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Research Paper

**HIGH CARBOHYDRATE DIET ENHANCES LEUKOTRIENE A₄ HYDROLASE
AND EXACERBATES ISOPROTERENOL-INDUCED MYOCARDIAL
DAMAGE**

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Abstract

Leukotriene A₄ hydrolase (LTA₄H) catalyzes production of the proinflammatory lipid mediator, leukotriene (LT) B₄, which is implicated in a number of inflammatory diseases. Cardiovascular diseases (CVD's) are often associated with severe inflammatory responses which determine the extent of myocardial damage. The aim of the study was to examine the influence of high carbohydrate diets (HCD) such as corn starch, rice and cassava in inducing inflammation and aggravating the isoproterenol (ISO)-induced myocardial damage in rats. Experimental animals were randomly allocated to eight groups with six animals in each group: Standard pellet, corn starch, rice, cassava, Standard pellet + ISO, cornstarch + ISO, rice +ISO, and cassava + ISO. Myocardial damage was induced by subcutaneous injection of ISO after 60 days of the experimental period. The inflammatory markers and leukotriene B₄ biosynthetic pathway was studied in detail. The analysis of high sensitivity-C reactive protein level and myeloperoxidase activity showed a significant increase after a HCD's, especially rice group. The activity of 5-LO and LTA₄H, expression of 5-Lipoxygenase activating protein and leukotriene B₄ receptor, and the level of leukotriene B₄ were increased in the HCD's, especially rice group. All these alterations were also analyzed in the ISO-induced myocardial damaged groups which showed that the damage was further aggravated in the rice fed ISO-induced myocardial damaged group. The extent of myocardial damage affects the severity of CVD. Study suggests that consumption of HCD's, especially rice enhances inflammation in normal as well as in ISO-induced myocardial damaged rats when compared to standard diet. This was evident by the increased activity of LTA₄H and expression of key protein and receptor in LTB₄ biosynthetic pathway, FLAP and BLT1. Thus HCD's, especially rice aggravates and extends the myocardial damage induced by ISO in experimental animals.

Key words: Inflammation, high carbohydrate diet, Leukotriene A₄ hydrolase, 5-lipoxygenase activating protein, Leukotriene B receptor.

INTRODUCTION

South Asian migrants living in several countries have higher death rates from coronary heart disease (CHD) at younger ages compared with the local population despite apparently lower

levels of conventional risk factors [1-5]. Deaths related to cardiovascular disease also occur 5 to 10 years earlier in South Asian countries than they do in Western countries. This has raised the possibility that South Asians exhibit a special susceptibility for acute myocardial infarction that is not explained by traditional risk factors [6].

The inflammatory process can cause myocardial damage, while agents causing inflammation contribute to the worsening and progression of cardiovascular diseases (CVD) leading to heart failure (HF). Leo have reported that habitual dietary pattern have a moderate influence on chronic, low-grade systemic inflammation [7]. Hu has studied the role of overall dietary patterns in predicting long-term risk of CVD [8]. The influence of diet on inflammation largely depends on the composition of diet. Reports by Bowen et al., showed that a mild pro-oxidative state accompanies meal ingestion, which results in raised circulating biomarkers of inflammation, adhesion, and endothelial dysfunction, all of which are factors in the development of CVD [9]. South Asian countries mainly consume a high carbohydrate diets (HCD).

Leukotriene (LT) are formed from the 5-lipoxygenase (5-LO) pathway and are known to have a variety of proinflammatory effects [10, 11]. In cellular biosynthesis of LTs, arachidonic acid is oxygenated by 5-LO, assisted by 5-LO activating protein (FLAP), into the unstable epoxide LTA₄. This intermediate may be hydrolyzed by LTA₄ hydrolase (LTA₄H) into LTB₄. LTB₄ exerts the biological effects through the receptors denoted as BLT₁. FLAP has an important role in the initial steps of leukotriene biosynthesis [12] Samuelsson have reported that LTB₄ activates leukocytes, leading to chemotaxis and increased adhesion of leukocytes to vascular endothelium, release of lysosomal enzymes such as myeloperoxidase and production of superoxide anions [11].

Isoproterenol (ISO) is a synthetic β -adrenoreceptor agonist and its subcutaneous injection induces myocardial damages in rats. It causes severe cellular damage and ultimately infarct like necrosis [13]. ISO induced cardiac alterations includes myocyte degeneration and necrosis, subsequent interstitial and perivascular fibrosis and myocardial hypertrophy especially of the left ventricle [14]. Therefore ISO can be used for studying the damages associated with CVD's.

Rice is the primary staple food of most Asian countries, while cassava is largely cultivated in tropical and subtropical countries and corn starch is consumed as a major source of carbohydrate. Higher consumption of white rice was associated with a significantly increased risk of type 2 diabetes, especially in Asian and US population [15, 16]. Wang et al. showed that consumption of whole rice diet was associated with increased inflammatory markers in pre-diabetes subjects [17]. Even though HCD's are associated with inflammation, the biological mechanisms that may underline are unclear. Understanding the association of HCD's and CVD is essential for the early intervention in the extension of the diseases. Hence, we hypothesized that HCD would upregulate 5-LO pathway by enhancing LTA₄H activity, thereby inducing inflammation. We further hypothesized that these detrimental effects could be different for different HC diets, especially white rice and cassava, which are important staple diets and known high GI foods. Thus, the present study examines the influence of HCD such as, rice and cassava in enhancing inflammation by upregulating LTA₄H activity in isoproterenol (ISO)-induced myocardial damage in rats.

