INTRODUCTION

A high-fat diet (HF) to rodents has proved to be a useful model of the putative effects of dietary fat in humans (1, 2). There is a large body of evidence that rats fed HF chronically produce obesity and insulin resistance (3, 4), which is similar to the effects of the western style diet on humans (5, 6). We already reported that visceral obesity is an independent determinant for decreased insulin responsiveness in skeletal muscle in vitro (7). Reducing visceral fat accompanies decreased triglyceride and insulin concentrations, and improving insulin resistance (8).

Previous studies also suggested that free fatty acids (FFA) influence insulin action. Infusion of lipid to rats for 5 hr reduced glucose infusion rate in hyperinsulinemic-euglycemic clamp study, which accompanied decreased glucose oxidation (9). Increased plasma FFA also decreased glucose uptake, glycolysis, glycogen synthesis, and carbohydrate oxidation in healthy men (10). The mechanisms that underlie these reactions might be explained by Randle theory. In the 1960s, Randle et al. introduced the concept of substrate competition, whereby enhanced availability and oxidation of FFA lead to impaired glucose metabolism (11).

Chronic HF is also known to increase $\beta$-oxidation in animal models (12, 13), and inhibition of $\beta$-oxidation by etomoxir increase insulin-stimulated glucose uptake (14). These results imply that dietary fat per se could be the independent factor to induce insulin resistance by chronic HF.

The purpose of this study was to determine whether chronic HF could induce insulin resistance independently of obesity. We measured insulin sensitivity using a euglycemic hyperinsulinemic clamp in high-fat and high-carbohydrate fed rats for 8 weeks.
libitum throughout the study period. Twenty rats were fed with a control diet (high-carbohydrate diet, HC) for 8 weeks, providing 66% energy as carbohydrate and 12% as fat. The other rats were fed with a HF for 8 weeks, providing 61% energy as fat and 24% as carbohydrate. The diets used in this study are described in Table 1.

Euglycemic hyperinsulinemic clamp study

The insulin and glucose clamp technique was performed according to Kim et al. (15) for 90 min. As briefly described, the measurements were done with the rats under anesthesia (40 mg/kg body weight pentothal sodium by intraperitoneal injection) after fasting for 6 hr. While one catheter was inserted into the left femoral artery for blood sampling, the other catheter was inserted into the right external jugular vein to infuse insulin (Velosulin, Novo Nordisk, Vagsvaerd, Denmark), [3-3H] glucose (NEN, Boston, MA, U.S.A.) and 25% glucose. After the collection of a basal-line sample, infusion of insulin and [3-3H] glucose was commenced at 120 pmol/kg and 8 μCi/kg for priming dose, respectively and then was maintained at 12 pmol/kg/min and 0.8 μCi/kg/min throughout the study, respectively. Glucose was infused (Gilson, Villiers Le Bel, France) at variable rates to adjust the glucose level at 4.5 mM and blood samples were taken at interval of 5 min for analysis of glucose concentration (YSI 1410, Yellow Springs, Ohio, U.S.A.). The blood samples for insulin and tritiated glucose were taken at 70, 80, and 90 min. Tritiated glucose was measured with a liquid scintillation counter (Wallac 1410, Pharmacia, Sweden). The glucose disappearance rate (Rd, mg/kg/min), glucose infusion rate (GIR), and hepatic glucose output (HGO) at steady state were calculated according to the methods by Kim et al. (15).

Measurement of fat mass and plasma insulin, FFA, and triglyceride

Visceral fat mass (VF) was determined by weighing excised fat in the intra-abdominal cavity. Subcutaneous fat mass (SF) was defined as fat in the subcutaneous area of the abdominal wall. Insulin concentration was determined by radioimmunoassay (NEN, Boston, MA, U.S.A.). The concentrations of plasma triglyceride and FFA were analyzed with diagnostic kits (Sigma, St. Louis, Missouri, U.S.A.).

Statistical analysis

All data are expressed as mean ± S.E.M. Statistical analyses were performed using the Student t-test and an analysis of covariance (ANCOVA), adjusting for percentage of VF to body weight (%VF). Simple linear regression analysis was used for the assessment of correlation between the factors. All statistical analyses were done using the SPSS program.

