Effects of Calorie-Restricted Low-Carbohydrate Diet on Glucose and Lipid Metabolism in Otsuka Long Evans Tokushima Fatty Rats

Nobukiyo Koide, Tomokazu Oyama, Yoh Miyashita, and Kouji Shirai

Department of Internal Medicine, Sakura Medical Center, School of Medicine, Toho University, Chiba, Japan.

Aim: We investigated the effects of a calorie-restricted low-carbohydrate diet on glucose and lipid metabolism, and body fat distribution, especially on the secretion of leptin and lipoprotein lipase from adipose tissue in Otsuka Long Evans Tokushima Fatty (OLETF) rats.

Methods: Forty-three week-old male OLETF rats were randomized into three groups (n=6 per group): the HC group (HC) was fed a diet with 60% carbohydrate; the LC group (LC) with 30% carbohydrate; and the P-HC group (P-HC) with 60% carbohydrate and pioglitazone (0.1%). The total calorie intake was restricted to 70% of the average intake from each diet (60 kcal/day). The diets were continued for 8 weeks.

Results: Similar decreases in body weight and serum glucose were observed in the three groups. Serum insulin concentration was significantly decreased in LC and P-HC compared to HC. Serum total cholesterol and triglycerides decreased significantly (p<0.05) in LC and P-HC compared to HC. The decrease of visceral fat area measured by computed tomography was greatest in LC among the three groups. At the end of the diet, leptin secretion from visceral adipose tissue and lipoprotein lipase (LPL) activity in subcutaneous adipose tissue were significantly higher in LC and P-HC compared to HC (p<0.05).

Conclusion: These results indicate that under calorie-restricted conditions, low carbohydrates are much more effective than high carbohydrates in improving insulin sensitivity.


Key words; OLETF rat, Visceral fat, Low carbohydrate diet, Leptin, Lipoprotein lipase

Introduction

Recently, lifestyle-related diseases, especially metabolic disorders due to the accumulation of visceral fat, have been increasing in Japan.1-3 The accumulation of visceral fat is known to be related to the development of insulin resistance4-5. Insulin resistance plays an important role in the development of obesity-associated metabolic disorders such as type 2 diabetes mellitus, hypertension and dyslipidemia. These metabolic disorders are related to a high incidence of coronary heart disease.6-7. Accumulation of visceral fat also causes imbalances of lipoprotein lipase (LPL) activity and various adipocytokines such as leptin, adiponectin and plasminogen activator inhibitor-1.8-10. These imbalances are also related to insulin resistance. Therefore, in addition to body weight reduction, normalization of LPL activity and adipocytokine balance are also important therapeutic goals in obesity with insulin resistance.

A low-calorie diet is the fundamental therapy for obesity with insulin resistance. The principle of this diet is to provide adequate amounts of proteins, vitamins and minerals, while restricting carbohydrates and fats as energy components; however, the optimal carbohydrate to fat ratio for the diet has not been established. Recent reports indicate that a low-calorie diet with low carbohydrates is beneficial to improve obesity and insulin sensitivity.11-13. We have previously reported that a low-carbohydrate low-calorie diet might be effective to reduce visceral fat and might improve insulin resistance in obese type 2 diabetes mellitus patients14.
Pioglitazone is known to improve insulin sensitivity by activating peroxisome proliferative activated receptor-γ\(^{15}\). This drug is often used with a high-carbohydrate low-calorie diet, because a fat-restricted diet is generally recommended according to the principles of diet therapy for diabetes mellitus. We are interested that which therapy is more effective for glucose and lipid metabolism, a low-carbohydrate low-calorie diet or a high-carbohydrate low-calorie diet with pioglitazone.

In this study, the effect of a low-carbohydrate low-calorie diet on glucose and lipid metabolism, and body fat distribution was compared to that of a high-carbohydrate low-calorie diet or a high-carbohydrate low-calorie diet with pioglitazone in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, which are a model of type 2 diabetes characterized by obesity and insulin resistance\(^{16, 17}\). Furthermore, we examined LPL activity and leptin secretion from adipose tissues after adaptation to the calorie-restricted low-carbohydrate diet.

### Materials and Methods

**Animals**

The experiments were performed in accordance with the guidelines for Animal Experiments of Toho University. Ten week-old male OLETF rats and Long-Evans Tokushima Otsuka (LETO) rats, which were used as age-matched controls for OLETF rats\(^{16}\), were kind gifts from Dr. Kazuya Kawano (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan). Rats were given commercial powdered food (Oriental Yeast Co., Tokyo, Japan) and water ad libitum for 33 weeks, and 43 week-old, rats were then used for the experiment. The animals were housed in stainless-steel wire-bottom cages in a temperature-controlled room (22 ± 2°C) with artificial lighting from 0600 to 1800.