MATERIALS AND METHODS

Animals

Female Swiss albino rats weighing 100-150g was used for the study. Rats were housed at standard laboratory conditions (25 \pm 2°C, relative humidity 50 \pm 10%, 12 h light/12 h dark photoperiod). They were fed with a high carbohydrate diets (HCD) [corn starch, white polished rice or cassava] (Table 1), 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 [18].

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 (STD, n=6)

Group II: Corn starch diet (CS, n=6)

Group III: Rice diet (R, n=6)

Group IV: Cassava diet (C, n=6)

Group V: Standard pellet diet + Isoproterenol (STD +ISO, n=6)

Group VI: Corn starch diet + Isoproterenol (CS+ISO, n=6)

Group VII: Rice + Isoproterenol (R+ISO, n=6)

Group VIII: Cassava + Isoproterenol (C+ISO, n=6).

Experimental induction of myocardial damage

Myocardial damage was induced by a subcutaneous injection of Isoproterenol (ISO, Sigma Aldrich, St. Louis, MO, USA) at a dose of 100 mg/kg body weight in normal saline on the 60th day, followed by a booster dose after 24 hours [19]. On the 62nd day the animals were sacrificed, blood samples and heart tissues were collected and transferred to ice cold containers for further biochemical estimations.

Estimation of glycemic index

Blood samples for the determination of glucose are taken prior to eating and at regular intervals after feeding the rats over the next several hours. The changes in blood glucose over time are plotted as a curve. The glycemic index is calculated as the area under the glucose curve after the test food is eaten, divided by the corresponding area after the control food is eaten. The value is multiplied by 100 to represent a percentage of the control food. Glucose was used as reference food.

Biochemical estimation

The level of hs-CRP in serum was estimated by using CRP-ultrasensitive Latex turbidometry kit according to the manufacturer's instruction (Spinreact, Spain). Activity of MPO was evaluated in the serum by the method of Richard *et al.* [20]. The concentration of myocardial phospholipids was studied by the method of Zilver-Smith and Davis [21]. Activities of Phospholipase A [22], Phospholipase C [23] and Phospholipase D [24]. The concentration of protein was estimated by Lowry *et al.* [25].

Estimation of the activity of 5-LO in monocytes

Activity of 5-LO was evaluated in the monocytes. Monocytes were separated from whole blood using histopaque (Sigma Aldrich, St. Louis, MO, USA) by the method of Hutch [26]. The level of 5-LO was determined in monocytes by the method of Axelrod *et al.* [27]. Briefly, the isolated monocytes were treated with phosphate buffer (pH 6.1, 0.2M) and arachidonic acid (Sigma Aldrich, St. Louis, MO, USA). The activity was measured spectrophotometrically at 238nm.

Estimation of the level of LTB₄

The LTB₄ level in the serum was measured as described by the manufacturer of an enzyme immunoassay kit (R&D systems, USA).

Estimation of LTA₄H activity

The activity of Lipoxygenase A4 hydrolase was assessed using ELISA method described by Engvall *et al.* [28]. Briefly, the cytosolic fraction was coated in 96 well plate and incubated overnight at 37°C. The wells were washed with PBS followed by 200µl of blocking buffer (0.2% gelatin with 0.05% Tween in PBS) was added and kept for 1h at room temperature. After incubation the wells were washed with PBS-Tween and 100µl of LTA₄H primary antibody (Cayman Chemicals) was added and incubated for 2h at room temperature. After incubation wells were washed with PBS-Tween and 200µl O-dianizidine (Sigma Aldrich) was added and incubated for 30min in dark at room temperature. Reaction was stopped by the addition of 5N HCl and the optical density was read at 450nm using ELISA plate reader (Multiscan, Fischer scientific).

Total RNA Isolation

Total RNA was isolated from the heart using TRI reagent (Sigma Aldrich) by the method described by Chomczynski and Sacchi [29].

Quantification of FLAP and BLT₁ by Reverse transcriptase PCR

The isolated RNA was used for RT-PCR to study the expression of FLAP (5-lipoxygenase activating protein), BLT₁ (Leukotriene B₄ receptor) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Total tissue RNA (2mg) was primed with 0.05mg oligo dT and

reverse-transcribed by omniscrypt RT using a cDNA synthesis kit (Qiagen). PCR was carried out using an Eppendorf thermocycler (model 5332). Primer sequences with accession number are given in Table 2. The PCR mixture contained 10mM-Tris (pH 8.3), 50mM-KCl, 1.5mM-Mg Cl₂, deoxy nucleoside triphosphate (dNTP) (20mM each), gene specific primers (0.5mM each) and Taq polymerase (0.025units/ml). After an initial denaturation step at 94°C, thirty-five amplification cycles were performed. A final extension step of 5min at 72°C was performed in order to complete the PCR. The amplified product was analyzed by electrophoresis on 2% agarose gel containing ethidium bromide. Then, the gels were subjected to densitometric scanning (Bio-Rad Gel Doc) to determine the optical density of each, and then normalized.

