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High Food Efficiency Ratio of Prepubertal Growth Period Leads to a Long-Term Susceptibility for Obesity and Insulin Resistance in Obesity-Prone and Obesity-Resistant Sprague Dawley Rats

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Abstract

Excessive body weight gain during the growth period of early life may predispose individuals towards obesity and metabolic disorder in later life. We investigated the possibility of using the food efficiency ratio as an early indicator for predicting susceptibility to diet-induced obesity and insulin resistance. Four-week-old, prepubertal, male Sprague Dawley rats were divided into obesity-prone and obesity-resistant groups based on food efficiency ratio values after five days on a high-fat diet. Metabolic parameters measured after 2, 6, and 10 weeks, and specific phenotypes were compared with each group. Obesity-prone rats had higher increases in body weight and fat mass compared to obesity-resistant rats over the study period. Obesity-prone rats became glucose intolerant early in this study and remained so throughout the experimental period, with increases in fat weight and leptin levels occurring first, followed by increases in insulin level. Gluconeogenesis and insulin resistance significantly increased in obesity-prone groups in which activities of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase were increased and glucokinase activity decreased. Higher food efficiency ratio at an early age was closely correlated with body fat accumulation, hyperleptinemia, and hyperinsulinemia of middle and elderly age. We suggest a high food efficiency ratio in prepubertal subjects may be a useful predictor of future obesity and insulin resistance.

Key words: adiposity, food efficiency ratio, insulin resistance, leptin, obesity-prone

Introduction

Obesity, a major health concern worldwide, is a key risk factor for the type 2 diabetes and cardiovascular disease (Ikenasio- Thorpe et al. 2007; Kang et al. 2014). Complex interactions among genetics, environment, physical inactivity, and inappropriate dietary habits contribute to the development of obesity and obesity-related metabolic disorders (Maximova et al. 2008). Genetic and adult lifestyle factors are known to contribute to obesity, but the early nutritional environment may have an important influence on the regulation of metabolic systems later in life. Obesity during growth period of infancy, childhood, and adolescence is very important because obesity tracks into adulthood, leading to elevated risks for insulin resistance and metabolic syndrome, including high blood pressure and abnormal glucose and lipid metabolism (Maximova et al. 2008; Gorski et al. 2006). Early identification of individuals at risk for becoming overweight or obese may help prevent childhood obesity and may provide long-term health benefits.

It was questioned why some easily become obese (obesity prone, OP) and others resist the development of obesity (obesity resistant, OR) when consumed the high energy diets. Although several hypotheses have been proposed such as physiological responsiveness, metabolic processes, and genetic differences (Alexander et al. 2006; Levin et al. 2005), the mechanisms underlying susceptibility to diet-induced obesity remain to be fully defined.

Rodent models have been used extensively to study the complex pathophysiology of obesity. Many findings on obesity and related disorders have come from studies with inbred mouse strains as well as transgenic and knockout mice (Sun et al. 2017; Scarpace & Zhang 2009). A large percentage of human obesity and type 2 diabetes follows a polygenic mode of inheritance, and high-fat (HF) diet-induced obese rat models may be useful surrogates for the study of these polygenic traits (Lin et al. 2000:

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Winzell &, Ahrén 2004: Ehrenberg et al. 2003). The differential responses to a HF diet within a group of animals with the same genetic background have led to the characterization of OP and OR phenotypes (Lin et al. 2000; Winzell &, Ahrén 2004). Similarly in human populations, some are susceptible to obesity, and others are not (Ehrenberg et al. 2003; Levine et al. 2005). OP and OR rats are considered among the best animal models of diet-induced obesity and recapitulate many key features of the human condition. Several studies (Ji & Friedman 2008; Giles et al. 2016) using OP and OR rats on a HF diet have shown differences in metabolic rate and neural regulation between two types and these differences may be major determinants for phenotype development. Although body weight changes were observed within a limited range of food intake, the OP rats showed a higher mean body weight than the OR rats (Leibowitz et al. 2007; Dourmashkin et al. 2006). Rats that gained more weight on a HF diet during growth period became more obese and insulin resistant later in life, compared with rats that gained less weight.

To develop factors that predict to the development of obesity later, it is essential to establish biomarkers indicating susceptibility before the onset of obesity in individuals. Most of studies (Giles et al. 2016; Leibowitz et al. 2007; Dourmashkin et al. 2006) divided OP and OR rats on the basis of body weight gain alone. In contrast, we divided OP and OR rats that have been selectively bred on the basis of their food efficiency ratio (FER) reflecting metabolic rate while on a high-calorie diet at either an early age with normal body weights or prior to the onset of obesity and then performed to predict susceptibility to diet-induced obesity and to validate this screening method. We also observed phenotypic differences between groups of OP and OR rats, classified based on the FER; performed oral glucose tolerance tests and measurements of several plasma biochemical parameters, endocrine hormones, and hepatic gene expression to determine whether the OP rats were more obese and insulin resistant than the OR rats on the same diet; and explored whether a higher FER during growth predicts obesity and abnormal glucose metabolism later in life.