**Adjustment of Diets**

OLETF rats were adapted to a calorie-restricted diet for 8 weeks. The calorie-restricted diet was 60 kcal/day, which is 70% of the average calorie intake of rats\(^{18}\). All rats were fasted overnight before the experiment was started. OLETF rats were randomly divided into 3 groups of six rats each. The first group was fed a high (60%) carbohydrate diet (HC). The second group was fed a low (30%) carbohydrate diet (LC). The third group was fed a pioglitazone-containing high-carbohydrate diet (0.1%) (P-HC)\(^{19}\). The characteristics of each group at baseline are shown in Table 1.

Food was prepared daily at 1800. The daily food intake was adjusted to 60 kcal/day. The protein: carbohydrate (PFC) ratios were approximately 30%:

| Table 1. Characteristics of each group at baseline |
|-----------------------------------------|-----------------|------------------|
|                                         | HC group        | P-HC group       |
| Body Weight (g)                         | 583 ± 23        | 608 ± 25         |
| FBS (mg/dL)                             | 287 ± 45        | 299 ± 47         |
| IRI (pmol/L)                            | 1,475 ± 124     | 1,458 ± 136      |
| TC (mg/dL)                              | 189 ± 22        | 215 ± 21         |
| TG (mg/dL)                              | 286 ± 54        | 203 ± 56         |
| HDL-C (mg/dL)                           | 51 ± 6          | 60 ± 7           |

FBS: fasting blood glucose, IRI: immunoreactive insulin, TC: total cholesterol, TG: triglycerides, HDL-C: high-density lipoprotein cholesterol

**Table 2.** Composition of high carbohydrate (HC) and low carbohydrate (LC) diets (expressed in % calories)

| Table 2. Composition of high carbohydrate (HC) and low carbohydrate (LC) diets (expressed in % calories) |
|-----------------------------------------|-----------------|------------------|
|                                         | HC (%)          | LC (%)           |
| Casein Na                               | 27.18           | 27.18            |
| L-Cystine                               | 0.18            | 0.18             |
| β-Cornstarch                            | 35.89           | 12.6             |
| α-Cornstarch                            | 10.0            | 10.0             |
| Sucrose                                 | 10.0            | 10.0             |
| Soy-bean oil                            | 0.99            | 0.0              |
| Cellulose powder                       | 5.7892          | 18.7292          |
| Mineral mix                             | 3.5             | 3.5              |
| Vitamin mix                             | 1.0             | 1.0              |
| Tartaric acid                           | 0.25            | 0.25             |
| Butyl hydroquinone                      | 0.0008          | 0.0008           |
| Palmitic acid                           | 0.93            | 0.0              |
| Oleic acid                              | 1.37            | 16.56            |
| Renic acid                              | 2.92            | 0.0              |

Oriental Yeast Co.Ltd.

10%: 60% for the HC diet and 30%: 40%: 30% for the LC diet. The diets were prepared by replacing carbohydrates in the diet with an isocaloric amount of fat (Table 2). The two diets contained equal amounts of protein and all of the essential minerals and vitamins required for rats.

**Body Weight Measurement and Blood Sampling**

After anesthetizing with pentobarbital (2 mg/kg), body weight (BW) was measured and blood sampled in the morning before and after test meal feeding. Theses samples were defined as fasting sera. Fasting blood glucose (FBS), total cholesterol (TC), triglyceride (TG) and high density lipoprotein-cholesterol (HDL-C) were assayed using a spectrophotometer (Hitachi Clinical Spectrophotometer 2015, Tokyo, Japan). Plasma
insulin concentrations (basal IRI) were measured by a radioimmunoassay\textsuperscript{20}. Each measurement of serum concentration was made in duplicate, and the mean value was used for each determination.

**Measurement of Subcutaneous and Visceral Fat Areas**
Before and after the 8-week experimental period, abdominal CT scans were performed to measure visceral and subcutaneous fat areas, using a Hitachi Pronto CT scanner\textsuperscript{21}. The scan was performed at the position of the navel after anesthetizing with pentobarbital (2 mg/kg).