Statistical Analysis

All statistical analysis in the present study was performed using SPSS/PC+, Version 17.0 (SPSS Inc., Chicago, IL, USA). A two way Factorial ANOVA followed by Bonferroni posthoc test for multiple comparisons was conducted to determine whether there was a significant interaction effect between diet and ISP-induced MI for each variable. $P < 0.05$ was considered to be significant.

RESULTS

The GI of the three diets is shown in Fig 1. The GI estimation of the three diets showed that the HCD's had higher GI compared to standard diet. The GI of rice diet was higher than cassava diet. The study showed that the level of hs-CRP and activity of MPO was increased after a HCD diet when compared to standard diet and among the HCD's, rice fed group showed the maximum significant ($p < 0.05$) increase when compared to corn starch or cassava fed groups. The level of hs-CRP and activity of MPO in the ISO-induced myocardial damaged groups were significantly ($p < 0.05$) higher in the HCD fed ISO-induced myocardial damaged groups. Among HCD fed ISO-induced myocardial damaged groups, rice fed ISO-induced myocardial damaged group showed significantly ($p < 0.05$) enhanced level of hs-CRP and activity of MPO (Table 3).

The analysis of myocardial phospholipids showed decreased concentration in the HCD group when compared to standard diet and among the HCD's, rice fed group showed the maximum significant ($p < 0.05$) decreased level when compared to corn starch or cassava fed groups. Significant ($p < 0.05$) decreased level of myocardial phospholipids was also seen after a HCD in the ISO-induced myocardial damaged groups. Rice fed ISO-induced myocardial damaged groups showed the maximum decreases phospholipid concentration among the HCD fed ISO-induced myocardial damaged groups (Fig 2).

The activities of phospholipases A, C, D (Fig 2), 5-LO (Table 4), LTA₄H (Fig 3), concentration of LTB₄ (Table 4) and the mRNA expressions of FLAP (Fig 4a) and BLT1

(Fig 4b) were increased in HCD groups in comparison with standard pelleted diet. And rice fed group showed the maximum significant ($p < 0.05$) increase when compared to corn starch and cassava fed group. Among HCD fed ISO-induced myocardial damaged groups, the rice fed group showed the maximum ($p < 0.05$) increase when compared to other ISO-induced myocardial damaged groups.

Table 1. Composition of diet

Ingredients (g/100g diet)	Low carbohydrate diet	High carbohydrate diet		
		Corn starch diet	Rice diet	Cassava diet
Available carbohydrate	60	75	75	75
Protein	22	15	15	15
Fat	3	4	4	4
Moisture	10	10	10	10

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

Table 2. Primer sequences of FLAP and BLT₁

Genes	Primer sequence	Accession number
FLAP	5' GAGCGGGTCTACACTGC 3' 5'GAAGCAGCGGGGAGATC 3'	NM_017260.2
BLT ₁	5'-GCATGTCCCTGTCTCTGTTG-3' 5'-CGGGCAAAGGCCTTAGTACG-3'	NM_021656.1
GAPDH	5'-GAAGGGCTCATGACCACAGT-3' 5'-GGATGCAGGGATGATGTTCT-3'.	NM_017008.4

Table 3. Concentration of hs-CRP and the activity of myeloperoxidase in rat serum

Groups	hs-CRP (mg/dl)	MPO (OD shift at 400nm/min/mg protein)
Std	0.122±0.006	14.06±0.51
CS	0.142±0.007*	17.01±0.63*
R	0.198±0.007*#	22.03±0.82*#
C	0.172±0.007*	19.99± 0.74*
Std+ISO	0.205±0.007	37.84±1.28
CS+ISO	0.222±0.008§	41.51±1.54 §
R+ISO	0.307±0.011§+	52.99±1.97§+
C+ISO	0.288±0.0105§	48.40±1.84§

Data are means± SEM of six rats in each group. Two-way ANOVA was used to test for interaction effects. All myocardial infarction groups were significantly different from diet groups (not shown). *P<0.05 vs Std, # P<0.05 vs CS and C, § P<0.05 vs Std+ISO, + P<0.05 vs CS+ISO and C+ISO.

Table 4. Concentration of myocardial membrane phospholipids, activity of 5-LO in monocytes and the level of LTB₄ in serum

Groups	Phospholipids (mg/100g tissue)	5-LO (OD shift/min/mg protein)	LTB ₄ (pg/ml)
Std	3.52±0.110	0.83±0.032	21.17±0.9
CS	2.85±0.090*	1.53±0.058*	26.45±1.2*
R	2.17±0.079*#	1.82±0.066*#	41.76±1.8*#
C	2.42±0.076*	1.01±0.037*	32.11±1.5*
Std+ISO	1.95±0.071	2.96±0.095	66.52±1.9
CS+ISO	1.65±0.064§	2.43±0.082 §	75.02±2.5§
R+ISO	1.12±0.045§+	4.70±0.158§+	99.92±3.4§+
C+ISO	1.58±0.060§	3.86±0.102§	88.76±3.1§

Data are means± SEM of six rats in each group. Two-way ANOVA was used to test for interaction effects. All myocardial infarction groups were significantly different from diet groups (not shown). *P<0.05 vs Std, # P<0.05 vs CS and C, § P<0.05 vs Std+ISO, + P<0.05 vs CS+ISO and C+ISO.

