THE EFFECT OF HIGH FAT LOW CARBOHYDRATE DIET ON BLOOD PRESSURE AND THE DEVELOPMENT OF CARDIAC HYPERTROPHY IN SPONTANEOUSLY HYPERTENSIVE RATS

by

Willy Soesanto Siauw

A thesis submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Master of Science

in

Nutrition

College of Health
The University of Utah
August 2009
Copyright © 2009 Willy Soesanto Siauw

All Rights Reserved
SUPERVISORY COMMITTEE APPROVAL

of a thesis submitted by

Willy Soesanto Siauw

This thesis has been read by each member of the following supervisory committee and by majority vote has been found to be satisfactory.

Chair: Thunder Jalili
To the Graduate Council of the University of Utah:

I have read the thesis of Soesanto Siauw in its final form and have found that (1) its format, citations, and bibliographic style are consistent and acceptable; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the supervisory committee and is ready for submission to The Graduate School.

Date

Thunder Jalili
Chair: Supervisory Committee

Approved for the Major Department

Eldon Wayne Askew
Chair/Dean

Approved for the Graduate Council

David S. Chapma
Dean of The Graduate School
The current dietary recommendation for a healthy heart and prevention of heart disease focuses mostly on low fat and high carbohydrate foods. However, previous studies have shown high fat diet may be able to attenuate cardiac hypertrophy and diets high in carbohydrate may increase blood pressure. These results raise the question as to the cardiovascular effect of a diet high in carbohydrate in patients with hypertension.

The purposes of this study are to: (1) observe the effect of a high fat low carbohydrate diet on the development of cardiac hypertrophy on SHR; and (2) determine whether a high fat low carbohydrate diet can reduce blood pressure on Spontaneously Hypertensive Rats (SHR).

Six-week-old male Wistar Kyoto (WKY) rats and SHR were fed with either high fat low carbohydrate (HFLC) or low fat high carbohydrate (LFHC, control) diet ad libitum for 10 weeks, after which, blood pressure were measured and insulin tolerance test (ITT) was conducted. Heart weight was standardized to tibia length to produce an index of cardiac hypertrophy.

It was found that blood pressure was significantly reduced in SHR fed with HFLC compared to LFHC diet without the attenuation or prevention of cardiac hypertrophy. ITT result suggested that the SHR was not insulin resistant and the diet only affected the WKY group where the WKY-LFHC group was more insulin resistant. In conclusion,
although blood pressure was reduced with the HFLC diet, it was still insufficient to prevent the pathological stimulus for the development of cardiac hypertrophy.
# TABLE OF CONTENTS

ABSTRACT ............................................................................................................ iv

INTRODUCTION ....................................................................................................... 1

MATERIALS AND METHODS ............................................................................... 3

- Animals and Diets .......................................................................................... 3
- Insulin Tolerance Test .................................................................................... 3
- Arterial Blood Pressure and Tissue Sampling ............................................... 5
- Mean Arterial Pressure ................................................................................... 5
- Statistical Analysis ......................................................................................... 5

RESULTS ............................................................................................................... 6

- Heart Weight, Body Weight, Tibia Length, Heart Rate, HOMA-IR Index ........... 6
- Index of Hypertrophy ...................................................................................... 6
- Blood Pressure ................................................................................................ 6
- Insulin Tolerance Test .................................................................................... 9

DISCUSSION ......................................................................................................... 11

REFERENCES ...................................................................................................... 17
INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death in the United States. In 2004, CVD was responsible for 36.3% of all deaths in the United States (1). According to the statistics released by the American Heart Association in 2007, approximately 80,700,000 American adults (33%) have one or more types of CVD (1). High blood pressure (hypertension) has been listed as the most common type of CVD (73,000,000 people) and it is one of the main causes of cardiac hypertrophy, which frequently leads to heart failure and possibly death (2).

It has been long recognized that ingesting high saturated fat and/or cholesterol diet has a potentially adverse effect on the heart (3, 4). As a consequence, the current dietary recommendation for a healthy heart and prevention of heart disease focuses mostly on food that has low fat, low cholesterol and high carbohydrate content (5). Contrary to the current dietary recommendation for the prevention of heart disease, recent studies using hypertensive rats fed with a high fat diet has been shown to either attenuate or prevent cardiac hypertrophy in these rats as well as having an improved contractile function (6, 7). It has also been reported that feeding a high carbohydrate (high simple sugar) diet to various rat models can elevate blood pressure (8-13). Moreover, clinical and experimental studies have reported that there is a negative correlation between hypertension and endothelial-dependent vasorelaxation with hypertension (14-16). In one of the study that observed the effect of high sucrose diet on hypertension, it was also
reported that arterial segments of rats fed with high sugar diet had a significant decrease in endothelial-dependent vasorelaxation compared to rats fed with low sugar diet (12). Thus, it is hypothesized that the mechanism of action of high sugar induced hypertension is due to the perturbation of endothelial-dependent vasorelaxation. These studies raise the question as to the cardiovascular effect of a diet high in carbohydrate and sugar in patients with hypertension.