Materials and Methods

1. Animals

All animal experiments were performed in compliance with the Korean Food and Drug Administration (KFDA) guidelines for care and use of laboratory animals (KCDC-12-026-1A). Sprague-Dawley (SD) rats were obtained from the KFDA and were provided ad libitum access to water and rat chow (Purina #5001 Chow, Dyets, Inc., Bethlehem, PA, USA). They were kept in metabolic cages at 22℃, with 50~60% relative humidity on a 12-h light/dark cycle. At 10 weeks of age, thirty of female SD rats were mated. The day after birth, litter sizes were adjusted to eight pups per dam. After 3 weeks of feeding from their mother, the offspring were weaned.

2. Experimental design and diet

The weaning rats were allowed to adapt to the chow diet for 1 week. At 4 weeks of age, 76 male offspring were weighed and fed a HF diet ad libitum for 5 days, according to a protocol modified from Dourmashikin et al. (2006). Body weight and food intake were recorded daily. For each rat, the FER was calculated from the amount of food consumed and the amount of weight gained after 5 days (Fig. 1). Based on the FER, the male rats were assigned to the OP or OR group (Fig. 1). The 24 rats in the highest FER tertile were as OP, and the 24 rats in the lowest FER tertile were designated as OR; the 24 rats in the middle tertile were eliminated from the study (Fig. 1). For the OP and OR groups, we used the AIN-93M HF diet with some

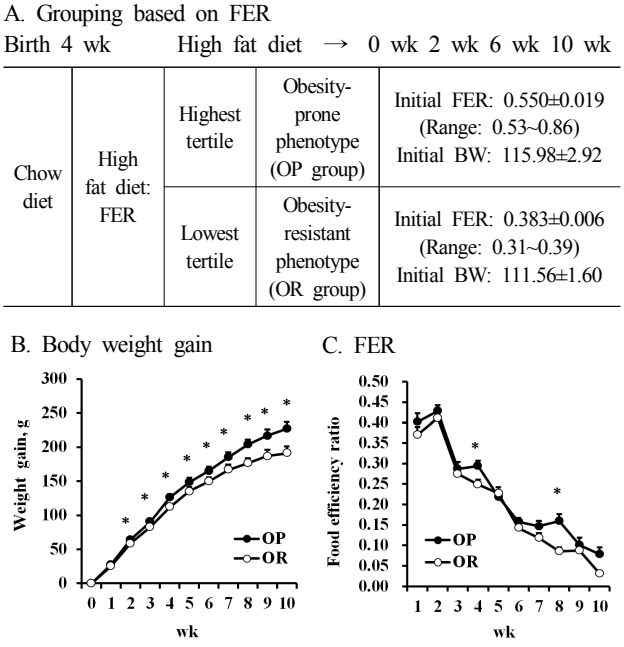


Fig. 1. Classification of OP and OR rats based on FER and change of cumulative body weight and FER. Data are expressed as means±SEM. Vales with an asterisk (*) were significantly different between groups at $p<0.05$.

modifications in fat and carbohydrate content (ICN, Irvine, CA, USA). The diet provided 3.90 kcal/g of total energy, with 43% from fat, 17% from protein, and 40% from carbohydrates (kcal %). The diet was composed of 31.4% (w/w) corn starch, 15.65% sucrose, 20% casein, 20.65% beef tallow, 1.75% corn oil, 0.1% cholesterol, 1% vitamin mix (AIN-93), 4% mineral mix (AIN-93), 0.25% choline bitartrate, and 0.2% L-cystine. Water was freely available throughout the experiment. Body weight was recorded weekly, and food intake was measured every 2~3 days for the duration of the experiment.

3. Blood and tissue collection

After 2, 6, and 10 weeks on the HF diet, the rats were fasted overnight and blood was collected by heart puncture. The plasma was separated by centrifugation at 3,000 rpm for 20 min at 4℃ and stored at −80℃ until analysis. The liver, adipose tissue, kidney and brain were excised, frozen immediately in liquid nitrogen, and stored at −80℃ until further analysis.

4. Biochemical analysis

Plasma concentrations of glucose (AM-201K), triglycerides (AM-157SK), cholesterol (AM-202K), and high-density lipoprotein (HDL)-cholesterol (AM203-3) were determined using an enzyme assay (Asan Pharmaceutical, Yongin, Gyeonggido, Korea). Low-density lipoprotein (LDL)-cholesterol was calculated using the formula of Friedewald et al. (1972). Free fatty acids were determined using a nonesterified free fatty acid kit (Wako, Osaka, Japan). Plasma insulin (Rat insulin ELISA kit; Shibayagi, Co., Ltd., Gunma, Japan), adiponectin (Mouse/rat adiponectin ELISA kit K1002-1; B-Bridge International, Inc., Sunnyvale, CA, USA), leptin (Mouse/rat leptin ELISA kit 022-LEP-E06; ALPCO Diagnostics, Salem, NH, USA), and acylated ghrelin (Ghrelin, Acylated Mouse/Rat EIA kit, ALPCO Diagnostics) were determined using commercial enzymatic assay kits. An insulin resistance index was calculated using homeostasis model assessment (HOMA) index as follows: (fasting serum insulin concentration, μU/mL) × (fasting serum glucose concentration, mM/L) ÷ 22.5 (Matthews et al. 1985). A high HOMA index denotes low insulin sensitivity and decrease the beta-cell function.

