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Change in Activity of the Sympathetic Nervous System in Diet-Induced Obese Rats

We investigated the change in activity of the sympathetic nervous system (SNS) in high-sucrose diet (HSD)-induced obese rats compared with controls. Power spectral analyses of R-R interval variability were performed to obtain the low frequency (LF, 0.04-0.699 Hz) and high frequency (HF, 0.7-3.0 Hz) powers. The percents of fat mass to body weight (%F/BW) and fat to muscle ratios (F/M) were significantly increased in HSD-fed rats. Plasma glucose, leptin, and triglyceride concentrations in rats fed with HSD were significantly increased. LF in normalized units (LFn), which represents both sympathetic and parasympathetic activities, was significantly increased whereas HF in normalized unit (HFn), which represents parasympathetic activity, was significantly decreased in HSD-fed rats. LF/HF, which represents sympathetic activity, was significantly increased in HSD-fed rats and was correlated with leptin (r=0.549, p<0.023), %F/BW (r=0.513, p<0.035), F/M (r=0.536, p<0.038), and triglyceride (r=0.497, p<0.042). When adjusted for leptin concentrations, however, LF/HF of HSD-fed rats was significantly decreased. In conclusion, HSD-induced obese rats showed increased LF/HF, which was significantly decreased by adjustment for leptin concentrations. We suggest that stimulating effect of leptin on SNS is reduced, which might play a role in induction of obesity by HSD.

Key Words: *Obesity; Sympathetic Nervous System; Spectrum Analysis; Rats*

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INTRODUCTION

Energy balance is regulated by the equilibrium between caloric intake and caloric expenditure. When caloric intake is greater than the expenditure, overweight, even obesity results (1). Energy balance is achieved by interactions between brain and peripheral tissues (1). The brain monitors the nutritional status of the body using peripheral afferent signals such as leptin and/or insulin and reacts to nutritional changes by modulating the activity of its neurohormonal efferent signaling (1-3). Leptin, produced in adipose tissue, is one of the satiety signals informing the brain of the status of nutritional storage. Increased production of leptin reduces food intake and increases resting energy expenditure by activating the sympathetic nervous system (SNS) (2, 4, 5). However, obese subjects with increased mass of adipose tissue also have increased plasma leptin concentrations (6, 7). Leptin is likely to interact with SNS activity and an abnormally high concentration of leptin in obese subjects implicate the aberrant autonomic nervous system (ANS) activity.

SNS is known to regulate energy expenditure (8, 9). Electrical stimulation of ventromedial hypothalamus, a part of SNS, increases metabolic rate by increasing lipid oxidation (10). Indeed, many previous studies have showed the reduction of sympathetic nervous activity in obese animals or humans (11-13). Although the causal relationship between decreased activity of SNS and obesity is not clear, the hypothesis in support of this is that sympathetic activity is decreased to induce obesity by reduction of energy expenditure. A single gene mutant (*ob/ob* and *db/db* mice, Zucker rats), which produces obesity without hyperphagia implicates that one or more components of energy expenditure in this models must be reduced (14, 15).

However, not all studies have provided evidences for reduced sympathetic nervous activity in obese subjects (16). Furthermore, several studies have reported increased activity of SNS. Especially, postmenopausal women with combined upper body obesity and visceral obesity have significantly higher cardiac ANS activities (17). Those who observed the overactivity of SNS have proposed the hypothesis that sympathetic activity is increased to prevent further weight gain.

Power spectral analysis, a non-invasive tool to assess R-R interval variability, has been used to investigate the cardiovascular control by ANS (18, 19). Periodic components of R-R interval variability tend to aggregate within several frequency bands. The high frequency (HF) fluctuation, centered at respiratory frequency, is mediated primarily by cardiac vagal efferent activity. The low frequency (LF) fluctuation reflect both sympathetic and vagal influence. Therefore, LF/HF usually represents sympathetic activity and HF represents parasympathetic activity (18, 19).