A few studies have been conducted to observe the effect of high fat low carbohydrate diet on the development of cardiac hypertrophy. These studies have reported that a diet high in fat and low in carbohydrate attenuates cardiac hypertrophy in hypertensive dahl salt-sensitive rats (6, 7). Surprisingly, these studies reported that blood pressure did not change despite the attenuation of cardiac hypertrophy, which suggests that there is another factor other than an increased workload or afterload (due to hypertension), that causes hypertrophy.

Based on current literature, it is hypothesized that a high fat low carbohydrate diet can attenuate or prevent the development of cardiac hypertrophy in two ways: reducing blood pressure and/or alter the biochemical signaling pathways in the cardiac myocyte that regulate cardiac hypertrophy in response to hypertension. To date, there have not been any studies that utilize spontaneously hypertensive rats (SHR), a clinically relevant model of hypertension and cardiac hypertrophy, to examine the effect of a high fat low sucrose diet. Therefore, the purposes of this study are to: (1) observe the effect of a high fat low carbohydrate diet on the development of cardiac hypertrophy on SHR; (2) determine whether a high fat low carbohydrate diet can reduce blood pressure on SHR.
MATERIALS AND METHODS

Animals and Diets

All protocols were approved by the University of Utah Institutional Animal Care and Use Committee. Thirty male SHR and 29 male control WKY were obtained from Charles River Laboratories and randomized to receive either the high fat low carbohydrate diet (HFLC, 60% fat, 20% protein 20% CHO in the form of 12% maltodextrin and 6% sucrose) or low fat high carbohydrate diet (LFHC, 10% fat, 20% protein, 70% CHO in the form of 31% corns starch, 4% maltodextrin 10, 35% sucrose) obtained from Research Diets, Inc., New Brunswick, New Jersey, USA. Please refer to table 1 for the complete break down of the ingredients in these diets. n=15 WKY, n=16 SHR were in the HFLC group and n=14 WKY, n=14 SHR was in the LFHC group. These rats were fed ad libitum for 10 weeks.

Insulin Tolerance Test

Rats were injected with two units of insulin per kg of body weight through the catheter and glucose levels were measured at 0 (12 hour fast), 10, 20, 30 and 60 minutes after insulin was injected using One Touch® glucose meter manufactured by LifeScan, Inc.
Table 1
Complete list of ingredients for the LFHC and HFLC diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>LFHC diet</th>
<th></th>
<th>HFLC diet</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gram</td>
<td>kcal</td>
<td>gram</td>
<td>kcal</td>
</tr>
<tr>
<td>Casein, 80 Mesh</td>
<td>200</td>
<td>800</td>
<td>200</td>
<td>800</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3</td>
<td>12</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Corn starch</td>
<td>315</td>
<td>1260</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>35</td>
<td>140</td>
<td>125</td>
<td>500</td>
</tr>
<tr>
<td>Sucrose</td>
<td>350</td>
<td>1400</td>
<td>68.8</td>
<td>275.2</td>
</tr>
<tr>
<td>Cellulose, BW200</td>
<td>50</td>
<td>0</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>25</td>
<td>225</td>
<td>25</td>
<td>225</td>
</tr>
<tr>
<td>Lard</td>
<td>20</td>
<td>180</td>
<td>245</td>
<td>2205</td>
</tr>
<tr>
<td>Mineral mix s10026</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Di-calcium phosphate</td>
<td>13</td>
<td>0</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>5.5</td>
<td>0</td>
<td>5.5</td>
<td>0</td>
</tr>
<tr>
<td>Pottassium citrate, 1 H2O</td>
<td>16.5</td>
<td>0</td>
<td>16.5</td>
<td>0</td>
</tr>
<tr>
<td>Vitamin mix V10001</td>
<td>10</td>
<td>40</td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>FD&amp;C yellow dye #5</td>
<td>0.05</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FD&amp;C blue dye #1</td>
<td></td>
<td></td>
<td>0.05</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1055.05</td>
<td>4057</td>
<td>773.85</td>
<td>4057</td>
</tr>
</tbody>
</table>
Arterial Blood Pressure and Tissue Sampling

A fluid filled catheter was inserted in the caudal artery of the anesthetized (using 3-5% isoflurane) rats. Blood pressure and heart rate were measured over ~ 20 cardiac cycles gap 60 minutes after regaining consciousness (Biopac Systems), after which rats were anesthetized again and sacrificed by removing the heart (17).

