Pharmacy Education (2024) 24(2) 121 - 126
https://doi.org/10.46542/pe.2024.242.121126

IAI SPECIAL EDITION

RESEARCH ARTICLE

Impact of a diet rich in carbohydrates, fats, and fructose on insulin resistance development

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Keywords
Carbohydrate
Fat
Fructose
Insulin resistance
Insulin tolerance test
Pancreatic beta cell

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Abstract

Background: Insulin resistance can be caused by carbohydrates, fats, and fructose. Insulin resistance is defined as a decrease in insulin's ability to stimulate the use of glucose for distribution to other parts of the body or a decrease in organ/cell response (fat tissue, liver, and muscle) to insulin. **Aim:** The goal of this study is to see how different diets high in fat, carbohydrates, and fructose affect the incidence of insulin resistance. **Methods:** The study was conducted for 60 days and used 24 male white rats (*Rattus norvegicus*) Wistar strain aged two months with 180-200 grams body weight. They were divided into four groups, namely the normal group, the high-carbohydrate-fat (CF) diet group, the Carbohydrate-Fat-Fructose (CFF) and the Carbohydrate-fat-Fructose-Drink (CFFD) diet group. Blood glucose levels, the oral glucose tolerance test (ITT), and histological features of pancreatic beta cells were all measured. **Results:** The CFF group had the highest blood glucose level of 111.25 mg/dl and the highest insulin resistance with an ITT value of 2.81, while the CF group had pancreatic beta cell (β) necrosis. **Conclusion:** According to the findings of this study, the CFF group had insulin resistance, while the CF group had pancreatic beta cell necrosis.

Introduction

Amidst growing worldwide health apprehensions, contemporary societal dietary habits have been subject to critical examination, specifically concerning ingesting foods that are abundant in carbohydrates, fats, and sugar-sweetened beverages. This nutritional predilection is motivated by an inclination towards fructose due to its perceived superior sweetness compared to glucose or sucrose, which results in an overconsumption of these macronutrients. Insulin resistance, a condition characterised by diminished insulin sensitivity in adipose tissue, liver, and muscles, is a notable outcome of these dietary practices. It contributes to impaired glucose metabolism and an elevated susceptibility to Type 2 diabetes. (Jastreboff *et al.*, 2016; Sunehag *et al.*, 2008).

Insulin resistance is distinguished by a compromised capacity of insulin to facilitate the uptake and utilisation of glucose, leading to the development of compensatory hyperinsulinemia and, ultimately, hyperglycemia. This dysregulation contributes to the development of metabolic syndrome, a cluster of conditions that elevates the risk of cardiovascular disease, stroke, and other health complications in addition to diabetes. (Castro *et al.*, 2014; Freeman *et al.*, 2023). An alarmingly high percentage of the world's population, ranging from 20 to 25, suffers from metabolic disorders. Metabolic Syndrome (MS) is a prevalent health concern that carries substantial implications, as evidenced by the substantial incidence rates documented by the Nutrition and Health Examination Survey (NHANES) and the International Diabetes Federation (IDF) in both the United States and Indonesia (Alberti *et al.*, 2018).

The precise mechanisms and progression of metabolic syndrome and insulin resistance, despite the well-established link between excessive consumption of carbohydrates, fats, and fructose, continue to be subjects of scientific inquiry. The lack of understanding in this area impedes the progress toward creating efficacious interventions and dietary recommendations

that guard against these health hazards. In light of this knowledge deficit, the primary objective of the present investigation is to clarify the effects of a diet abundant in these macronutrients on the progression of insulin resistance. This study will investigate, using a controlled rat model, how fat, carbohydrates, and fructose ingestion induce the development of insulin resistance.

To determine the effects of dietary components on insulin signaling pathways and glucose metabolism, the research will employ an extensive array of methodologies, including Blood Glucose Level (BGL) measurements, the Insulin Tolerance Test (ITT), and an evaluation of cell necrosis in beta (β) cells. A diet high in carbohydrates, fats, and fructose significantly exacerbates the development of insulin resistance and contributes to the onset of metabolic syndrome, according to the study's hypothesis, which is supported by an examination of these parameters.

Through in-depth investigation, this study aims to elucidate the manner in which particular dietary constituents impact the pathophysiology of insulin resistance and the metabolic complications that accompany it. The study seeks to provide significant contributions to the understanding of the dietary factors that contribute to metabolic disorders and to society-wide efforts to combat their increasing prevalence by elucidating the mechanisms that underlie these associations. To reduce the burden of metabolic diseases and enhance overall health, the ultimate goal of this research is to establish a foundation for more informed dietary recommendations and interventions.