5. Oral glucose tolerance test

After 2, 6, and 10 weeks on a HF diet, an oral glucose tolerance test was performed in the OP and OR rats. The rats were deprived of food overnight and were given an oral dose of glucose (2 g/kg body weight) the next morning. Blood samples were obtained from the tail at 0, 30, 60, 90, and 120 min after glucose administration, and the blood glucose concentration was measured using a Super Glucocard II analyzer (ARKRAY, Inc., Kyoto, Japan). To measure insulin tolerance, the area under the curve (AUC) from baseline was calculated using SigmaPlot 8.0 (SPSS Inc., Chicago, IL, USA).

6. Hepatic lipid content

The livers were homogenized, and tissue lipids were extracted in a chloroform-methanol solution according to the method of Bligh & Dyer (1959). After the addition of 0.9% NaCl, the phases were separated by centrifugation, and the lower phase was collected for evaporation. Phosphate-buffered saline (pH 7.4) containing 1% Triton X-100 was added to dissolve the remaining pellet. Hepatic triglyceride and total cholesterol concentrations were measured using the same commercial kits as those used for the blood samples.

7. Insulin secretion from isolated islets

We evaluated the insulin-secreting capacity of islets isolated from individual rats (Fujimoto et al. 1998). At 10 weeks, islets were isolated from rats in the OP and OR groups (n=3 per group) by collagenase digestion. Islets were cultured for 48 h in RPMI 1640 medium containing 5.6 mM/L glucose, 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 mg/mL) in an atmosphere of 5% CO_2 at 37℃ (Winzell & Ahrén 2008). After 48 h, 20 islets were added to each well of a 96-well plate and incubated for 90 min at 37℃ in Krebs-bicarbonate buffer (115 mM NaCl, 5 mM KCl, 24 mM $NaHCO_3$, 1 mM $CaCl_2$,1 mM $MgCl_2$, and 3 g/L bovine serum albumin) containing glucose (5.5 mM or 16.7 mM) balanced with a mixture of 95% O_2~ 5% CO_2, pH 7.4. Released insulin was measured by enzyme immunoassay (Rat insulin ELISA kit; Shibayagi, Co., Ltd., Gunma, Japan).

8. Glucose metabolizing enzyme activities

Glucokinase (GK) activity was estimated by subtracting the hexokinase activity measured at 0.5 mM glucose from that measured at 200 mM glucose. Hexokinase activity was measured in the cytosolic fractions by following the production of glucose-6-phosphate in an assay coupled to the reduction of nicotinamide adenine dinucleotide in the presence of excess glucose-6-phosphate dehydrogenase (Pilkis SJ 1975). One unit of GK was defined

as the enzyme activity resulting in the formation of 1 μM of glucose-6-phosphate/min per mg protein. The activity of glucose-6-phosphatase (G6Pase) was assayed according to the method of Baginski et al. (1974), with a slight modification. After the reaction with G6Pase, the liberated inorganic phosphate in an aliquot of supernatant was determined using a commercial reagent (Phosphor B-test; Waco) based on the molybdenum blue method. One unit of G6Pase was defined as the enzyme activity resulting in the formation of 1 μM of phosphate/min per mg protein. The activity of phosphoenolpyruvate carboxykinase (PEPCK) was assayed according to the method of Bentla et al. (1976), with a slight modification. One unit of PEPCK was defined as the enzyme activity resulting in the formation of 1 μM of NAD^+/minpermgprotein.

9. RNA extraction and analysis of mRNA expression

Total RNA was extracted from tissues using TRI reagent (Molecular Research Center, Inc., Cincinnati, OH, USA) according to the manufacturer's instructions. RNA expression was quantified by real-time quantitative PCR using SYBR green PCR reagents (Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 7900 HT sequence detection system (Applied Biosystems). The relative quantitation was calculated by analyzing the changes in SYBR green fluorescence during PCR, according to the manufacturer's instructions. The data are expressed as $2^{-\Delta\Delta C_T}$ normalized to 18S rRNA and mean ΔC_T normalized to the OP group. Rat-specific gene primers were used for the sterol response element binding protein-1c (SREBP-1c; forward, 5'-ggagccatggattgcaca -3'; reverse, 5'-aggaaggcttccagagagga-3'); steroyl CoA desaturase -1 (SCD-1; forward, 5'-gtgatgttccagaggaggta-3'; reverse, 5'-ccgagattgaatgttcttgt-3'); medium chain acyl-CoA dehydrogenase (MCAD; forward, 5'-gcaaagaaatcgcagaggtc-3'; reverse, 5'-aagttggccactggattgag- 3'); fatty acid synthase (FAS; forward, 5'-tgcaactgtgcgttagccacc-3'; reverse, 5'-tgtttcaggggagaagagacc-3'); peroxisomal acyl CoA oxidase (AOX; forward, 5'-cacaatcgccatacgataca-3'; reverse, 5'-ctcaggcagttcactcaggt-3'); HMG CoA reductase (HMG-CoAR; forward, 5'-ggtggtgggaaccttct-3'; reverse, 5'-cacgccccttgaacaccta-3'); cholesterol 7α-hydroxylase (CYP7A1; forward, 5'-caagtgtccccctctaga-3'; reverse, 5'-actcaatatcatgtagtggtggcaaa-3'); LDL receptor (LDLR; forward, 5'-caggccgatgcattcctgact-3'; reverse, 5'-agttcatccgagccattttca-3'); GK (forward, 5'-agcagatccacaacatcctaagc-3'; reverse, 5'-tcctgcggagcacatatggc-3'); G6Pase (forward, 5'-gaaggccaagagatggtgtga-3'; reverse, 5'-tgcagctcttgcggtacatg-3'); PEPCK (forward, 5'-cccaggaagtgaggaagtttgt-3'; reverse, 5'-ggagccgtcgcagatgtg-3'); and 18S rRNA (forward, 5'-gtcgtaccactggcattgtg‐3'; reverse, 5'-ctctcagctgtggtggtgaa-3').

