

Effect of High Carbohydrate Diet on Complete Freund's Adjuvant Induced Inflammation in Rats

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Chronic low grade inflammation is an essential pathological feature of a variety of non-communicable diseases (NCDs). These diseases have now superseded infectious diseases where the burden of disease is concerned. One of the important modifiable factors contributing to chronic disease is food high in carbohydrate. This study was planned to study the role of high carbohydrate diet on a model of inflammation induced by Complete Freund's adjuvant (CFA) in male Wistar rats. Animals were divided into 3 groups of 10 rats each. Group I fed with standard diet serving as control; Group II fed with high carbohydrate diet (HCD) and Group III fed with standard diet serving as disease free normal group. CFA was injected subcutaneously into the hind paw 4 weeks after starting the diet into groups I and II only. Diet was continued for up to 21 days after CFA injection. Digital plethysmometer measured the paw volume. Blood obtained before euthanasia served for estimating cytokines and oxidative stress parameters. A rise in paw edema was seen in control and high carbohydrate diet groups up to day 21. In the high carbohydrate group there were high serum cytokine levels and significant depletion of antioxidant enzymes. The authors conclude that, a high carbohydrate diet contributes significantly to the process of inflammation which has now been established as a significant factor in the causation of NCDs. Therefore it would be prudent to restrict carbohydrates in our diet.

Keywords: Carbohydrate, Complete Freund's Adjuvant, Cytokines, Inflammation, Oxidative stress.

Inflammation has been recognized for decades as a vital component of host defence. However, scientists have now come to realize that inflammation which serves a vital function in tissue repair and immunosurveillance can be catastrophic in its chronic low grade form. A host of chronic non-communicable diseases (NCDs) such as type 2 diabetes mellitus (T2DM), metabolic syndrome (MetS), cardiovascular disease (CVD) and non-alcoholic fatty liver disease (NAFLD) have chronic low grade inflammation as an essential pathological feature¹.

Inflammation as described in terms of its classic features of tumor, rubor, dolor and calor is beneficial in the short term in bringing about tissue repair. Metabolic disorders where chronic inflammation plays a major role show that prolonged inflammation is not beneficial though the mediators involved in classic inflammation and in metabolic disease are the same².

Over the past 40 years the world has experienced a changing trend in disease patterns. The burden of disease which was dominated by infectious diseases in developing countries has now

been superseded by NCDs. According to the WHO, NCDs kill 17 million people before the age of 70 and 40 million people annually which account for 70% of deaths the world over. Over the next 20 years NCDs are going to cost the global economy US\$47 trillion and are responsible for pushing millions of people to the brink of poverty^{3,4}.

Urbanization, increased marketing and affordability have led to the consumption of refined grains, sugar sweetened beverages, cakes, biscuits and confectionery which increase the risk of development of chronic disease. Modern society has become unhealthy when compared to our healthy and robust hunter gatherer ancestors as a result of lack of physical exercise and consumption of junk food which is highly processed^{4,5,6}.

The susceptibility to these diseases is determined by an amalgam of genetic and environmental factors. Industrializing countries with a growing economy have a large population of people who have migrated from rural to urban areas as a result of which, their lifestyles and diets have changed. These changes may have unveiled a susceptibility to these new diseases. Modern society has started to follow diets rich in sugar and refined food stuffs and poor in dietary fiber content⁷.

From the literature reviewed it can be concluded that, one of the important modifiable risk factors contributing to chronic disease is food which is high in carbohydrate and that chronic inflammation happens to be an essential pathophysiological feature of chronic NCDs. Therefore the objective of this study was to evaluate the role of high carbohydrate diet in a model of inflammation induced by Complete Freund's adjuvant (CFA) in male Wistar rats.

MATERIALS AND METHODS

Complete Freund's adjuvant of 1mg/ml concentration was purchased from Sigma Aldrich, Saint Louis, Missouri, USA. Digital plethysmometer was purchased from Orchid Scientific and Innovative India Pvt. Ltd. Nashik, Maharashtra, India. Colorimetric kit for measuring Thiobarbituric acid reactive substances (TBARS) was purchased from Bioassay Systems, USA. ELISA kits for measuring catalase and superoxide dismutase (SOD) were purchased

from MyBioSource, Inc, San Diego, CA, USA. ELISA kits for measuring tumor necrosis factor alpha (TNF α) and interleukin 1 beta (IL-1 β) were purchased from Krishgen Biosystems, Mumbai, India.

Diet was produced from locally available ingredients except zero carbohydrate whey protein manufactured by Isopure which was purchased from Amazon.

Animals

Adult male Wistar rats (weighing 150-200g) obtained from Central Animal Facility of the institution were used in the present study. Animals were housed under standard conditions. The animal experiment was reviewed and approved by the Institutional Animal Ethics Committee (Letter no.7/A dated 18/05/2016). Animal handling and experiments were performed according to the guidelines put forward by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Animal Procedures

The animals were divided into 3 equal groups (n = 10 per group).