The excised heart was then placed into a 0.9% warm saline solution to let it continue beating for 10 seconds to remove all the blood in the heart chambers. The heart was then transferred to a cold saline solution for trimming of the excess tissue that is not part of the heart. For an accurate measurement of weight, the heart was blotted to remove excess saline.

Mean Arterial Pressure

The mean arterial blood pressure (MAP) was calculated using the formula:

$$\text{MAP} = \frac{(2 \times \text{diastolic pressure}) + \text{systolic pressure}}{3}$$

Statistical Analysis

A one-way analysis of variance (ANOVA) was utilized to determine if there is significant difference among the groups using SPSS version 16. $P$-value is set at 0.05 for significance. When a significant value was obtained, a post hoc test is conducted using LSD (Least Significance Difference) to determine where within the individual groups the differences are.

A two tailed Pearson correlation coefficient was also conducted to test if there is any relationship between two factors. $P<0.05$ is set for significance.
RESULTS

Heart Weight, Body Weight, Tibia Length, Heart Rate, HOMA-IR Index

No significant difference was observed in heart weight, body weight, heart rate and Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index. The only significant difference was observed in the tibia length (Table 2). HOMA-IR index was calculated to see whether the rats fed with high fat diet will develop insulin resistance since it is more reliable than the fasting glucose/insulin ratio and Quantitative Insulin Sensitivity Check Index (QUICKI) for assessing insulin resistance (18).

Index of Hypertrophy

Cardiac mass was standardized to tibia length (cardiac mass, mg: tibia length, mm) to create an index of cardiac hypertrophy. The SHR groups had greater cardiac hypertrophy than WKY groups, regardless of diet treatment $p<0.01$ (Figure 1).

Blood Pressure

Both groups of SHR (HFLC and LFHC) had higher systolic, diastolic and mean arterial blood pressure (MAP) than WKY (HFLC and LFHC) $p<0.01$. The WKY groups had similar blood pressure in all categories. Systolic, diastolic and MAP of SHR fed HFLC diet were significantly lower compared to SHR fed with LFHC diet $p<0.01$ (Figure 2).
Table 2

Characteristics of rats fed HFLC and LFHC at the end of 16 weeks of feeding

<table>
<thead>
<tr>
<th></th>
<th>WKY-LFHC</th>
<th>SHR-LFHC</th>
<th>WKY-HFLC</th>
<th>SHR-HFLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>14</td>
<td>14</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>Heart wt, mg</td>
<td>1101 ±26</td>
<td>1289 ±25</td>
<td>1167 ±24</td>
<td>1315 ±23</td>
</tr>
<tr>
<td>Body wt, g</td>
<td>326 ±6</td>
<td>341 ±6</td>
<td>341 ±6</td>
<td>355 ±5</td>
</tr>
<tr>
<td>Tibia length, mm</td>
<td>39.1 ±0.2</td>
<td>39.0 ±0.2b</td>
<td>40.1 ±0.2b</td>
<td>39.6 ±0.2a</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>370 ±8</td>
<td>385 ±8</td>
<td>353 ±8</td>
<td>390 ±7</td>
</tr>
<tr>
<td>HOMA-IR index</td>
<td>27.8 ±4.5</td>
<td>26.5 ±4.4</td>
<td>25.5 ±4.2</td>
<td>21.1 ±4.0</td>
</tr>
<tr>
<td>Plasma insulin, ng/ml</td>
<td>5.9 ±0.48a</td>
<td>5.6 ±0.48a,c</td>
<td>4.5 ±0.45b,c</td>
<td>3.8 ±0.43b</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Means in a row with superscripts without common letter differ, \( P<0.05 \).

Figure 1. Terminal heart weight-to-tibia length ratios of SHR and WKY fed HFLC and LFHC. Values are means ± SEM. Different letters denote significant difference \( P<0.01 \).
Figure 2. Systolic, diastolic and mean arterial blood pressure. Values are means ± SEM.
Different letters denote significant difference $p<0.01$. 
The correlation between MAP and cardiac hypertrophy was significant, $r = 0.721$, $r^2 = 0.52$, $P<0.01$, indicating that higher blood pressure was a major factor driving development of cardiac hypertrophy (Figure 3). However, in spite of lower blood pressure, SHR-HF did not have any change in developed hypertrophy vs. SHR-LF.