10. Statistical analysis

Data are expressed as the means±SEM (standard error of mean). Differences between group means were determined by Student's *t*-test using the Statistical Analysis Systems statistical software package version 9.1 (SAS Institute, Cary, NC, USA). Differences were considered statistically significant at $p<0.05$.

Results and Discussion

1. Food intake, weight gain, and food efficiency ratio

Many studies (Leibowitz et al. 2006; Nam et al. 2015; Levin et al. 1997) have reported a correlation between early weight gain and long-term weight gain on a high fat or energy-rich diet. These studies used body weight gain only as a measure for distinguishing obesity-resistant rats from obesity-prone rats with differential long-term weight gain without reflecting food intake during growth period. In contrast, we distinguished OP and OR rats based on the FER (Fig. 1), which takes into consideration both dietary intake and subsequent body weight gain. Specifically, prepubertal rats were classified based on daily body weight gain and daily food intake during the first 5 days on a HF diet. When beginning an experiment, the FER significantly differed between the OP and OR rats after 5 days on a HF diet because of a higher weight gain per diet intake in the OP rats, but the body weight or diet intake was not different between the OP and OR rats, respectively (Table 1). Changes in body weight between the two groups during the experimental period are shown in Fig. 1 and Table 1. There was no significant difference in initial body weight between the two groups at the beginning of the study. However, they began to diverge after 2 weeks, such that the OP rats became significantly heavier than the OR rats at all subsequent time points (Table 1, Fig. 1, $p<0.05$), and this difference was maintained for up to 10 weeks. Total body weight gain was also markedly higher in OP rats. Despite of marked changes in body weight following the introduction of high fat diet, diet intake was not significantly different except for 2 weeks between the two groups (Table 1). The FER decreased with age in both OP and OR rats (Fig. 1, Table 1), but the OP rats had a higher FER comred with the OR rats, suggesting a difference in the metabolic response to the diet between the two groups (Table 1, Fig. 1).

Table 1. Body weight, food intake and organ weight

Group	OP			OR		
	2 week	6 week	10 week	2 week	6 week	10 week
Initial BW (g)	112.38±3.68[1)]	111.47±4.39	121.76±5.41	110.93±3.74	108.17±2.36	114.64±2.66
Final BW (g)	192.36±5.36	277.68±5.73^{a*}	328.77±17.42$^{a\#}$	181.32±2.93	259.62±3.07b*	299.26±5.52$^{b\#}$
BW gain (g)	79.99±5.24	203.95±24.06^{a*}	219.84±12.42^{a}	70.39±1.91	151.45±2.71b*	186.37±4.24$^{b\#}$
Diet intake (g/day)	11.05±0.14	13.03±0.35*	14.46±0.36$^{a\#}$	11.67±0.27	12.68±0.12	15.08±0.43^{b}
Initial FER	0.528±0.014^{a}	0.539±0.024^{b}	0.572±0.039^{a}	0.391±0.012^{b}	0.369±0.030^{b}	0.389±0.013^{b}
Final FER	0.330±0.022^{a}	0.341±0.022^{b}	0.226±0.009$^{a\#}$	0.305±0.007^{b}	0.290±0.006^{b}	0.205±0.004$^{b\#}$
Liver (g)	7.97±0.30	8.56±0.35	10.12±0.88	8.01±0.35	8.59±0.32	9.22±0.40
Fat (g)	5.79±0.63	13.52±1.77*	23.93±3.00$^{a\#}$	5.14±0.48	10.72±0.85*	17.07±1.40$^{b\#}$
Adiposity (%)	3.00±0.29	4.81±0.56*	6.90±0.53$^{\#}$	2.82±0.24	4.12±0.30*	5.65±0.39$^{\#}$
Kidney (g)	1.77±0.07	1.90±0.07	2.12±0.13	1.74±0.04	2.01±0.06	2.02±0.08$^{\#}$
Brain (g)	1.88±0.04	1.90±0.05	1.95±0.05	1.84±0.04	1.84±0.03	1.88±0.06

[1)] Mean±SEM.
Means with different superscript letters were significantly different between groups on week 2, 6 and 10 at $p<0.05$.
Means with an asterisk (*) were significantly different within the same group compared with the value on 2 weeks at $p<0.05$.
Means with a number sign ($^{\#}$) were significantly different within the same group compared with the value on 6 weeks at $p<0.05$.