**Assay of Leptin in Adipose Tissue**
At the endpoint of the calorie-restricted diet, leptin concentrations secreted from subcutaneous and visceral adipose tissues were measured separately by the following methods. Fifty mg of minced adipose tissue in 1 mL of Dulbecco’s modified Eagle’s medium (DMEM) containing 20% fetal bovine serum was incubated at 37°C for 40 min. The amount of leptin extracted in the medium was determined by an enzyme-linked immunosorbent assay (ELISA) using a commercial rat leptin kit (Morinaga Biochemical Industries, Japan)\textsuperscript{22}. Adipose tissue of the mesenterium was used as visceral adipose tissue.

**Assay of LPL Activity in Adipose Tissue**
At the end of the calorie-restricted diet, heparin-released LPL activities from subcutaneous and visceral adipose tissues were determined separately by the following methods. One hundred mg of minced adipose tissue in 1 mL of DMEM containing 10 U/mL heparin was incubated at 37°C for 45 min, and then TritonX-100 emulsified-triolein as a substrate was added to the medium. LPL activity in the medium was determined using the concentration of free fatty acid released from the substrate to medium\textsuperscript{23}. Adipose tissue of the mesenterium was used as visceral adipose tissue.

**Statistical Analysis**
Data were analyzed by ANOVA using the software program StatView 4.0 (Abacus Concepts, Berkeley, CA, USA). Fisher’s protected least significant difference (PLSD) test was used when there was a significant difference among groups. Significance was set at a $P$ value of 0.05.

**Results**

**Change of BW by Energy-Restricted Diet**
BW changes after 8 weeks of diet are shown in

---

![Fig. 1](image_url)

**Fig. 1.** Changes in mean body weight after a calorie-restricted diet for 8 weeks.
Data are expressed as the mean ± S.D. of six rats.

**Effect of Calorie-Restricted Diet on Glucose Metabolism**
After 8 weeks of diet, FBS decreased significantly and similarly in all groups (Fig. 2A). Basal IRI also showed a significant reduction in all groups (Fig. 2B). The decrease of basal IRI in LC was significantly greater than that in HC, and almost equal to that in P-HC.

**Effect of Calorie-Restricted Diet on Lipid Metabolism**
After 8 weeks of diet, LC showed marked lowering of TC and TG to almost the same levels as P-HC, and the decreases in the two groups were significantly greater ($p<0.05$) than those in HC (Fig. 3A and B). HDL-C level was slightly decreased in HC, and slightly increased in LC and P-HC. There were no significant differences in the change of HDL-C level among the three groups (Fig. 3C).

**Change of Body Fat Distribution by Energy Restricted Diet**
Visceral fat areas of OLETF rats were greater than those of LETO rats (Table 3). These results indicated that fat distribution in OLETF rats was visceral-dominant at baseline.
After 8 weeks of diet, the subcutaneous and visceral fat areas in the three groups were significantly reduced. Changes in the subcutaneous fat area are shown in Fig. 4A. Reduction of the subcutaneous fat area was significantly greater in HC than in P-HC. Changes in the visceral fat area over the 8-week study are shown in Fig. 4B. The decrease of visceral fat area was greatest in LC among the three groups, while the decrease was almost the same in P-HC and HC.

**Leptin Secretion and LPL Activity in Adipose Tissue**
At the end of the 8-week calorie-restricted diet, spontaneous leptin secretion and heparin-released LPL
activity were examined in subcutaneous and visceral adipose tissue. In subcutaneous adipose tissue, no significant change in leptin secretion was observed among the three groups (Fig. 5A); however, in visceral adipose tissue, leptin secretion in LC was significantly higher than in HC (p<0.05), and almost the same as that in P-HC (Fig. 5B).

LPL activity in subcutaneous adipose tissue in LC was significantly higher than in HC but almost equal to that in P-HC (Fig. 6A); however, activity in visceral adipose tissue was not remarkably different among the three groups (Fig. 6B).

**Discussion**

In the present study, all three groups of OLETF rats showed significant body weight reduction. This finding confirmed that calorie restriction was almost perfectly implemented in OLETF rats.

Under calorie restriction, LC, HC and P-HC showed almost the same reduction of FBS. For basal IRI, however, P-HC showed the greatest reduction followed by LC. Decrease of serum IRI together with lowering of serum glucose is generally consistent with improvement of insulin sensitivity. These results therefore suggest that when the restricted diet was made isocaloric, a low-carbohydrate diet might be a more effective treatment for insulin resistance than a high-carbohydrate diet. Previous reports indicate that dietary fiber has the potential to improve glucose and lipid metabolism. In this study, the intake of dietary fiber in LC was greater than that in HL or P-HC. This difference in dietary fiber intake also might affect the results; however, dietary fiber shows mainly a blood sugar-lowering effect by delaying carbohydrate digestion and absorption. The mechanism of insulin reduction in LC may therefore be chiefly due to a decrease in insulin requirements by the restriction of carbohydrates. The decrease of TG and increase of HDL-C in LC indirectly support that insulin sensitivity was improved to a greater extent in LC than in HC, and LC may have almost equal potential to improve insulin re-