Methods

Design

Male white rats were used in this study. Padjadjaran University's Research Ethic Commission granted a Code of Ethics. A letter of approval with the number 1163/UN6.KEP/EC/2023 has granted permission to use rats in this Insulin Resistance study. Rattus norvegicus of the Wistar breed, two months old and weighing 180-200 grams. There were 24 rats divided into four groups: normal, carbohydrate-fat (CF), carbohydrate-fat-fructose (CFF), and carbohydrate-fat and fructose-drink (CFFD). The test animals were fed normal food and high carbohydrate food with the composition shown in Table I (Sulaeman *et al.*, 2022).

Table I: Ingredients of normal and high carbohydrate and fat

Ingredients	Normal food (g/1000g)	High carbohydrate-fat food (g/1000g)
Wheat flour	172	172
Rice flour	0	189
Corn flour	102	204
Fish flour	370	130
Mung bean flour	336	114
Beef tallow	0	171
Vegetable oil	20	20

Assessment

Insulin resistance test with Insulin Tolerance Test (ITT)

The insulin tolerance test was performed on day 60. The animal subject's glucose level was measured using a blood sample drawn from the tail (T_0). Following the administration of 0.01 g/kgbw insulin intraperitoneally, blood glucose levels were monitored every 15 minutes for an hour. The Glucometer from Easy Touch was the device used to measure blood glucose levels. ITT is a slope or curve gradient score multiplied by 100 from the natural algorithm of linear regression curve in glucose towards time (Susilawati, 2019).

Blood glucose level test

A blood glucose level test was performed on day 0, day 15, day 30, day 45, and day 60. Before injecting with a syringe tip, the tail tip must be cleaned with a 70% alcohol solution. The blood from the tail was inserted into the glucose strip, which was attached to the glucometer Easy Touch. The result was displayed on the monitor (Samsuri *et al*., 2020).

Pancreatic histology

The rat pancreas was removed and histologically examined. Trimming, sampling, clearing, dehydration, embedding, paraffin infiltration, sectioning, and eosin hematoxylin dying towards beta cells are among the procedures to be examined. The number of beta cells that die was a parameter (Hermawati *et al.*, 2020).

Data analysis

Data obtained from the research was processed by ANOVA method (One Way ANOVA) and Post Hoc Test LSD using SPSS 26.0 version of 2020.

Results

The Insulin Tolerance Test (ITT) is an essential procedure utilised to assess insulin resistance through the evaluation of tissue sensitivity to insulin. Insulin resistance is characterised by a compromised physiological response in which tissues demonstrate an abnormally low response to insulin stimulation. The comprehensive results of this assessment are systematically documented in Table II.

The groups that consumed diets enriched with Carbohydrates-fats (CF), Carbohydrates-fats-fructose (CFF), and Carbohydrates-fats-fructose-drink (CFFD) had higher average blood glucose levels than those that consumed a normal diet, as shown in Table III. Significantly, despite being elevated, these levels remain within the clinically recognised normal glucose range. An examination of the blood glucose levels in the CFF group and the normal diet group reveals a statistically significant distinction, as evidenced by an ANOVA, specifically at the T_{60} time point, with a *p*-value below 0.05. On the contrary, there are no statistically significant differences in blood glucose levels observed between the CF and CFFD groups and the control group (*p*-values exceed 0.05).

Table II: Insulin Tolerance Test (ITT) score on day 60

	ITT score ±SD
Group	**Day 60**
Normal	9.13±0.33
CF	4.33±1.51*
CFF	2.81±0.43*
CFFD	5.176±2.34*

Notes: (*) significant difference towards normal group($p < 0.05$); CF: Carbohydrate-Fat; CFF: Carbohydrate-Fat- Fructose; CFFD: Carbohydrate-Fat-Fructose Drink

Table III: Mean level of blood glucose from day 0 to day 60

Test group	Mean percentage of blood glucose ± SD					
	Day 0	Day 15	Day 30	Day 45	Day 60	Increase (%)
Normal	94.50±7.33	88.00±8.64	92.00±6.73	92.00±5.60	94.75±4.57	0.25%
CF	94.75±2.22	94.75±5.91	100.25±4.27	103.75±5.26ǂ	101.50±4.99	6.75%
CFF	95.00±15.25	103.25±8.58ǂ	107.50±8.58ǂ	106.00±4.40ǂ	111.25±9.57ǂ	16.25%
CFFD	93.5±3.70	102.25±9.64ǂ	104.75±7.50ǂ	108.50±7.05ǂ	101.25±6.65	7.75%