There is controversy regarding the changes of SNS in obese subjects, and whatever the changes, aberrant SNS activity might have an influence on development of obesity and vice versa. Therefore the elucidation of the changes of SNS in obese subjects might provide basic knowledge for the understanding of pathophysiology of obesity and its related conditions and treatment. Rats are commonly used laboratory animals but few studies have been done on the changes of SNS activities in obese rats, especially induced by diet.

In this study, we have analyzed R-R interval variability using power spectral analysis in controls and diet-induced obese rats to elucidate the changes in SNS activities.

MATERIALS AND METHODS

Animals and diet

Male Wistar rats weighing 70-80 g were purchased from SLC (Japan) and housed in an animal care unit of College of Medicine, Yeungnam University for one week until the diet regimen started. Food and water were accessed ad libitum. The rats were randomly assigned as control or palatable diet group and were fed with laboratory chow or a high-sucrose diet (HSD) for 10 weeks, respectively. The standard laboratory chow diet provided 67% energy as carbohydrate, 25% as protein, and 9% as fat. Palatable HSD provided 68% energy as carbohydrate, 17% as protein, and 13% as fat. Body weight was measured once a week.

Spectral analysis of heart rate variability

For measuring activities of SNS, we used power spectral analysis of R-R interval variability. Lead I electrocardiogram (ECG) signal was obtained and digitized at 1,000 Hz with a physiologic recorder (Biopac systems, Santa Barbara, CA, U.S.A.) and stored on the disk of a personal computer as a text file. The text file was imported to a script language program written with a software command set (Spike2 version 3, Cambridge Electronic Design Limited, U.K.), which was used for the power spectral analysis of R-R interval variability. ECG was recorded continuously for the 5 min period. R-R interval was detected from peaks of QRS complex in the ECG and then equidistant time series of R-R interval was constructed at 10 Hz by interpolating with a cubic spline function. DC trend was eliminated by subtracting a linear regression equation from the time series. Power spectra of R-R interval variability were obtained by use of a fast Fourier transform (FFT) algorithm. ECG recording for 5 min usually provided a 256-sec block available for the analysis, from which four 102.4 sec segments overlapping by 50% were extracted, smoothed with a raised cosine window, and admitted to FFT to yield a frequency resolution of 0.01 Hz. Power spectral density functions derived from all the segments were averaged to produce the final spectrum, from which very low frequency (VLF) (0-0.039 Hz), LF (0.04-0.699 Hz) and HF (0.7-3 Hz) power were obtained (20). Total powers (σ^2) were calculated as sum of the three frequency bands.

Normalized LF and HF power were calculated as follows.

$$LFn = LF/(\sigma^2\text{-}VLF) \times 100$$

$$HFn = HF/(\sigma^2\text{-}VLF) \times 100$$

Fat and muscle masses and plasma biochemicals

After the monitoring of ECG, the rats were sacrificed by carbon dioxide inhalation and blood samples for insulin, glucose, and leptin analysis were withdrawn by cardiac puncture. Perirenal and epididymal fat masses and soleus and gastrocnemius muscle masses were measured. Plasma insulin and leptin concentrations were measured by radioimmunoassay (Linco Research, St. Charles, MO, U.S.A.) and glucose, triglyceride and free fatty acids were measured by enzymatic colorimetric methods.

Statistics

The results were expressed as mean±SE. The difference between the two groups was examined with Student's t-test. Simple linear regression analysis was used for the assessment of correlation between the factors. All statistical analyses were performed using the SPSS program.

RESULTS

Body weight, fat and muscle masses

Rats fed with HSD had significantly increased body weight by 19% for ten weeks compared with controls

(Table 1). Soleus and gastrocnemius muscles were weighed for lean body mass. While the muscle mass of gastrocnemius was significantly increased in rats with HSD, it was not the case as for the soleus. Overall, the added weights of soleus and gastrocnemius muscles were increased by 10% in rats with HSD. Both perirenal and epididymal fat masses were significantly increased and the added weights of the two were increased by 88% in rats fed with HSD compared with controls. The percents of fat mass to body weight (% F/BW) and fat mass to muscle mass ratio (F/M) were significantly increased in rats fed with HSD by 66% and 67%, respectively (Table 1). Therefore the difference in the body weight between the two groups was mostly attributable to increased fat mass rather than lean body mass.