RESULTS

Rats fed with the HF show insulin resistance and increased fat mass

Rats fed with HF gained more body weight compared with those fed with HC for 8 weeks. While VF was increased by HF, it was not the case as for the SF. The percentage of VF to body weight (%VF) was also increased by the HF (Table 2).

Plasma glucose and triglyceride concentrations in rats fed with HF were significantly increased by 8% and 25%, respectively. There was no difference in plasma insulin and FFA concentrations between the two groups (Table 3).

Rd of rats fed with HF was decreased significantly, while HGO was increased. Correlation analysis between Rd and various indicators (body weight, VF, %VF, SF, and %SF) showed inverse relationship between Rd and these factors except %SF (Table 4).

Rats fed with HF show insulin resistance even after controlling %VF compared to those fed with HC

%VF was chosen as the most reliable indicator for fat mass (16, 17) and showed wider range in HF (2.7 to 7.2) than HC (2.2 to 5.0) in this study (Fig. 1). To determine

Table 1. Composition of diet used in the experiment

|                      | High-carbohydrate | High-fat    |
|----------------------|-------------------|-------------|
| Corn starch          | 610               | 310         |
| Casein               | 200               | 200         |
| Cellulose            | 80                | 80          |
| Soybean oil          | 50                | 50          |
| Lard                 | 0                 | 300         |
| Vitamin mix          | 20                | 20          |
| Mineral mix          | 40                | 40          |

Table 2. Body weights and fat masses in rats fed either with high-carbohydrate (HC) or with high-fat diet (HF)

|                      | HC (n=20)         | HF (n=20)       |
|----------------------|-------------------|-----------------|
| Body weight (BW, g)  | 242.3 ± 3.91      | 259.8 ± 2.73    |
| Fat masses           |                   |                 |
| Visceral fat (VF, g) | 8.7 ± 0.51        | 11.3 ± 0.75     |
| %VF to BW            | 3.6 ± 0.19        | 4.3 ± 0.27      |
| Subcutaneous fat (SF, g) | 5.0 ± 0.18   | 5.8 ± 0.43 |
| % SF to BW           | 2.1 ± 0.07        | 2.2 ± 0.16      |

The results are presented as mean ± S.E. Data of HC and HF were examined with Student t-test. *p<0.05, **p<0.01, and ***p<0.001 vs HC.
whether dietary fat induces insulin resistance regardless of visceral obesity, we compared Rd and HGO between groups after matching %VF in both groups and using an ANCOVA to adjust for %VF. Firstly, to match %VF, %VF from the lowest value of the HF (2.7) to the highest value of the HC (5.0) was selected. The average %VF was 3.7 and 3.8 in the HC and the HF, respectively (Fig. 1), and %SF and plasma biochemicals were not significantly different (Table 5). However, Rd in the HF was significantly decreased by 14% and HGO was significantly increased by 110% (Fig. 2). Furthermore, when adjust for %VF by ANCOVA, Rd for HF (9.3 ± 0.2) was significantly decrea-

Table 3. Characteristics of rats fed either with the high-carbohydrate (HC) or with the high-fat diet (HF) in basal and clamp studies

|                  | HC (n=20) | HF (n=20) |
|------------------|-----------|-----------|
| Glucose (mM/L)   | 3.9 ± 0.1 | 4.2 ± 0.1*|
| Insulin (pM/L)   | 152 ± 8.5 | 153 ± 12.1|
| Triglyceride (mg/dL) | 28 ± 2.0 | 35 ± 2.2*|
| Free fatty acids (mEq/L) | 521 ± 48 | 553 ± 37 |

The results are presented as mean ± S.E. Comparison between groups were examined with Student t-test. *p<0.05 and †p<0.001 vs HC. Rd, glucose disappearance rate; GIR, glucose infusion rate; HGO, hepatic glucose output rate