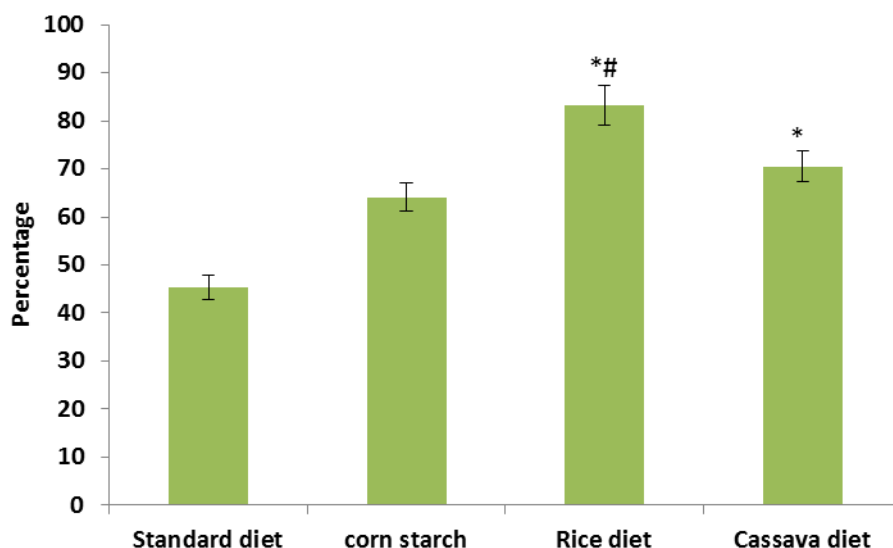


Fig 1. Glycemic index of the diets was estimated using glucose as reference. Data are means \pm SEM. *P<0.05 vs Std, # P<0.05 vs CS and C.

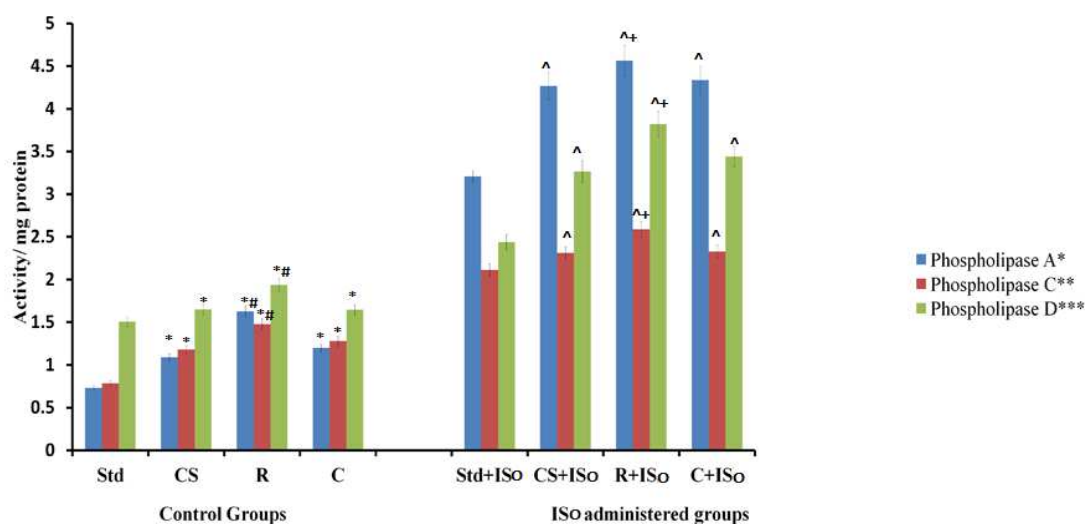


Fig 2. Effect of HCD on the activities of Phospholipase A, Phospholipase C and Phospholipase D in rat myocardium.

Data are means \pm SEM of six rats in each group. Two-way ANOVA was used to test for interaction effects. All cardiac damage groups were significantly different from diet groups (not shown). *P<0.05 vs Std, # P<0.05 vs CS and C, ^ P<0.05 vs Std+ISO, + P<0.05 vs CS+ISO and C+ISO.