Table 1. Body weight, muscles, and fat masses in control rats and rats fed with HSD

	Control (n=9)	High-sucrose (n=10)
Initial body weight	102±1.7	100±1.2
Final body weight	419±10.2	476±5.7†
Muscle mass (S+G)	2.1±0.05	2.3±0.07†
Soleus (S)	0.12±0.01	0.13±0.004
Gastrocnemius (G)	1.9±0.05	2.2±0.07*
Fat mass (P+E)	13.9±1.2	26.1±1.8†
Perirenal (P)	6.9±0.99	14.2±1.2†
Epididymal (E)	7.0±0.4	12.0±0.7†
%Fat mass/body weight	3.3±0.2	5.5±0.1†
Fat mass/muscle mass	6.8±0.3	11.3±0.8†

Results are presented as mean±SE
*p<0.05, †p<0.01, †p<0.001 for high-sucrose vs. control

Table 2. Plasma biochemicals in control rats and rats fed with HSD

	Control (n=9)	High-sucrose (n=10)
Glucose (mM)	7.8±0.1	8.8±0.3*
Insulin (ng/mL)	3.0±0.4	3.7±0.2
Free fatty acids (mEq/L)	533±59	671±77
Triglyceride (mg/dL)	81±6.1	206±13.7†
Leptin (ng/mL)	9.6±1.8	21.6±1.5†

Results are presented as mean±SE
*p<0.05, †p<0.001 for high-sucrose vs. control

Plasma biochemicals and their correlation with fat mass

Plasma glucose, leptin, and triglyceride concentrations in rats with HSD were significantly increased by 12%, 125%, and 154%, respectively, compared with controls. There was no significant difference in plasma insulin or free fatty acids concentrations between the two groups (Table 2). Correlation between the two indicators of fat mass (% F/BW and F/M) and various biochemical factors were assessed. %F/BW significantly correlated with leptin, triglyceride, and glucose but not with free fatty acids or insulin. F/M had almost the same correlation coefficient with %F/BW for various biochemicals (Table 3).

Spectral analysis of R-R interval variability

We obtained various data through spectral analysis of R-R variability. Heart rate and R-R interval of control group were 397±6.9 beats/min (bpm) and 151±2.5 msec, respectively, which were not significantly different compared with those of HSD groups. Total and VLF power in HSD group were not significantly different compared with controls. LF and HF in HSD group were not significantly different from those of controls, but when transformed to normalized units, there was a significant difference between the two groups. LF in normalized units (LFn) of HSD group was significantly increased while HFn was significantly decreased compared with controls. LF to HF ratio was significantly increased by 49% in HSD group (Table 4). Correlation analyses were performed between the parameters of R-R interval variability and various factors associated with obesity. LF/HF was significantly associated with leptin, %F/BW, F/M, and triglyceride. Free fatty acid or insulin was not significantly correlated with LF/HF (Table 5). When adjusted for plasma leptin concentrations, however, LF/HF of HSD group was significantly decreased compared with controls (Fig. 1).

DISCUSSION

Body weight of rats fed with HSD for 10 weeks was

Table 3. Simple correlation coefficients for percents of fat mass to body weight (%F/BW) and fat mass to muscle mass ratio (F/M) and the indicative variables in pooled experimental cases in all groups (n=19)

		Glucose	Insulin	Leptin	Free fatty acids	Triglyceride
%F/BW	r	.583	.257	.920	.414	.785
	p<	.018	.320	.000	.078	.000
F/M	r	.582	.207	.912	.305	.771
	p<	.018	.424	.000	.204	000

r: correlation coefficient, p: p-value

Table 4. Spectral analysis of R-R interval variability in control and HSD-induced obese rats