**Insulin Tolerance Test (ITT)**

ITT was conducted to detect if insulin resistance developed when rats were fed a high fat diet. WKY-LFHC had significantly higher blood glucose at all time intervals after insulin injection vs. other groups (Figure 4). WKY-HFLC, SHR-LFHC, and SHR-HFLC had similar glucose responses during the ITT.

![Figure 3. Relationship between MAP and cardiac hypertrophy. Values are means ± SEM (mg.mm)](image-url)
Figure 4. Blood glucose response after insulin injection. Values % blood glucose from baseline ± SEM (represented as percentage from the mean). *P<0.05 between WKY-LFHC and all other groups
DISCUSSION

It was hypothesized that by feeding the spontaneously hypertensive rats (SHR) a high fat low carbohydrate diet, cardiac hypertrophy will be attenuated or reduced due to a decrease in blood pressure. The results confirmed that a low carbohydrate (low sugar) / high fat diet decreased hypertension but cardiac hypertrophy was not affected. Contrary to the Okere et al. studies that reported that a HFLC diet attenuates cardiac hypertrophy without a decrease in blood pressure in Dahl salt-sensitive (DSS) rats (6, 7), the present data showed opposite results. While systolic, diastolic and mean arterial pressure for SHR fed with HFLC diet was significantly reduced compared to SHR-LFHC, there was no change in developed cardiac hypertrophy.

Several possibilities may explain the discrepancies between the results of the present study and Okere et al. First of all, Okere et al. used DSS rats and SHR was utilized as models for the present study. DSS rats have different genetic background and hypertension is induced by ingestion of salt while in SHR, it is a spontaneous occurrence of hypertension. It is possible that cardiac hypertrophy can be attenuated via two mechanisms: reducing blood pressure and/or altering the biochemical signaling pathways in the cardiac myocyte that regulate cardiac hypertrophy in response to hypertension. One could speculate that the DSS rats responded through the latter mechanism where proteins responsible for the development of cardiac hypertrophy are down regulated by a decrease in insulin stimulation. Insulin has been shown to activate the Akt-mammalian target of
rapamycin (mTOR) pathway that is essential for cardiomyocyte growth in rats (19-22) and clinical studies have also observed a positive correlation between plasma insulin and cardiac hypertrophy (23, 24). Lastly, the specific compositions of the diet might differ between the present study and Okere et al. However, in that study, the ingredients for the diets were not reported. Accordingly, it should be standard practice to report diet composition in such studies so that a comparison of results will be more meaningful.

Although the hypothesis that HFLC diet will reduce blood pressure was satisfied, this reduction however was not sufficient to attenuate the development of cardiac hypertrophy (table 2). These data indicate that an 11 mmHg reduction in blood pressure is not sufficient to attenuate cardiac hypertrophy. In other words, even though the level of blood pressure in SHR fed with HFLC is lower than SHR fed with LFHC, it is still sufficient to provide an adequate pathological stimulus to initiate cardiac growth. However, since hypertension is one of the main causes of cardiac hypertrophy, perhaps the prolonged decrease in blood pressure will be beneficial in reducing cardiac hypertrophy or dysfunction in the long run. Thus, it might be worthwhile to conduct another study with a longer treatment period to determine if sustained decrease in blood pressure would reduce hypertrophy or attenuate the development of heart failure in the long term.

It is possible that any rat fed with HFLC diet will develop insulin resistance (27, 28). Insulin resistance may have an effect on the signal transduction pathways responsible for cell proliferation and development of cardiac hypertrophy, notably the Akt and mammalian target of rapamycin (mTOR) pathway (25). Since insulin is required for the activation of Akt-mTOR pathway and subsequent cell proliferation and cardiac
growth (25), status of insulin resistance and concentration of plasma insulin may be an important variable in determining risk of hypertrophy. Accordingly, an Insulin Tolerance Test (ITT) was conducted and HOMA-IR index was calculated to determine whether the SHR were insulin resistant, and if a HFLC diet exacerbates this condition. There was no significant difference in blood glucose after insulin was injected in the WKY-HFLC, SHR-HFLC and SHR-LFHC groups. However, the WKY-LFHC group was significantly more insulin resistant (shown by the higher glucose levels after insulin injection) than the other groups (figure 4). Therefore, it can be concluded that the diet affected only the WKY but not the SHR groups. This might be due to the higher carbohydrate content, specifically sucrose, in the LFHC compared to HFLC diet (35% vs. 6%) that causes the WKY-LFHC group to be more insulin resistant. In agreement with this speculation, previous studies have also reported that WKY rats fed with high sucrose diet will develop insulin resistance or hyperinsulinemia (26, 27). However, the results for HOMA-IR index suggest that there were not any difference in terms of insulin sensitivity between all the groups. The plasma sample collected for insulin measurement was taken randomly (after ~4-6 hours fast); thus, this might affect the accuracy for the HOMA-IR index calculation since it takes into consideration of fasting insulin level. The inaccurate HOMA-IR index might explain for contradicting result observed between the ITT and HOMA-IR index.