*Milliequivalents of ester hydrolysed/ min/mg protein

**Millimoles of phosphoryl formed/min/mg protein

***Millimoles of choline formed/min/mg protein

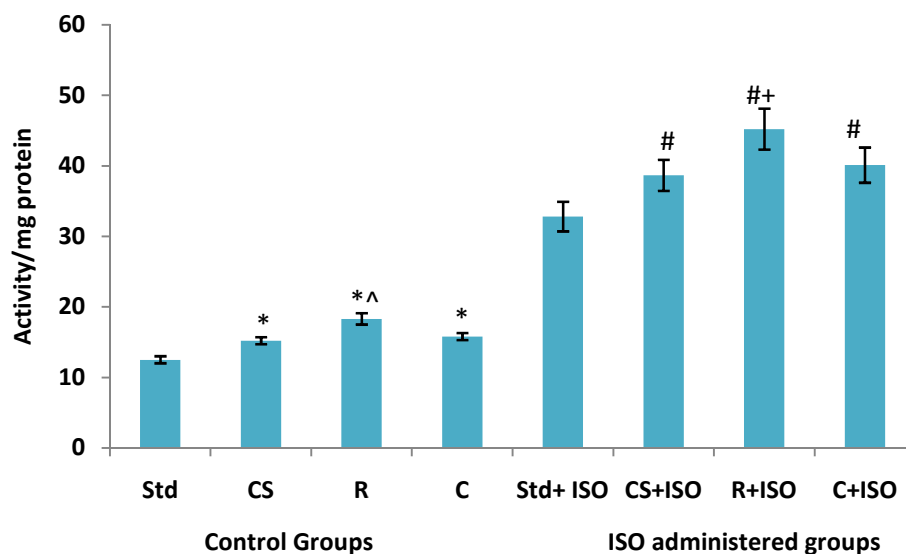


Fig 3. Effect of HCD on the activity of leukotriene A4 hydrolase in cytosol of the myocardium. Data are means \pm SEM of six rats in each group. Two-way ANOVA was used to test for interaction effects. All cardiac damage groups were significantly different from diet groups (not shown). * $P < 0.05$ vs Std, ^ $P < 0.05$ vs CS and C, # $P < 0.05$ vs Std+ISO, + $P < 0.05$ vs CS+ISO and C+ISO.

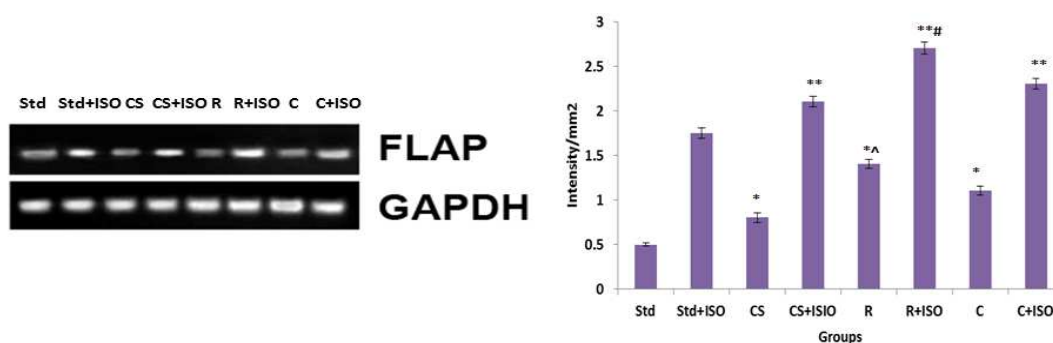


Fig 4a. Effect of HCD on the mRNA expression of FLAP. Expression of FLAP was analyzed in the myocardium by agarose gel electrophoresis and the intensities of the bands were compared with that of the intensities of GAPDH bands expressed in the samples. Intensities of the bands were quantified using Biorad gel doc and plotted. Data are means \pm SEM of six rats in each group. Two-way ANOVA was used to test for interaction effects. All cardiac damage groups were significantly different from diet groups (not shown). * $P < 0.05$ vs Std, ^ $P < 0.05$ vs CS and C, ** $P < 0.05$ vs Std+ISO, # $P < 0.05$ vs CS+ISO and C+ISO.

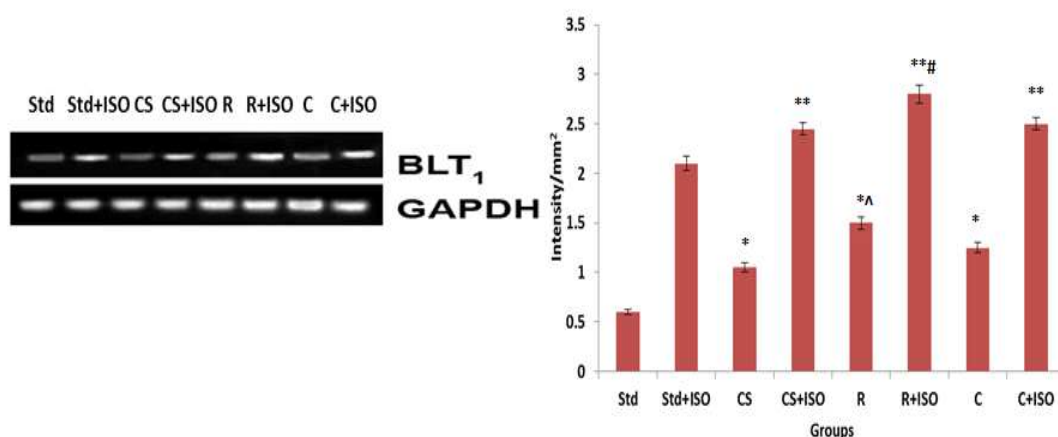


Fig 4b. Effect of HCD on the mRNA expression of BLT₁.

Expression of BLT₁ was analyzed in the myocardium by agarose gel electrophoresis and the intensities of the bands were compared with that of the intensities of GAPDH bands expressed in the samples. Intensities of the bands were quantified using Biorad gel doc and plotted. Data are means \pm SEM of six rats in each group. Two-way ANOVA was used to test for interaction effects. All cardiac damage groups were significantly different from diet groups (not shown). * $P < 0.05$ vs Std, ^ $P < 0.05$ vs CS and C, ** $P < 0.05$ vs Std+ISO, # $P < 0.05$ vs CS+ISO and C+ISO.