	Control (n=9)	High-sucrose (n=10)
Heart rate (beats/min)	397±6.9	382±9.4
R-R interval (msec)	151±2.5	158±3.9
TP ($msec^2$)	24.6±9.8	16.5±2.5
VLF ($msec^2$)	9.6±5.9	3.5±0.8
LF ($msec^2$)	8.6±2.8	9.0±1.6
LFn	58.5±2.7	67.1±2.8*
HF ($msec^2$)	5.8±1.5	4.0±0.5
HFn	41.6±2.7	32.9±2.8*
LF/HF	1.48±0.2	2.2±0.3*

Results are presented as mean±SE
TP: total power, VLF: very low frequency power, LF: low frequency power, LFn; normalized low frequency power, HF: high frequency power, HFn: normalized high frequency power
*$p<0.05$ for high-sucrose vs. control

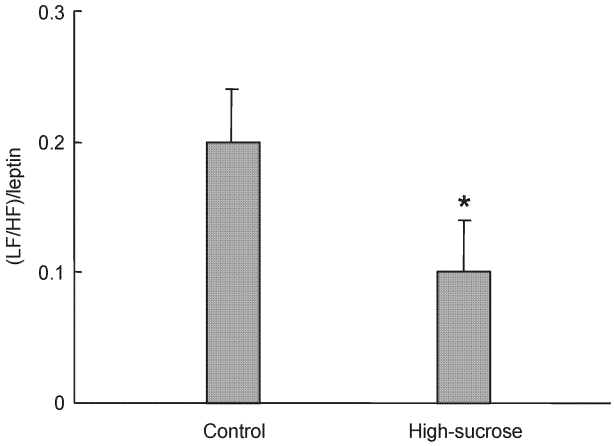

Fig. 1. LF to HF ratio after the adjustment for plasma leptin concentration. LF: low frequency power, HF: high frequency power. *$p<0.05$ for high-sucrose vs. control.

59 g (19%) more than controls. The gain of body weight was accompanied by major increases in the weights of both perirenal and epididymal fat masses by 88% and 71% above controls, respectively, while gastrocnemius muscle mass was increased only by 16% in HSD group compared with controls. Therefore, HSD for 10 weeks in rats produced visceral obesity (accumulation of fat mass in peritoneal cavity), which led to an increase of circulating leptin and triglyceride concentrations (21). Correlation analysis revealed the association between fat mass and these factors. The significant increase of glucose concentration in our results might be caused by elevated plasma triglyceride which is known to increase the hepatic glucose production and triglyceride accumulation in skeletal muscle and pancreas to induce glucose intolerance in obese subjects (22).

Spectral analysis of R-R variability revealed increased sympathetic responsiveness and decreased parasympathetic responsiveness in diet-induced obese rats compared with controls. Spectral analysis showing increased LF/HF ratio, an index of sympatho-vagal balance, also supported the increased function of sympathetic activity in obese rats. These results are in accordance with previous studies. In healthy humans, LF/HF is correlated with body fat mass and waist to hip ratio (23). In postmenopausal women with combined upper body obesity and visceral obesity, significantly higher cardiac sympathetic activities were noted (17). Pima indians also have increased muscle sympathetic activities which is positively correlated with percent body fat (24). Obese animals, such as Wistar fatty rats and fat-fed rats show elevated plasma norepinephrine concentrations (25, 26). In addition, diminished cardiovascular responsiveness to vagal stimulation in obese rats was also reported (27).

The mechanisms responsible for the increased SNS activity in obese subjects are not clear yet, but several physiological factors associated with obesity and its complications are known to regulate the SNS activity. Obesity is associated with hyperinsulinemia and it is known that insulin infusion can induce the increase of sympathetic activities (28, 29). Furthermore, leptin, an adipocyte-derived hormone, also increases sympathetic activities. According to the previous studies, direct injection of leptin into ventromedial hypothalamus, which regulates sympathetic activities, increases both norepinephrine and epinephrine concentrations in a dose-dependent manner (30). Thus, increased leptin in our study could be responsible for elevated sympathetic activity. Moreover, leptin showed a positive correlation with LF/HF. It is possible that increased leptin might cause increased sympathetic activities through ventromedial hypothalamus (1-3). Sympathetic hyperactivity