Group I- Rats fed with standard diet injected with CFA serving as control

Group II- Rats fed with high carbohydrate diet (HCD)

Group III - Rats fed with standard diet not injected with CFA serving as disease free normal group

The respective diets (composition elaborated in Table 1) were started 4 weeks before induction of inflammation and continued throughout the study period. All diets were fed on *ad libitum* basis. Diets were prepared as per the principles mentioned in the literature^{8,9}.

Induction of inflammation

Inflammation was induced by subcutaneously injecting 0.1ml of CFA (1mg/ml concentration) at the back surface of right hind paw. CFA was injected into animals belonging to Group I and II, but not Group III. The diet was continued for 21 days following the injection of CFA. Paw volume of injected hindpaw was measured on days 0, 3, 7, 14 and 21 using a digital plethysmometer¹⁰

Serum analysis

Blood was obtained by cardiac puncture after anesthetizing the animals using intraperitoneal thiopentone on day 21 following which animals were euthanized by thiopentone overdose. Blood

was allowed to clot, following which serum was separated by centrifuging it at 2000 rpm for 15 min and the obtained serum was used for estimation of cytokines and oxidative stress parameters.

Assessment of Inflammation and Oxidative stress

Estimation of inflammatory mediators

The serum levels of inflammatory mediators like TNF α and IL-1 β were estimated quantitatively using ELISA kits as per the directions mentioned in the manufacturer's protocol.

Estimation of Oxidative stress parameters

The serum levels of TBARS were measured using a colorimetric kit and antioxidant enzymes namely SOD and catalase were estimated quantitatively using ELISA kits as per the directions mentioned in the manufacturer's protocol.

Data analysis

The data was analyzed using statistical software Graph Pad Prism (GraphPad Software, Inc. La Jolla, California, USA). To assess the differences between the groups One-way analysis of variance (ANOVA) was carried out which was followed by Dunnett's post hoc analysis. A p value of < 0.05 was considered significant.

Table 1. Composition of diets

Constituents / 100g of diet	Standard diet	High carbohydrate diet
Wheat flour	50g	-
Maida	-	32g
Sugar (Sucrose)	-	32g
Whey protein	25g	26g
Ghee	05g	05g
Bran	20g	05g

Table 2. Effect of high carbohydrate diet on Complete Freund's adjuvant (CFA) induced paw edema

Mean \pm SEM(ml)	Control	HCD	Normal	F value	P value
Day 0	1.071 \pm 0.037	1.08 \pm 0.042	1.057 \pm 0.034	0.091	0.9128
Day 3	1.729 \pm 0.041	2.162 \pm 0.043 ****	1.124 \pm 0.026****	186.9	< 0.0001
Day 7	1.518 \pm 0.042	1.696 \pm 0.067*	1.16 \pm 0.024****	31.9	< 0.0001
Day 14	1.793 \pm 0.038	2.2 \pm 0.059****	1.032 \pm 0.027****	183.4	< 0.0001
Day 21	2.095 \pm 0.038	2.270 \pm 0.053**	1.185 \pm 0.02****	213.2	< 0.0001

Analysis by ANOVA followed by Post Hoc Dunnett's test.

* indicates P < 0.05; ** indicates P < 0.01; *** indicates P < 0.001; **** indicates P < 0.0001

SEM: Standard error of mean; HCD: High carbohydrate diet

RESULTS

The present study was planned to evaluate the role of high carbohydrate diet in a model of inflammation induced by CFA in male Wistar rats. All results have been expressed as mean \pm standard error of mean (SEM).

Effect of high carbohydrate diet on CFA induced paw edema

A rise in paw edema was seen both in control and high carbohydrate diet groups as compared to day 0. There was a slight fall on day 7 in both groups which reflects the natural course of paw edema development following CFA injection following which there was a rise up to day 21. But, the rise in paw edema from day 3 onwards was significantly more in the HCD group compared to the control group as depicted in Table 2.

Effect of high carbohydrate diet on inflammatory cytokines

Analysis of inflammatory cytokines namely TNF α and IL-1 β in the serum revealed that, in the HCD group there was a significant increase in levels of both cytokines compared to the control group as depicted in Table 3.

Effect of high carbohydrate diet on oxidative stress parameters

Analysis of TBARS and antioxidant enzymes in the serum revealed that, in the HCD group there was a significant increase in the level of TBARS and significant depletion of antioxidant enzymes superoxide dismutase and catalase compared to control group as depicted in Table 4.

DISCUSSION

The present study was planned to evaluate the role of high carbohydrate diet on a model of inflammation induced by CFA in male Wistar rats. The results of the present study prove that, feeding a high carbohydrate diet over a period of 7 weeks adds to the inflammation produced by CFA, which is sustained over a period of 21 days. Apart from this, there is an increase in inflammatory cytokines, susceptibility to lipid peroxidation and depletion of antioxidant defenses in rats fed a high carbohydrate diet.

The strengths of the present study are with respect to the ingredients of the high carbohydrate diet and the parameters evaluated in the study.