DISCUSSION

Dietary patterns play a key role in the progression of many inflammatory diseases and MI is preceded by severe inflammatory responses. The extent of the inflammatory response following damage to cardiac tissue is a key prognostic factor for heart disease. The severity of diet in inducing inflammation depends upon the composition of the diet. In the study, the standard commercial pelleted diet containing 60% carbohydrate was compared with 75% of corn starch, rice or cassava as a source of carbohydrate. Our investigation revealed that 75% corn starch, rice or cassava diet could induce inflammation by the acceleration of 5-LO pathway. Inflammation particularly prolonged and persistent inflammation, can also damage normal tissue [30]. Rice induced higher inflammation than cassava. This may be attributed to the high GI of rice. The rice we used was refined white rice, since this is widely used. Radhika et al. have reported that refined grains which have only the endosperm (starch) have an adverse effect on the cardio metabolic factors including glucose intolerance and diabetes [31]. HCD was seen to aggravate the inflammation that occurred during the ISO-induced myocardial damage, with greater inflammation shown by rice fed group.

CRP is the most examined inflammation marker in relation to CVD and substantial evidence indicates that baseline hs-CRP level is an independent predictor of cardiovascular events [32, 33]. MPO is a hemoprotein expressed in neutrophils that has recently been implicated in the pathogenesis of cardiovascular disease and may be elevated in patients with acute coronary syndromes [34-38]. Several studies have investigated the diagnostic and prognostic role of MPO in coronary artery disease [39]. Release of MPO may precede myocardial injury and identify patients earlier who are at risk for cardiac ischemia or thrombosis [35]. Thus both hs-CRP and MPO are good indicator of inflammation occurring during the myocardial damage. Our study showed an increased concentration of hs-CRP and enhanced activity of MPO after a HCD's when compared to standard diet with a more effect shown by rice fed group. Also among the ISO-induced myocardial damaged groups, rice fed ISO-induced myocardial damaged group showed the maximum increased concentration of hs-CRP and enhanced activity of MPO. These results indicate that the intake of HCD diet causes inflammation in the myocardium with rice group showing the maximum inflammation when compared to cassava or corn starch diet. HCD's

especially rice also aggravated the inflammation caused by the ISO- induced myocardial damaged groups.

The study showed a decreased concentration of myocardial phospholipids in the HCD fed group and also in HCD fed ISO-induced myocardial damaged groups when compared to standard diet group and standard diet fed ISO-induced MI groups. Daniel *et al.*, have reported that AA are liberated from the membrane phospholipids by the action of cytosolic phospholipase A₂ [40]. Similarly our study also showed increased activities of phospholipase A, C and D in the HCD fed and HCD fed ISO-induced myocardial damaged groups when compared to standard diet fed and standard diet fed ISO-induced MI groups. The decreased level of phospholipids in HCD fed and HCD fed ISO- induced myocardial damaged group might be due to the increased degradation of membrane phospholipids by phospholipases for the liberation of AA. Among the HCD's, rice fed and rice fed ISO- induced myocardial damaged group showed the maximum increased activities of phospholipase A, C and D and decreased level of phospholipids when compared to cornstarch or cassava fed and cornstarch or cassava fed ISO- induced myocardial damaged groups respectively.

Reports shows that 5-LO and FLAP act on AA formed from the membrane phospholipids to liberate LTB₄ [10, 11]. Similarly, our data also revealed an increased activity of 5-LO and increased mRNA expression of FLAP in the HCD fed and also in HCD fed ISO- induced myocardial damaged groups when compared to the standard diet groups. Maximum 5-LO activity and FLAP expression was seen in the rice fed group when compared to cassava or corn starch and rice fed ISO- induced myocardial damaged group when compared to cassava and cornstarch fed ISO- induced myocardial damaged groups. This is in agreement with the study by Anna *et al.*, which showed that the variants of *ALOX5AP* encoding FLAP are associated with greater risk of myocardial infarction and stroke [41].

LTB₄ represents the most powerful pro-inflammatory product of 5-LO [42]. LTB₄ exerts its proinflammatory responses through its BLT₁ receptors. The LTB₄ synthesis pathway is seen elevated during inflammation in ISO induced MI [43]. In our study, the level of LTB₄ was increased in the HCD fed group when compared to the standard diet fed group and also in HCD fed ISO-induced myocardial damaged groups when compared to standard diet fed ISO-induced myocardial damaged groups. Reports showed that endothelial cells and smooth muscle cells respond with BLT₁ upregulation, thereby enhancing the LT signaling [44-46]. The assessment of the BLT₁ receptor in the present study revealed an increase mRNA expression of BLT₁ in the HCD fed and HCD fed ISO- induced myocardial damaged groups when compared to standard diet fed and standard diet fed ISO- induced myocardial damaged groups. Among the HCD, rice fed showed the greater level of LTB₄ and maximum expression of BLT₁ when compared to cassava or cornstarch fed groups and rice fed ISO-induced myocardial damaged group when compared to cassava or cornstarch fed ISO- induced myocardial damaged groups.

It can be concluded that consumption of HCD especially carbohydrate obtained from white rice induced inflammation by upregulating the 5-LO inflammatory pathway and thereby rendering the heart more vulnerable for ISO-induced myocardial damage. The severity of the inflammation during myocardial damage may influence the recovery period of the myocardium from damage.