Table 4. Simple correlation coefficients for glucose disappearance rate (Rd) and the indicative variables in pooled experimental cases in both groups (n=40)

|       | BW    | VF    | %VF to BW | SF    | %SF to BW |
|-------|-------|-------|-----------|-------|-----------|
| Rd    | -0.413| -0.437| -0.438    | -0.401| -0.312    |
| p     | 0.015 | 0.009 | 0.009     | 0.017 | 0.068     |

The results are presented as mean ± S.E. BW, body weight; VF, visceral fat mass; SF, subcutaneous fat mass

Table 5. Characteristics of the rats fed either with the high-carbohydrate diet (HC) or with the high-fat diet (HF) after matching the percentage of visceral fat mass to body weight

|                  | HC (n=18) | HF (n=14) |
|------------------|-----------|-----------|
| % Visceral fat mass to BW | 3.7 ± 0.2 | 3.8 ± 0.2 |
| % Subcutaneous fat mass to BW | 2.1 ± 0.1 | 2.0 ± 0.2 |
| Plasma biochemicals |
| Glucose (mmol/L) | 3.9 ± 0.1 | 4.3 ± 0.1 |
| Insulin (pmol/L) | 147 ± 9.0 | 147 ± 13.2|
| Triglyceride (mg/dL) | 29.3 ± 2.1 | 35.7 ± 2.7 |
| Free fatty acids (mEq/L) | 551 ± 50 | 601 ± 40 |

The results are presented as mean ± S.E.

Fig. 1. Percentage of visceral fat mass to body weight (%VF) in rats fed either with the high-carbohydrate diet (HC) or with the high-fat diet (HF). Dotted lines represent upper and lower limit for %VF matching. Open square: mean ± S.E. in %VF matching.

Fig. 2. Glucose disappearance rate (A) and hepatic glucose output rate (B) as a function of percentage of visceral fat mass to body weight (%VF) in rats fed either with the high-carbohydrate diet (HC, open circle) or with the high-fat diet (HF, closed circle). Open and closed square represent mean ± S.E. in HC and HF, respectively. Comparison between groups were examined with Student t-test. *p<0.001 vs HC.
sed (p<0.01) compared to HC (10.5±0.2). HGO was significantly increased in HF (2.3±0.3 and 4.4±0.2 in HC and HF, respectively, p<0.01, ANCOVA).

DISCUSSION

Insulin resistance by chronic HF is known to be associated with increased visceral fat mass (3, 18). However, a large body of evidence suggests that FFA is also one of the most important factors to modulate glucose metabolism (9, 10, 12). Therefore we hypothesized that not only the increased visceral fat mass, but also dietary fat per se could be one of the independent factors to induce insulin resistance by chronic HF.

Here we have showed that HF for 8 weeks produced insulin resistance as assessed by a euglycemic hyperinsulinemic clamp study, which was accompanied by increased visceral fat mass. According to the previous studies, increased visceral fat mass contributes FFA to the liver to increase circulating triglyceride and hepatic glucose production rates (19). Compatible with previous studies, plasma triglyceride and HGO were also increased in the present study. Simple linear regression analysis revealed that significant association of glucose disposal rate with visceral fat mass. These results imply that increased visceral fat mass could be one of the causes for insulin resistance in chronic HF.

In order to determine whether dietary fat could induce insulin resistance independent of obesity, we matched visceral fat mass of the HF fed rats with that of the HC fed rats and compared insulin-stimulated glucose disappearance rate between the two groups. As expected, the result showed that rats fed with the HF produced insulin resistance compared with those fed with the HC despite the same visceral fat mass. Many short-term infusion studies proved that an increased lipid availability might produce insulin resistance through a decreased glucose oxidation (9, 10). However, influence of chronic HF on glucose metabolism have been explained exclusively by obesity (3, 4, 19) and few study has investigated the effects of other factors independent of obesity. The mechanisms involved in this reaction could be explained by 1) glucose-fatty acid cycle like short-term infusion study (11) and/or 2) changes in fatty acid profile of membrane induced by chronic dietary fat modification (20) but further studies are needed to clear them.

Finally, we suggest dietary fat induces insulin resistance in rats fed with chronic HF independent of visceral obesity.

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