2. Fat content

Next, we have measured organ weight and adiposity because OP rats had a significantly higher body weight than OR rats after 2, 6 and 10 weeks on a high fat diet. Liver, kidney, and brain weights did not differ between the groups from 2 weeks through 10 weeks. However, the absolute visceral fat pad weight increased over time through (Table 1, $p<0.05$) and was significantly higher in the OP rats than in the OR rats ($p<0.05$). Adiposity, calculated as the percentage of visceral fat in body weight of the OP rats was respectively higher than the OR rats at 2, 6, and 10 weeks, reflecting the increased body fat content (Table 1). There was no difference in the intake of diets between OP and OR groups, but the increase in body weight and body fat could be attributed to the low metabolic efficiency in the body. It can be inferred that OP rats seem to be possessed a weight regulatory system that either does not sense the energy value of a high fat diet accurately or has a blunted homeostatic response that impairs the ability to defend against weight gain and became obese adult rats.

3. Oral glucose tolerance test

It was demonstrated in this study that OR and OP rats have been shown to be associated with phenotypic differences when fed the same high fat diet. The question to be addressed here is whether these OP rats relative to the OR rats exhibit disturbances that are expected to contribute to their adult obesity and insulin resistance. The OGTT has been widely applied in the evaluation of the pathophysiological progression of diabetes, insulin resistance, impaired β-cell function (Retnakaran et al. 2008). Glucose tolerance was significantly impaired in the OP rats compared with the OR rats after 2, 6, and 10 weeks on the HF diet (Fig. 2). The AUC values at 2, 6, and 10 weeks were also significantly increased in the OP rats compared with the OR rats (Fig. 2, $p<0.05$). These results are indicative of inhibited insulin sensitivity in OP rats compared with OR rats. The OP rats with high body weight and visceral fat have greater glucose intolerance than OR rats with low quantities of them. It is becoming abundantly clear that increase of body weight and accumulation of body fat centrally are associated with glucose intolerance and insulin insensitivity.

4. Plasma levels of glucose, insulin, insulin resistance index, leptin and ghrelin

Insulin resistance can be influenced by factors such as genetics, age, exercise, dietary nutrients, medications and body fat distribution (Olefsky et al. 1973). The development of diet induced obesity is associated with hyperinsulinemia and insulin resistance as well as hyperleptinemia (Levin et al. 2004). We observed these biochemical parameters which are related with glucose intolerance and insulin resistance. The plasma levels of glucose, insulin,

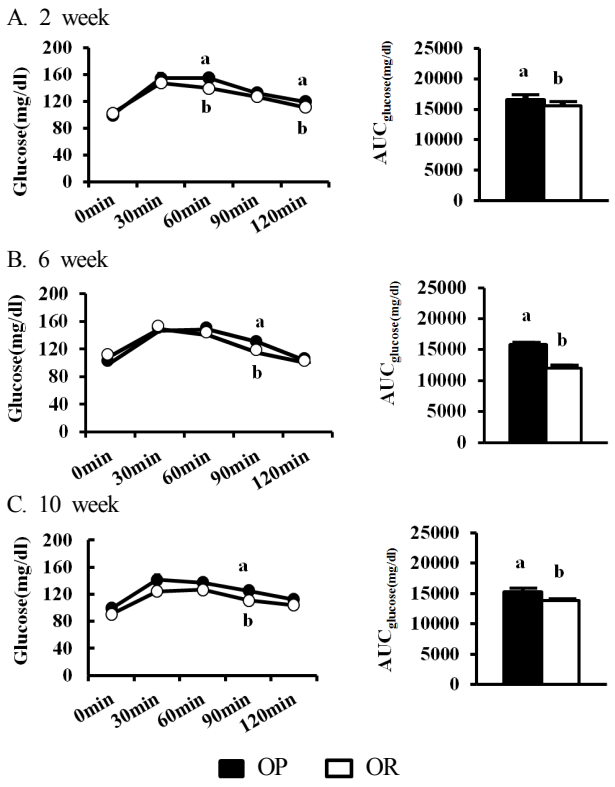
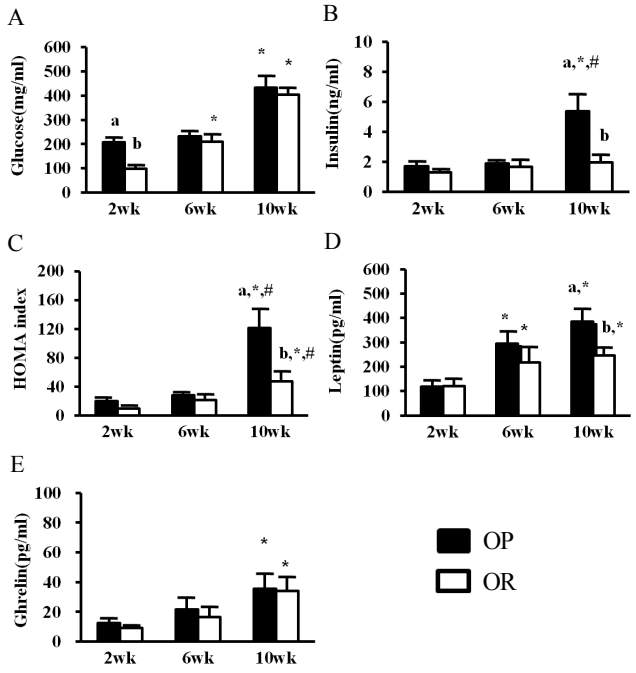


Fig. 2. Glucose tolerance test in OP and OR rats at 2, 6, and 10 weeks. Data are expressed as means±SEM. Means with different letters were significantly different at $p<0.05$.