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REFERENCES

- [1] Anand, S.S., Yusuf, S., Vuksan, V., Devanesen, S., Teo, K.K., Montague, P.A., Kelemen, L., Yi, C., Lonn, E., Gerstein, H., Hegele, R.A., McQueen, M., 2000, Difference in risk factors, atherosclerosis, and cardiovascular disease between ethnic groups in Canada: the Study of Health Assessment and Risk in Ethnic groups (SHARE), *Lancet*, 356, 279-284.
- [2] Yusuf, S., Reddy, S., Ounpuu, S., Anand, S., 2001, Global burden of cardiovascular diseases, part II: variations in cardiovascular diseases by specific ethnic groups and geographic and prevention strategies, *Circulation*, 104, 2855-2864.

- [3] Enas, E.A., Yusuf, S., Mehta, J., Prevalence of coronary artery disease in Asian Indians, *Am J Cardiol*, 1992; 70: 945-949.
- [4] McKeigue, P.M., Marmot, M.G., 1988, Mortality from coronary heart disease in Asian communities in London, *BMJ*, 297, 903.
- [5] Balarajan. R., 1991, Ethnic differences in mortality from ischaemic heart disease and cerebrovascular disease in England and Wales, *BMJ*, 302, 560-564.
- [6] Prashant, J., Shofiqul, I., Prem, P., Srinath, R., Prabhakaran, D., Khawar, K., Mrigendra, R.P., Sirajul, H., Shanthi, M., Sumathy, R., Salim Y, 2007, Risk Factors for Early Myocardial Infarction in South Asians Compared With Individuals in Other Countries, *JAMA*, 297, 286-294.
- [7] Galland. L., 2010, Diet and Inflammation, *Nutr Clin Pract*, 25, 634-640.
- [8] Hu, F.B., 2002, Dietary patterns analysis: a new direction in nutritional epidemiology, *Curr Opin Lipidol*, 13, 3-9.
- [9] Bowen, P.E., Borthakur, G., 2004, Postprandial lipid oxidation and cardiovascular risk, *Curr Atheroscler Rep*, 6, 477-484.
- [10] Ford-Hutchinson, A.W., 1990, Leukotriene B₄ in inflammation, *Crit Rev Immunol*, 10, 1-12.
- [11] Samuelsson. B., 1983, Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation, *Science*, 220, 568-575.
- [12] Dixon, R.A., Diehl, R.E., Opas, E., Rands, E., Vickers, P.J., Evans, J.F., Gillard, J.W., Miller, D.K., 1990, Requirement of a 5-lipoxygenase-activating protein for leukotriene synthesis, *Nature*, 343, 282-284.
- [13] Hamid, S., Arash, K., Afagh, G., Nasrin, M.D., Alireza, G., 2012, Acute treatment with metformin improves cardiac function following Isoproterenol induced myocardial infarction in rats, *Pharmacol Res*, 641,476-484.
- [14] Daniela, G., Dietmar, E., Heribert, S., Michael, P., Daniel, G., Gunter, B., Frank, M., Riegger, A.J., Eckhard, P.K., 1998, Development of heart failure following Isoproterenol administration in the rat: role of the renin angiotensin system, *Cardiovas Res*, 37, 91-100.
- [15] Hu EA, Pan A, Malik V, Sun Q. White rice consumption and risk of type 2 diabetes: meta-analysis and systematic review. *BMJ*. 15 (2012) 344: e1454.
- [16] Sun Q, Spiegelman D, van Dam RM, Holmes MD, Malik VS, Willett WC, Hu FB. White rice, brown rice, and risk of type 2 diabetes in US men and women. *Arch. Intern. Med*. 14 (2010) 961-969.
- [17] Wang B, Medapalli R, Xu J, Cai W, Chen X, He JC, Uribarri J. Effects of a whole rice diet on metabolic parameters and inflammatory markers in prediabetes. *e-SPEN journal*. 8 (2013) e15-e20.
- [18] Hume, C.W., 1972, *The UFAW Handbook on the care and management of laboratory animals*, Edinburg/London:Churchill Livingstone.
- [19] Wexler, B.C., Greenberg, B.P., 1978, Protective effects of clofibrate on Isoproterenol-induced myocardial-infarction in arteriosclerotic and non-arteriosclerotic rats, *Atherosclerosis*, 29, 373-395.
- [20] Richard, K., Ralph, S., Huch, H., Evan, B., Jamiska, M., Maje, M., Shelton, 1972, Guinea pig heterophil and eosinophil peroxidase, *Arch Biochem Biophys* 148: 1972; 452-65.
- [21] Zilversmith, D.B., and Davis, A.K., 1950, Micro determination of plasma phospholipids by trichloroacetic acid precipitation, *Lab Clin Med*, 35, 155-160.
- [22] Rimon, A., and Shapiro, B., 1959, Properties and specificities of pancreatic phospholipases A, *The Biochemical Journal*, 71, 620-623.
- [23] Kleiman, J.H., and Lands, W.E.M., 1969, Purification of phospholipases C from *Bacillus cereus*, *Biochem Biophys Acta*, 187, 477-85.
- [24] Aoyagi T, Matsui T. The Cardiomyocyte as a Source of Cytokines in Cardiac Injury. *J. Cell. Sci. Ther*. 2012(2011): 003.