Table 5. Simple correlation coefficients for LF to HF ratio and the indicative variables in pooled experimental cases in all groups (n=19)

		Leptin	%F/BW	F/M	Triglyceride	FFA	Insulin
LF/HF	r	.549	.513	.536	.497	.420	.327
	p<	.023	.035	.038	.042	.093	.235

LF: low frequency power, HF: high frequency power, %F/BW: percents of fat mass to body weight, F/M: fat mass to muscle mass ratio, FFA: free fatty acid, r: correlation coefficient, *p*: *p*-value

may be accompanied by a reciprocal inhibition of parasympathetic activity.

Increased leptin was supposed to decrease food intake and delay or stop the increase of fat mass (5). However, according to our result the difference of body weight between controls and diet-induced obese rats increased over time. In order to determine the function of leptin to increase sympathetic activity, we adjusted LF/HF for plasma leptin concentration and found that the adjusted LF/HF was significantly lower in the obese rats. These results imply the development of leptin resistance in obese rats in this model. In line with this notion are the previous studies which showed decreased cardiovascular responses to intracerebroventricular leptin in high fat-fed obese rats compared with controls (31).

The reasons for controversy over the changes of the SNS activities is not clear with current study, but we speculate that the severity of leptin resistance might be the answer. Further studies regarding pattern of changes of the sympathetic activities and leptin resistance which depend on gaining weight with time might solve this controversy.

In conclusion, HSD-induced obese rats showed increased LF/HF, suggesting enhanced cardiac sympathetic activity. However when adjusted for leptin concentration, sympathetic activity is significantly decreased in obese rats. We suggest that stimulating effect of leptin on sympathetic activity is reduced, which might play a role in induction of obesity by HSD.