Notes: ǂ significant different towards normal group ($p < 0,05$); CF: Carbohydrate-Fat; CFF: Carbohydrate-Fat- Fructose; CFFD: Carbohydrate-Fat-Fructose Drink

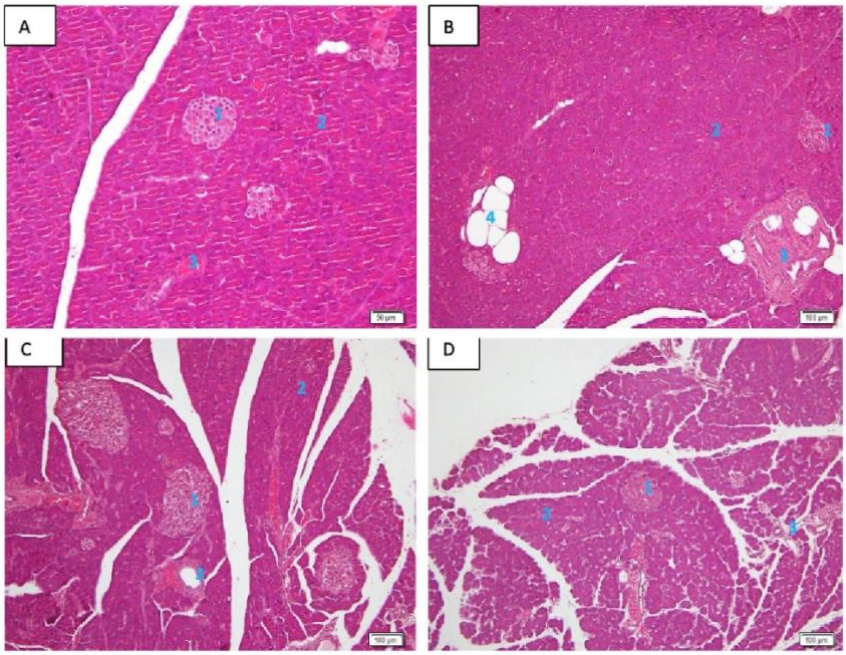
The results emphasise that the CFF diet is significantly correlated with elevated blood glucose levels, which are roughly 16.25 % greater than those observed in the control group. This is an astute observation that highlights the detrimental effects of fructose when it is ingested alongside carbohydrates and fats. In contrast, despite consuming comparable dietary components to the CFF group, the CFFD group displays an unusual fructose distribution pattern as a result of its liquid formulation. This factor may potentially contribute to a marginal, yet significant, elevation in blood glucose levels, specifically 7.75%. The aforementioned observations are crucial as they provide insight into the variable impact that the form and combination of dietary components, specifically fructose, can have on blood glucose levels and, consequently, insulin sensitivity and resistance. A comprehensive comprehension of these dietary impacts is critical in order to formulate precise nutritional approaches that alleviate the likelihood of developing insulin resistance and related metabolic disorders.

Table IV shows pancreas histology results after 60 days of induction. The less number of cells in langerhans, mass of cell α, mass of cell β, and langerhans island diameter, means the pancreatic function is decreased. ANOVA analysis shows total cells summary in per langerhans and and mass of cell α are significantly different in CF, CFF, CFFD group towards the normal group, mass of cell β is also significantly different in group C and CFF towards normal group ($p < 0.05$) meanwhile the diameter of langerhans island (μm^2) does not have a significant difference among test groups towards normal group ($p > 0.05$). Figure 1 shows that pancreatic histology in group CF underwent cell necrosis with the presence of vacuolation, total number of cells in langerhans, mass of cell α, mass of cell β are slight and significantly different with normal group.

Table IV: Total number of langerhans cells, mass of cell α, mass of cell β, and diameter of langerhans island (μm²)

Group	Total cell number per langerhans	Mass cell α	Mass cell β	Diameter of langerhans island
Normal	211.00 ± 69.44	61.00 ± 4.53	149.00 ± 64.91	209.17 ± 137.79
CF	50.00 ± 2.55*	22.00 ± 0.57*	30.00 ± 1.98*	138.92 ± 38.73
CFF	154.00 ± 27.44*	37.00 ± 9.62*	127.00 ± 31.96	188.92 ± 20.16
CFFD	55.00 ± 15.84*	8.00 ± 1.70*	47.00 ± 14.14*	185.75 ± 44.18