- [25] Radhika G, Sumathi C, Ganesan A, Sudha V, Henry CJK, Mohan VB: Glycaemic index of Indian flatbreads (rotis prepared using whole wheat flour and 'atta mix' added whole wheat flour. *Br. J. Nutr.* 103 (2010) 1642-1647.
- [26] Mollerung, H., and Bregmeyer, H.U., 1964, *Methods of enzyme analysis*, H J Bregmeyer (ed), NewYork, Academic, pp 415.
- [27] Lowry, O.H., Rosebrough, N. J. and Farr, A.L. 1951, Protein measurement with the folin phenol reagent, *J Biol chem*, 193, 265-275.
- [28] Huch, H.Y., Pearce, S.F., Tersher, I. M., Schindler, I. L., Silverstain, R.L. 1996, *Blood* (8th ed.), 265-271.
- [29] Axelrod, B., Cheesbrough, T.M., Laakso, S. 1981 *Methods in Enzymology*, 71, 441-453.
- [30] Engvall, E., and Perlman, P. 1971, Enzyme-Linked Immunosorbent Assay, (ELISA) Quantitative Assay of Immunoglobulin G, *Immunochemistry*, 8, 871-874.
- [31] Chomcynski, P., and Sacchi, N., 1987, Single-Step Method of RNA Isolation by Acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction, *Anal Biochem*, 162, 156-59.
- [32] Danesh, J., Wheeler, J.G., Hirschfield, G.M., Eda, S., Eiriksdottir, G., Rumley, A., Lowe, G.D., Pepys, M.B., Gudnason, V., 2004, C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease, *N Engl J Med*, 350, 1387-1397.
- [33] Blake, G. J., and Ridker, P.M. 2002, Inflammatory bio-markers and cardiovascular risk prediction, *J Intern Med*, 252, 283-294.
- [34] Apple, F.S., Wu, A.H., Mair, J., Ravkilde, J., Panteghini, M., Tate, J., Pagani, F., Christenson, R.H., Mockel, M., Danne, O., Jaffe, A.S., 2005, Future biomarkers for detection of ischemia and risk stratification in acute coronary syndrome, *Clin Chem*, 51, 810-824.
- [35] Baldus, S., Heeschen, C., Meinertz, T., Zeiher, A.M., Eiserich, J.P., Münzel, T., Simoons, M.L., Hamm, C.W., 2003, Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes, *Circulation*, 108, 1440-1445.
- [36] Buffon, A., Biasucci, L.M., Liuzzo, G., D'Onofrio, G., Crea, F., Maseri, A, 2002 Widespread coronary inflammation in unstable angina, *N Engl J Med*, 347, 5-12.
- [37] Eiserich, J.P., Baldus, S., Brennan, M.L., Ma, W., Zhang, C., Tousson, A., Castro, L., Lusis, A.J., Nauseef, W.M., White, C.R., Freeman, B.A., 2002, Myeloperoxidase, a leukocyte-derived vascular NO oxidase, *Science*, 296, 2391-2394.
- [38] Podrez, E.A., Schmitt, D., Hoff, H.F., Hazen, S.L., 1999, Myeloperoxidase-generated reactive nitrogen species convert LDL into an atherogenic form in vitro, *J Clin Invest*, 103, 1547-1560.
- [39] Wetzstein, C.J., Shern-Brewer, R.A., Santanam, N., Green, N.R., White-Welkley, J.E., Parthasarathy, S., 1998, Does acute exercise affect the susceptibility of low density lipoprotein to oxidation? *Free Radic Biol Med*, 24, 679-682.
- [40] Zhang, R., Brennan, M. L., Fu, X., Aviles, R.J., Pearce, G.L., Penn, M.S., Topol, E.J., Sprecher, D.L., Hazen, S.L., 2001, Association between myeloperoxidase levels and risk of coronary artery disease, *JAMA*, 286, 2136-2142.
- [41] Poeckel, D., and Funk, C.D., 2010, The 5-lipoxygenase/leukotriene pathway in preclinical models of cardiovascular disease, *Cardiovasc Res*, 2010, 86 (2), 243-53.
- [42] Anna, H., Andrei, M., Gudmar, T., Solveig, G., Helga, J., Unnur, T., Nilesh, J.S., Gudmundur, G., 2004, The gene encoding 5-lipoxygenase activating protein confers risk of myocardial infarction and stroke, *Nature Genetics*, 36, 233 -239.
- [43] Panicker, S., Swathy, S.S., John, F., Madambath, I., 2012, Impact of selenium on the LTB₄ synthesis pathway during Isoproterenol- induced Myocardial infarction in experimental rats. *Inflammation*, 35(1), 74-78.
- [44] Back, M., Bu, D.X., Branstrom, R., Sheikine, Y., Yan, Z.Q., Hansson, G.K., 2005, Leukotriene B₄ signaling through NF-kappaB-dependent BLT1 receptors on vascular smooth muscle cells in atherosclerosis and intimal hyperplasia, *Proc Natl Acad Sci USA*, 2005, 102, 17501-17506.

- [45] Pettersson, A., Sabirsh, A., Bristulf, J., Kidd-Ljunggren, K., Ljungberg, B., Owman, C., Karlsson, U., 2005, Pro- and anti-inflammatory substances modulate expression of the leukotriene B₄ receptor, BLT1, in human monocytes, *J Leukoc Biol*, 77, 1018-1025.
- [46] Qiu, H., Johansson, A.S., Sjostrom M, Wan M, Schroder O, Palmblad J, Haeggstrom JZ, 2006, Differential induction of BLT receptor expression on human endothelial cells by lipopolysaccharide, cytokines, and leukotriene B₄, *Proc Natl Acad Sci USA*, 103, 6913-6918.