Fig, 3. The levels of plasma biochemical parameters in OP and OR rats. All data are expressed as means±SEM. Means with different letter was significantly different between groups on week 2, 6 and 10 at $p<0.05$. Means with an asterisk (*) were significantly different within the same group compared with the value on 2 weeks at $p<0.05$. Means with an number sign (#) were significantly different within the same group compared with the value on 6 weeks at $p<0.05$.

leptin, ghrelin and insulin resistance index (HOMA index) increased over time in both of OP and OR rats (Fig. 3). The plasma glucose level was significantly higher in the OP rats compared with the OR rats at 2 weeks ($p<0.05$); although the glucose levels tended to be higher in the OP group at 6 and 10 weeks, the differences were not significant (Fig. 3). At 10 weeks, the plasma insulin level and insulin resistance index (HOMA index) were significantly higher in the OP rats compared with the OR rats (Fig. 3, $p<0.05$). The plasma leptin level tended to increase with fat accumulation in both OP and OR rats (Fig. 3) and was significantly higher in the OP rats compared with the OR rats at 10 weeks (Fig. 3, $p<0.05$). The plasma levels of ghrelin, which is hunger hormone, tended to increase in the OP rats, but not significantly changed (Fig. 3). Overall, OP rats showed insulin resistance as results of the elevated fasting insulin levels and increased HOMA index (Fig. 3) and areas under both their plasma glucose curves after a glucose load (Fig. 2). The increase in adiposity (Table 1) of OP rats on the high fat diet was associated with a clearly abnormal insulin response to glucose. Leptin is also positively correlated with adiposity, and its level may be induced by factors related to insulin and glucose levels (Aas et al. 2009). Consistent with previous studies (Yura et al. 2005; Tulipano et al. 2004; Björnholm et al. 2007), the OP rats became obese and exhibited hyperleptinemia over the course of the present study. This suggests that the release of leptin may be positively correlated with not only increased body fat but also elevated insulin levels and insulin resistance, developing many features of the metabolic syndromes.

5. Glucose-stimulated insulin secretion (GSIS) from isolated islets

GSIS is the principal mechanism of insulin secretion (Wortham & Sander 2016). Since the triggering pathway is responsible for initiating insulin secretion in response to a threshold level of glucose, glucose is the key stimulus for insulin secretion, with the rate of its metabolism by the β-cell determining

the insulin secretory response. In GSIS experiment, after 48 h of culture in a medium containing 5.6 mM glucose, isolated islets were challenged at either a physiological glucose concentration (5.5 mM) or at a high glucose concentration (16.7 mM) for 90 min. An increase from 5.5 to 16.7 mM glucose in the medium caused significantly increased insulin secretion in both OP and OR rats (Fig. 4, $p<0.05$). At both 5.5 and 16.7 mM glucose, insulin secretion was significantly higher in the OP rats compared with the OR rats (Fig. 4, $p<0.05$). In addition, the islets of OP rats secreted more insulin in both the basal state and glucose-stimulation state at 10 weeks, compared with OR rats. These results were consistent with increased plasma insulin levels in OP rats. The increase of insulin secretion is thought to be that one of the defining characteristics of compensating β-cells is a sensitized insulin secretory response to glucose, wherein more insulin is secreted relative to glucose concentration. It was suggest that islets have the ability to adapt to a high fat content in the diet, but tend toward an insulin-resistant state.

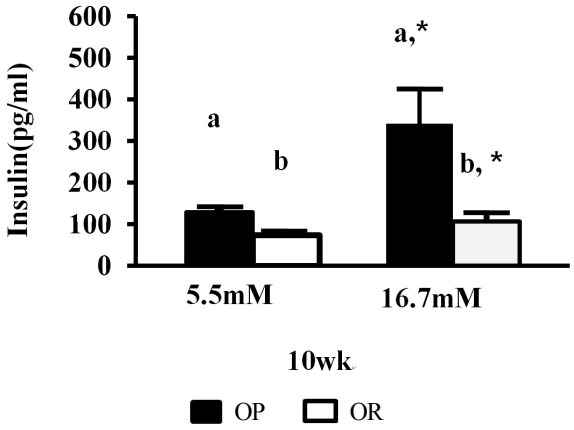


Fig. 4. The levels of glucose stimulated insulin secretion in islets of OP and OR rats. All data are expressed as means± SEM. Means with different letter was significantly different between groups on week 10 at $p<0.05$. Means with an asterisk (*) were significantly different within the same group between 5.5 mM glucose and 16.7 mM glucose after glucose stimulation at $p<0.05$.