---

**Table 3.** Visceral or subcutaneous fat areas of each group at baseline

<table>
<thead>
<tr>
<th></th>
<th>LETO</th>
<th>OLETF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HC group</td>
<td>LC group</td>
</tr>
<tr>
<td>Visceral fat (mm²)</td>
<td>784 ± 23</td>
<td>2,854 ± 34</td>
</tr>
<tr>
<td>Subcutaneous fat (mm²)</td>
<td>389 ± 32</td>
<td>116 ± 21</td>
</tr>
</tbody>
</table>

**Fig. 4.** Changes in the mean subcutaneous fat area (A) or visceral fat area (B) after a calorie-restricted diet for 8 weeks. Data are expressed as the mean ± S.D.
Fig. 5. Leptin-secreting potential from subcutaneous adipose tissue (A) or visceral adipose tissue (B) at the end of a calorie-restricted diet for 8 weeks.
Data are expressed as the mean ± S.D. performed in triplicate.

Fig. 6. Lipoprotein lipase (LPL) activity in subcutaneous adipose tissue (A) or visceral adipose tissue (B) at the end of a calorie-restricted diet for 8 weeks.
Data are expressed as the mean ± S.D. performed in triplicate.
sistance as P-HC.

The visceral fat area in LC decreased to a significantly greater extent than in HC or P-HC during the diet. The difference between LC and HC can be explained by the lower insulin requirement in LC than in HC. Hyperinsulinemia is reported to be associated with visceral fat accumulation and a high level of fasting insulin is reported to predict visceral fat accumulation28). Accordingly, low insulin secretion in LC probably facilitated the reduction of visceral fat accumulation. Hence, greater improvement of serum glucose and lipid metabolism in LC than in HC may be explained by the greater decrease of visceral fat in LC than in HC. In P-HC, the decrease of the visceral fat area was significantly lower than in LC. Pioglitazone increases the number of small adipocytes and induces the apoptosis of large adipocytes; therefore, in P-HC, the change in qualitative characteristics of adipocytes in visceral fat may be stronger than the quantitative change.

After the calorie-restricted diet, the potential of leptin secretion from visceral adipose tissue and LPL activity in subcutaneous adipose tissue in LC were higher than in HC, while LC and P-HC had almost the same levels. These results suggest that a low-carbohydrate diet is superior to a high-carbohydrate diet in enhancing the potential of leptin secretion and LPL activity, and these beneficial effects are almost equal to those obtained by administering pioglitazone with high carbohydrates. The mechanism of these changes is unclear. Probably, a low-carbohydrate diet and pioglitazone could promote mRNA expression of LPL in subcutaneous adipose tissue, because the expression of the LPL gene was decreased in subcutaneous adipose tissues30). It is reported that after a hypocaloric diet, no significant change in mRNA expression of leptin in subcutaneous adipose tissue was observed31). The expression of the leptin gene also could be promoted in visceral adipose tissue by a low-carbohydrate diet and pioglitazone. Recent reports suggest that leptin may improve insulin sensitivity32, 33). LPL activity is considered to be a marker of insulin sensitivity34, 35). From these findings, we consider that the greater leptin secretion and higher LPL activity in LC compared to HC may account for the favorable effects on glucose and lipid metabolism observed in LC and P-HC. Accordingly, it is speculated that a low-calorie diet with low carbohydrates may achieve almost the same level of leptin secretion and LPL activity as a low-calorie high-carbohydrate diet and pioglitazone together.

In summary, a calorie-restricted low-carbohydrate diet was superior to a high-carbohydrate diet in reducing visceral fat and enhancing leptin secretion and LPL activity in adipose tissues. It may be concluded that in obese patients treated by calorie restriction therapy, low carbohydrates are much more effective than high carbohydrates in improving insulin sensitivity.

Acknowledgments

We thank Dr. Kazuya Kawano (Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan) for OLETF rats and LETO rats.

References

12) Foster GD, Wyatt HR, Hill JO, McGuckin BG, Brill C,


22) Ogino T, Moralejo DH, Kose H, Yamada T, and Matsumoto K: Serum leptin concentration is linked to chromosomes 2 and 6 in the OLETF rat, an animal model of type 2 diabetes with mild obesity. Mamm Genome, 2003; 65:1207-1211