Plasma insulin concentration was found to be higher in the LFHC (both WKY and SHR) group, which was in line with the hypothesis. Since an increase in plasma insulin suggests that there will be higher activation of the Akt-mTOR signaling pathway, it was expected that the SHR-LFHC group will have a severe cardiac hypertrophy. However, ITT data revealed similar levels of insulin sensitivity between SHR LFHC and SHR
HFLC, and cardiac hypertrophy was also similar between both SHR groups. Taken together, these data do not support the notion that a LFHC diet would alter insulin sensitivity and/or exacerbate cardiac hypertrophy in the SHR through the activation of Akt-mTOR pathway in the 10-week period examined in this study. However, the long-term effects of such a diet on insulin sensitivity and hypertrophy are unknown.

Many previous studies have reported that the SHR will develop abnormalities in glucose metabolism such as hyperinsulinemia, reduced insulin sensitivity and decreased insulin clearance by the liver due to the hypertension (28, 29). Only one previous study has demonstrated that there is no difference in insulin resistance between WKY and SHR (30). Conversely, the finding of the present study does not concur with that by the previous investigators. In fact, the ITT result suggested that SHR has better insulin sensitivity than the WKY.

In the current study, ITT was conducted to determine the status of insulin resistance in the rats. In contrast, previous studies that have reported the development of insulin resistance in SHR utilized methods other than ITT. Abnormal glucose metabolism in SHR was first reported in 1978 by Yamori et al. and they conducted glucose tolerance test (GTT) to study the status of insulin resistance (31). Since then, many studies have utilized different methods to test for insulin resistance such as the ability of phenylisopropyladenosine to modulate basal and maximal insulin-stimulated glucose uptake (32), euglycemic hyperinsulinemic clamp technique (33) and incubation of adipocytes with 3-O-d[Methyl-(3)H] glucose with and without insulin (34). Furthermore, some of the studies were specific to certain target tissue such as the adipocytes (33), liver (29), and muscles (32) and they yield different results.
Furthermore, since insulin resistance was inferred from the relationship between circulating insulin and glucose, certain methods conducted by previous investigators to determine insulin resistance might alter glucose metabolism on the rats. Consequently, this might influence the measurements of plasma glucose and determination of insulin resistance. In some cases rats were restrained (31, 35), which can induce stress and increase the circulating blood glucose levels. Also, in some studies, rats were subjected to general anesthesia during testing (35, 36) which will significantly increase blood glucose level (37). In the current study, ITT was conducted 2 hours after the rats regain consciousness (to ensure the anesthesia are completely worn off) and blood sample was taken when the rats were in a relaxed (resting) state. Therefore, the different methods employed, as well as the different target tissues used in determining the status of insulin resistance conducted by the previous studies and the present study, may explain the absence of insulin resistance observed in the present study.

Another factor that might elucidate the inconsistency in results in terms of insulin resistance is the possible adaptation developed by the SHR in the current study. This adaptation allowed the SHR to have regular insulin response and might be contributed by certain ingredient(s) or nutrient(s) found in the diet since the diet composition from the present study may not be identical to those from previous studies. However, further investigations are still required to determine if the diet is indeed responsible for this adaptation.

To conclude, no studies have shown the effect of HFLC diet on blood pressure and the subsequent effect on the development of cardiac hypertrophy in SHR. The present data suggest that HFLC diet lowers blood pressure without suppressing or
preventing the development of cardiac hypertrophy in SHR. Although blood pressure was reduced, it was still sufficient to produce a pathological stimulus for the development of cardiac hypertrophy. Despite of the hypertrophy, the SHR fed with HFLC diet might be protected from developing heart failure or will develop heart failure later in life due to the lowered blood pressure. In addition, it was found that the SHR may be protected from developing insulin resistance under LFHC diet when compared to WKY, a finding which has not been previously reported.
REFERENCES