The key ingredients used in the high carbohydrate diet are sucrose (which contains glucose and fructose) and maida (highly refined wheat flour) which are being extensively used in the present day and age especially in the production of junk food which is a major contributor to NCDs like T2DM, obesity and ischaemic heart disease. Hence, conclusions drawn from this study are relevant to the human population though, the study has been done in rats.

Another strength of the study is the fact that, it has not just confined itself to the measurement of a parameter like paw edema which is visible to the naked eye but, has taken the study to a higher level by evaluating the effects of a high carbohydrate diet on markers like inflammatory cytokines and oxidative stress parameters.

In the present study, levels of serum cytokines, namely TNF- α and IL-1 β have been found to be increased in the HCD group. Similar findings were also reported by a study which evaluated the effect of carbohydrates like fructose and sucrose on the same cytokines¹¹.

The elevation of cytokines is caused not just by sugar, but also a refined carbohydrate like bread which contains maida which was proven by a study where in white bread was found to acutely activate nuclear factor kappa light chain enhancer of activated B cells (NF- κ B)¹². NF- κ B is a transcription factor which controls the expression of genes involved in inflammation. It is important to note that, NF- κ B increases the synthesis of pro-inflammatory cytokines namely IL-1, IL-6 and TNF- α ¹³.

Apart from increased susceptibility to lipid peroxidation as shown by the increased

Table 3. Effect of high carbohydrate diet on inflammatory cytokines

Mean \pm SEM	Control	HCD	Normal	F value	P value
Serum IL-1 β (pg/ml)	652.5 \pm 9.73	945.1 \pm 10.89****	113.7 \pm 2.62****	2421	< 0.0001
Serum TNF α (pg/ml)	37.54 \pm 2.96	48.75 \pm 2.47**	15.71 \pm 0.65 ****	55.22	< 0.0001

Analysis by ANOVA followed by Post Hoc Dunnett's test

* indicates $P < 0.05$; ** indicates $P < 0.01$; *** indicates $P < 0.001$; **** indicates $P < 0.0001$

SEM: Standard error of mean; HCD: High carbohydrate diet; IL-1 β : Interleukin 1 beta;

TNF α : Tumor necrosis factor alpha

Table 4. Effect of high carbohydrate diet on oxidative stress parameters

Mean \pm SEM	Control	HCD	Normal	F value	P value
Serum SOD (U/ml)	140 \pm 1.33	91.49 \pm 1.37 ***	231.7 \pm 14.06****	75.51	< 0.0001
Serum catalase (pg/ml)	60.87 \pm 2.24	25.35 \pm 3.835 ****	244.3 \pm 2.12 ****	1707	< 0.0001
Serum TBARS (iM)	14.79 \pm 0.90	18.11 \pm 0.25 **	4.480 \pm 0.46 ****	136.7	< 0.0001

Analysis by ANOVA followed by Post Hoc Dunnett's test

* indicates $P < 0.05$; ** indicates $P < 0.01$; *** indicates $P < 0.001$; **** indicates $P < 0.0001$

SEM: Standard error of mean; HCD: High carbohydrate diet; SOD: Superoxide dismutase; TBARS: Thiobarbituric acid reactive substances

serum TBARS levels, the present study also found significant depletion of antioxidant enzymes namely SOD and catalase in the HCD group. Similar findings were found in a study, in which rats were fed high sucrose diet for 2 weeks. Significant increase in the plasma TBARS levels and decreased SOD levels were found in the hearts of the rats which were fed a high sucrose diet¹⁴.

Apart from the mechanisms documented in the present study, there are other mechanisms by which carbohydrates can contribute to inflammation and oxidative stress as documented in the literature.

Fructose can induce an accumulation of advanced glycation end-products and the oxidative degradation of fructose adducts can lead to production of free radicals¹⁵.

A diet rich in sucrose / fructose can induce inflammation by altering the gut microbiota. There is ultimately an increase in gram negative bacteria leading to excess lipopolysaccharide (LPS) release. This LPS release causes an increase in mRNA expression levels of toll like receptors (TLRs) TLR2 and TLR4¹¹. TLR4 binds to LPS of bacterial cell walls of gram negative bacteria. This binding activates a signaling pathway, leading to activation of the NF- κ B pathway which in turn activates cytokines, chemokines and other effectors of innate immunity¹⁶.

An acutely high glucose causes alterations in osmolarity leading to activation of NF- κ B. Exposure to high glucose for more prolonged times causes changes in antioxidant defences and activation of protein kinase C (PKC), which potentiates activation of NF- κ B¹⁷.

CONCLUSION

From the results of the present study it can be concluded that, a high carbohydrate diet contributes significantly to the process of inflammation by causing a rise in inflammatory cytokines, increased susceptibility to lipid peroxidation and depletion of antioxidant defenses. Inflammation has now been firmly established as an essential pathophysiological component in the pathogenesis of non - communicable diseases. Hence, it would be prudent to restrict carbohydrates in our diet.

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