.68±1.29[b]	32.12±1.19[b]	87.34±3.2[b]
ISP	129.10±4.8[d]	25.84±0.96[d]	56.75±2.11[d]	106.72±3.9[d]
CS+I	146.07±5.4[e]	23.63±0.88[e]	64.66±2.40[e]	115.48±4.3[e]
R+I	158.00±5.8[f]	20.62±0.76[f]	79.51±2.96[f]	167.58±6.2[f]
C+I	148.48±5.5[e]	21.73±0.81[e]	65.83±2.45[e]	122.27±4.5[e]

Values are expressed as Mean ±SEM.
[a,b,c,d,e,f] Mean values within a column with unlike superscript letters were significantly different (P<0·05).

Table 4: Concentration of Total cholesterol, free fatty acids and triglycerides levels in heart

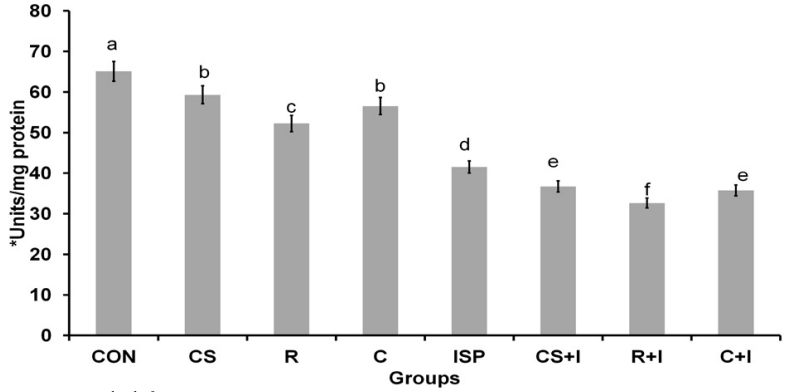
Groups	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL+VLDL cholesterol (mg/dl)
CON	164.84±6.14[a]	149.04±5.55[a]	47.88±1.78[a]
CS	176.51±6.57[b]	156.40±5.82[b]	53.47±1.99[b]
R	183.00±6.81[c]	163.18±6.07[c]	59.60±2.18[c]
C	177.73±6.62[b]	159.15±5.92[b]	53.53±1.99[b]
ISP	262.36±9.77[d]	216.56±8.06[d]	77.31±2.88[d]
CS+I	276.49±10.3[e]	227.49±8.47[e]	87.70±3.26[e]
R+I	292.30±10.8[f]	237.98±8.86[f]	96.46±3.55[f]
C+I	281.40±10.4[e]	231.36±8.61[e]	89.54±3.33[e]

Values are expressed as Mean ±SEM.
[a,b,c,d,e,f] Mean values within a column with unlike superscript letters were significantly different (P<0·05).

Table 5: Activities of HMG CoA reductase, malic enzyme and Glucose-6-phosphate dehydrogenase in liver

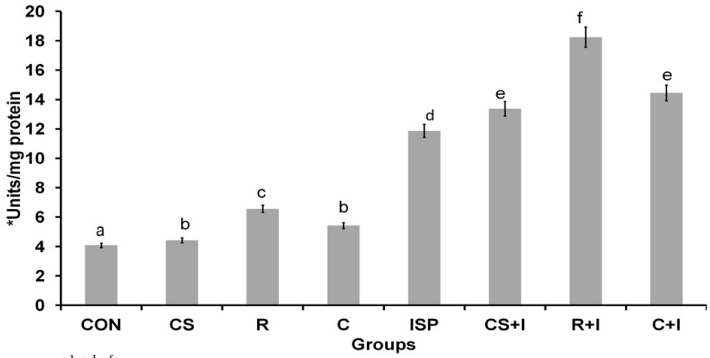
Groups	HMG CoA reductase*	Malic enzyme**	Glucose-6-phosphate Dehydrogenase**
CON	4.64±0.17[a]	24.44±0.91[a]	21.38±0.79[a]
CS	4.19±0.15[b]	29.85±1.11[b]	26.83±0.99[b]
R	3.59±0.13[c]	38.07±1.41[c]	31.72±1.18[c]
C	4.04±0.15[b]	33.65±1.25[b]	28.34±1.05[b]
ISP	1.40±0.04[d]	70.24±2.61[d]	43.60±1.62[d]
CS+I	1.16±0.04[e]	79.28±2.95[e]	49.31±1.83[e]
R+I	1.01±0.03[f]	85.92±3.13[f]	58.57±2.18[f]
C+I	1.15±0.04[e]	81.84±3.04[e]	51.43±1.92[e]

Values are expressed as Mean ±SEM.
[a,b,c,d,e,f] Mean values within a column with unlike superscript letters were significantly different (P<0·05).
*HMG CoA:Mevalonate. Lower ratio indicates higher activity.
**Enzyme which causes an increase in OD/1 min/g protein



Values are expressed as Mean ±SEM. [a,b,c,d,e,f] Mean values within a column with unlike superscript letters were significantly different (P<0·05).*µmoles of p-nitrophenol liberated per minute

Figure1. Activity of paraoxonase 1 in serum



Values are expressed as Mean ±SEM. [a,b,c,d,e,f] Mean values within a column with unlike superscript letters were significantly different (P<0·05).*Enzyme concentration required to inhibit the chromogen production by 50% in one minute.

Figure 2. Activity of Xanthine oxidase in serum

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