6. Plasma and hepatic lipid compositions

Regardless of increased body weight and visceral fat weight, the lipid levels of plasma and liver did not differ among groups except plasma triglyceride. In plasma, the levels of free fatty acids, total cholesterol, HDL-cholesterol, and LDL-cholesterol did not change significantly between the OP and OR rats at 2, 6, and 10 weeks but triglyceride at 10 weeks was significantly increased in OP rats compared to those of OR rats, reflecting somewhat increased body weight and adiposity (Table 2). Hepatic triglyceride (295.36±20.98 vs. 285.80±29.24 mg/g liver) and cholesterol (84.98±8.86 vs. 83.91±5.24 mg/g liver) contents did not differ between the OP and OR rats at 2 and 10 weeks. Although the lipid composition of liver and blood did not change dramatically, the elevated plasma triglyceride ($p<0.05$) are directed toward white adipose tissue and changes occur in adipocyte size, which leads to changes in its function, and an increase in secretion of leptin.

7. Hepatic mRNA expression and activities of glucose metabolizing enzymes

Hepatic glucose metabolizing enzyme activities and gene expression also changed in the OP rats. The GK mRNA level was significantly increased in the OR group compared with the OP group at 10 weeks (Fig. 5A). At 2 and 10 weeks, the GK activity, important in the catabolism of glucose, was significantly decreased in the OP rats (Fig. 5B) and G6Pase and PEPCK activities, important in the production of glucose through gluconeogenesis, were significantly increased in the OP rats compared

Table 2. Plasma biochemical parameters

Group	OP			OR		
	2 week	6 week	10 week	2 week	6 week	10 week
Triglyceride (mg/dL)	36.15±6.56[1)]	41.18±10.68	251.45±49.58[a*#]	37.65±5.29	47.79±8.54	142.65±49.68[b]
Cholesterol (mg/dL)	49.96±3.33	50.17±4.52	77.07±6.84[*#]	45.48±4.29	61.78±5.23[*]	71.02±3.64[*]
HDL-Cholesterol (mg/dL)	14.04±0.86	17.61±2.05	15.67±1.06	11.28±1.65	18.93±2.38[*]	15.03±0.81[*]
Free fatty acid (μEq/L)	463.3±16.0	467.8±65.0	420.4±89.4	524.5±43.6	527.8±43.6	514.7±32.0[*#]

[1)] Mean±SEM.

Means with different superscript letters were significantly different between groups on week 2, 6 and 10 at $p<0.05$.

Means with an asterisk (*) were significantly different within the same group compared with the value on 2 weeks at $p<0.005$.

Means with a number sign (#) were significantly different within the same group compared with the value on 6 weeks at $p<0.05$.

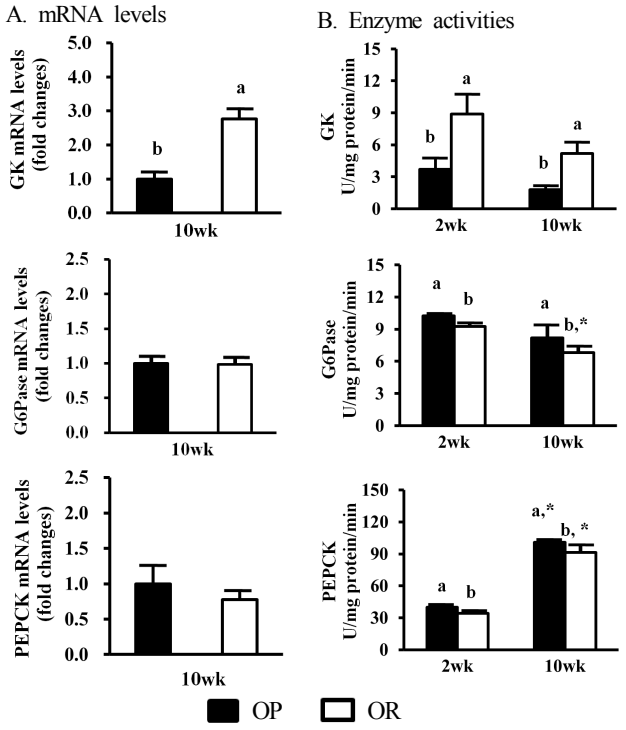


Fig. 5. The mRNA expression and activities of glucose metabolizing enzyme in the liver of OP and OR rats. Data are expressed as means±SEM. Means with different letter was significantly different between groups on week 2 and 10 at $p<0.05$. Means with an asterisk (*) were significantly different within the same group compared with the value on 2 weeks at $p<0.05$. Means with an number sign(#) were significantly different within the same group compared with the value on 6 weeks at $p<0.05$.

with the OR rats (Fig. 5B). The significant decreases in GK activity and gene expression, and the significant increases in G-6-Pase and PEPCK activities were resulted to the increased glucose output through increased gluconeogenesis in the liver and then led to insulin resistance. For all that the significant changes in glucose metabolism-related enzyme activity and mRNA expression, plasma glucose levels did not differ between the two groups. The primary signal for insulin secretion is an elevation in the blood glucose concentration, which induces the release of stored insulin through increased PDX-1 expression (Shao et al. 2009). In contrast, chronic exposure of isolated islets to a high glucose or palmitic acid concentration may reduce PDX-1 and insulin expression (Cerf et al. 2005). These changes appeared to induce improper glucose homeostasis, with over-secretion and decreased clearance of insulin (Kotronen et al. 2008; Shao et al. 2009; Cerf et al. 2005). In this study, plasma insulin levels and glucose-induced insulin secretion were significantly increased in OP rats, meaning that insulin production and secretion were not inhibited. It is thought to be an adaptive mechanism by increased insulin production and secretion in pancreatic β-cells. Tthe elevated circulating insulin level in OP rats also possibly due to the decreased clearance of insulin from liver and whole body insulin resistance.