Notes: (*) significant different towards normal group(p<0,05). CF: Carbohydrate-Fat; CFF: Carbohydrate-Fat- Fructose; CFFD: Carbohydrate-Fat-Fructose Drink



Notes: **A:** Normal group (HE x200); **B:** Carbohydrate and fat group (HE x100); **C:** Carbohydrate, fat and fructose group (HE x100); **D:** Carbohydrate, fat, and fructose drink group (HE x100); 1: langerhans island; 2: pancreatic gland; 3: ductus pancreaticus; 4: vacuolation

Figure 1: Pancreas histology results using hematoxylin eosin dye

Discussion

This study aims to examine the intricate relationship between dietary composition and the onset of insulin resistance, which is a significant predisposing factor to Type 2 diabetes mellitus. By employing the Insulin Tolerance Test (ITT) to assess insulin sensitivity, the findings reveal that an insulin-sensitising diet rich in carbohydrates, fats, and fructose (CFF) substantially reduces insulin sensitivity in comparison to a standard diet. This is supported by a decreased ITT score and a statistically significant *p*-value in the analysis of variance ($p < 0.05$). This discovery aligns with prior research that emphasizes the detrimental impacts of high-fructose diets on metabolic health. (Adriawan *et al.,* 2014).

It is noteworthy that diets consisting exclusively of carbohydrates and fats (CF) or fructose in the form of a beverage (CFFD) did not exhibit a substantial deviation in insulin sensitivity when compared to the control group. This observation may indicate that solid fructose combined with fats and carbohydrates has a distinct metabolic effect, which merits additional research. The variability in fructose dosage among participants in the CFFD group highlights the intricate nature of dietary research and emphasises the importance of meticulously evaluating the form and composition of foods.

The study posits a molecular mechanism by which an excessive consumption of dietary fat and fructose induces the synthesis of fatty acyl CoA, diacylglycerol, and ceramide. It is established that these metabolites induce Protein Kinase C (PKC) activation, which subsequently hinders the insulin signaling pathway through inhibition of Insulin Receptor Substrate (IRS)

phosphorylation and subsequent interactions with PI3K and AKT. This disturbance is of the utmost importance because it obstructs the translocation of GLUT-4 transporters to the plasma membrane, a critical procedure necessary for the cellular uptake of glucose from the blood (Adriawan *et al*., 2014). An abundance of studies establish a connection between dietary components and impaired glucose transport and insulin signaling, lending support to this proposed pathway (Mathew & Tadi, 2020).

The investigation delves deeper into the systemic ramifications of modified diets by employing histological examination of the pancreas. Significant alterations were noted in the Langerhans island diameter, cell mass, and total cell count when comparing the CF, CFF, and CFFD groups to the control group. The significance of these structural alterations in the pancreas cannot be overstated, as they indicate a compromised ability to efficiently produce and regulate insulin. Further complicating matters is the presence of vacuolation and minor cell necrosis in the CF group, which suggests possible cellular dysfunction and distress that could contribute to hyperglycemia and insulin resistance.

Additionally, the research paper examines the detrimental consequences of chronic hyperglycemia, which include hyperinsulinemia and beta-cell insensitivity, which serve to worsen insulin resistance. In insulin-resistant states, beta cell necrosis results from altered caspase pathways and ceramide accumulation, which impairs the body's capacity to generate insulin, a characteristic feature of Type 2 Diabetes Mellitus (Donath *et al.*, 2005).

Although necrosis was not observed in the CCF group, the decrease in Langerhans island diameter and cell mass indicates a nuanced form of activity degradation. The group in question exhibited a notable rise in fasting blood glucose levels, which is noteworthy considering the absence of obvious pancreatic necrosis. This underscores the complex and diverse characteristics of insulin resistance, which encompass not only pancreatic beta cells but also alpha (α) cells, which are responsible for glucagon synthesis in fasting states.

Conclusion

In the study, the escalation of blood sugar levels was sequentially noted in groups CFF>CF>CFFD>N. The highest increase in insulin resistance, as indicated by the reduction measured by the Insulin Tolerance Test (ITT), followed the order of groups CFF>CF>N>CFFD. Furthermore, the greatest incidence of necrosis in pancreas beta cells was observed in the sequence of groups CF>CFFD>CFF>N. This pattern of changes suggests a need for future research to explore the underlying mechanisms driving these differential responses in glucose metabolism and beta-cell health across the studied groups.

Acknowledgement

The authors gratefully acknowledge the financial support provided for this research by the Center of Research and Community Services (CRCS) of Bhakti Kencana University. Their contribution has been invaluable in facilitating the progress and completion of this scientific study.

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