8. Hepatic gene expression related to lipid metabolism

Biochemical parameters related to obesity and metabolic disorders were significantly different between the OP and OR groups in our study. However, the mRNA levels of genes related to fatty acid synthesis and fatty acid oxidation were not significantly different between the OP and OR groups (Fig. 6). Recent studies have suggested that a reduced capacity for fat oxidation may contribute to a propensity for diet-induced obesity in rats, via accelerated weight gain and increased partitioning of fats into storage (Ji & Friedman 2008: Frihauf et al. 2016). Except plasma triglyceride, we did not observe any significant changes of the levels of cholesterol and free fatty acid in plasma and the lipid deposits and lipid metabolism-related gene expression in liver between the OP and OR rats. Nevertheless, OP rats showed greater fat mass and plasma triglyceride, with fat accumulation becoming more apparent with time on the HF diet.

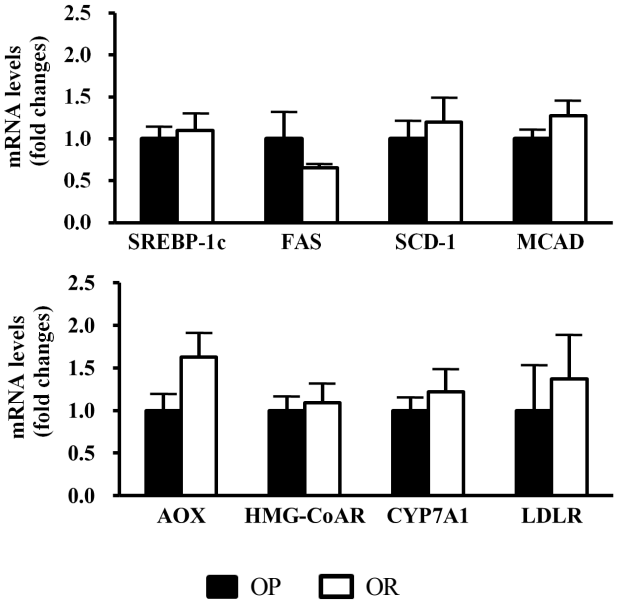


Fig. 6. Hepatic gene expression related to lipid metabolism. Data are expressed as means±SEM. mRNA expression was analyzed by quantitative RT-PCR from hepatic tissues.

Conclusion

Early identification of individuals at risk for becoming overweight or obese may help prevent obesity later in life and may provide long-term health benefits. The aim of this study was to identify early biomarkers detectable prior to the onset of obesity that can predict the propensity for future diet-induced obesity and insulin resistance in obesity-prone and obesity-SD rats fed a chronic HF diet and to validate this screening method. Obesity-prone or obesity-resistant rats are considered among the best animal models of diet-induced obesity and recapitulate many key features of the human condition. We divided OP and OR rats that have been selectively bred on the basis of their food efficiency ratio (FER) reflecting metabolic rate while on a high-calorie diet at either an early age with normal body weights or prior to the onset of obesity and then measured differences in several biochemical parameters between the OP and OR rats, to determine the usefulness of the FER as a forecast of future obesity and metabolic disorders. There was no significant difference in the average body weight or diet intake between the OP and OR rats at the first time they were classified. However, the OP rats showed significantly greater weight gain from week 2 to week 10 on the HF diet, compared with the OR rats. After 2 weeks on the HF diet, the FER still defined the distinction between the OP and OR groups, and this characteristic difference was consistently sustained from week 6 through the end of the experimental period. It has been demonstrated that OP and OR rats have been shown to be associated with phenotypic differences and metabolic changes when fed the same high fat diet and the OP rats showed more obesity-related signs compared with the OR rats. Following the introduction of a high fat diet, OP rats become obese and developing many features of the metabolic syndromes, including glucose intolerance, insulin resistance, hypertriglyceridemia and hyperleptinemia, as evidenced by increases in the insulin level, leptin level, insulin resistance (HOMA index), glucose-stimulated insulin secretion of pancreatic islets, and the activities and mRNA expression of hepatic gluconeogenesis related enzyme. These results indicate that OP rats are more sensitive to the stimulatory effect of dietary fat. Increases in these parameters were found to be correlated with body fat accumulation and body weight gain. The high FER during the prepurbertal period was also closely associated with visceral fat accumulation over time in both OP and OR rats. Therefore, high FER measured during early life would be a potent biomarker, which can predict a propensity for future obesity and insulin resistance. Future studies in obesity-prone rats classified based on the FER should address the prevention of adult-onset obesity by dietary intervention.

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