REFERENCES

1. Tataranni PA. *From physiology to neuroendocrinology: a reappraisal of risk factors of body weight gain in humans. Diabetes Metab 1998; 24: 108-15.*
2. Bray GA, York DA. *The MONA LISA hypothesis in the time of leptin. Recent Prog Horm Res 1998; 53: 95-117.*
3. Friedman JM. *Leptin, leptin receptors, and the control of body weight. Nutr Rev 1998; 56(2 Pt 2): s38-46; discussion s54-75.*
4. Dunbar JC, Hu Y, Lu H. *Intracerebroventricular leptin increases lumbar and renal sympathetic nerve activity and blood pressure in normal rats. Diabetes 1997; 46: 2040-3.*
5. Tang-Christensen M, Havel PJ, Jacobs RR, Larsen PJ, Cameron JL. *Central administration of leptin inhibits food intake and activates the sympathetic nervous system in Rhesus macaques. J Clin Endocrinol Metab 1999; 84: 711-7.*
6. Buettner R, Newgard CB, Rhodes CJ, O'Doherty RM. *Correction of diet-induced hyperglycemia, hyperinsulinemia, and skeletal muscle insulin resistance by moderate hyperleptinemia. Am J Physiol Endocrinol Metab 2000; 278: E563-9.*
7. Suga A, Hirano T, Inoue S, Tsuji M, Osaka T, Namba Y, Miura M, Adachi M. *Plasma leptin levels and triglyceride secretion rates in VMH-lesioned obese rats: a role of adiposity. Am J Physiol 1999; 276: E650-7.*
8. Bray GA. *Sympathetic nervous system, adrenergic receptors, and obesity. J Lab Clin Med 1999; 134: 4-6.*
9. Flechtner-Mors M, Ditschuneit HH, Yip I, Adler G. *Sympathetic modulation of lipolysis in subcutaneous adipose tissue: effects of gender and energy restriction. J Lab Clin Med 1999; 134: 33-41.*
10. Ruffin M, Nicolaidis S. *Electrical stimulation of the ventromedial hypothalamus enhances both fat utilization and metabolic rate that precede and parallel the inhibition of feeding behavior. Brain Res 1999; 846: 23-9.*
11. Matsumoto T, Miyawaki T, Ue H, Kanda T, Zenji C, Moritani T. *Autonomic responsiveness to acute cold exposure in obese and non-obese young women. Int J Obes Relat Metab Disord 1999; 23: 793-800.*
12. Pacak K, McCarty R, Palkovits M, Cizza G, Kopin IJ, Goldstein DS, Chrousos GP. *Decreased central and peripheral catecholaminergic activation in obese Zucker rats. Endocrinology 1995; 136: 4360-7.*
13. Piccirillo G, Vetta F, Fimognari FL, Ronzoni S, Lama J, Cacciafesta M, Marigliano V. *Power spectral analysis of heart rate variability in obese subjects: evidence of decreased cardiac sympathetic responsiveness. Int J Obes Relat Metab Disord 1996; 20: 825-9.*
14. Blouquit MF, Geloen A, Koubi H, Edwards D, Gripois D. *Decreased norepinephrine turnover rate in the brown adipose tissue of pre-obese fa/fa Zucker rats. J Dev Physiol 1993; 19: 247-51.*
15. Trayhurn P. *The development of obesity in animals: the role of genetic susceptibility. Clin Endocrinol Metab 1984; 13: 451-74.*
16. Young JB, Macdonald IA. *Sympathoadrenal activity in human obesity: heterogeneity of findings since 1980. Int J Obes Relat Metab Disord 1992; 16: 959-67.*
17. Gao YY, Lovejoy JC, Sparti A, Bray GA, Keys LK, Partington C. *Autonomic activity assessed by heart rate spectral analysis varies with fat distribution in obese women. Obes Res 1996; 4: 55-63.*
18. Kuwahara M, Yayou K, Ishii K, Hashimoto S, Tsubone H, Sugano S. *Power spectral analysis of heart rate variability as a new method for assessing autonomic activity in the rat. J Electrocardiol 1994; 27: 333-7.*
19. Piccirillo G, Vetta F, Viola E, Santagada E, Ronzoni S, Cacciafesta M, Marigliano V. *Heart rate and blood pressure variability in obese normotensive subjects. Int J Obes Relat Metab Disord 1998; 22: 741-50.*
20. Wada T, Ono K, Hadama T, Uchida Y, Shimada T, Arita M. *Detection of acute cardiac rejection by analysis of heart rate variability in heterotopically transplanted rats. J Heart Lung Transplant 1999; 18: 499-509.*
21. Banerji MA, Faridi N, Atluri R, Chaiken RL, Lebovitz HE. *Body composition, visceral fat, leptin, and insulin resistance in Asian Indian men. J Clin Endocrinol Metab 1999; 84: 137-44.*

22. Gower BA, Nagy TR, Goran MI. *Visceral fat, insulin sensitivity, and lipid in prepubertal children. Diabetes 1999; 48: 1515-21.*
23. Paolisso G, Manzella D, Ferrara N, Gambardella A, Abete P, Tagliamonte MR, De Lucia D, Furgi G, Picone C, Gentile S, Rengo F, Varricchio M. *Glucose ingestion affects cardiac ANS in healthy subjects with different amounts of body fat. Am J Physiol 1997; 273: E471-8.*
24. Tataranni PA, Cizza G, Snitker S, Gucciardo F, Lotsikas A, Chrousos GP, Ravussin E. *Hypothalamic-pituitary-adrenal axis and sympathetic nervous system activities in Pima Indians and Caucasians. Metabolism 1999; 48: 395-9.*
25. Kaufman LN, Peterson MM, Smith SM. *Hypertension and sympathetic hyperactivity induced in rats by high-fat or glucose diets. Am J Physiol 1991; 260: E95-100.*
26. Yamakawa T, Tanaka S, Tamura K, Isoda F, Ukawa K, Yamakura Y, Takanashi Y, Kiuchi Y, Umemura S, Ishiiu M, Sekihara H. *Wistar fatty rat is obese and spontaneously hypertensive. Hypertension 1995; 25: 146-50.*
27. Bunag RD, Krizsan D, Itoh H. *Diminished cardiovascular responsiveness to vagal stimulation in obese rats. Am J Physiol 1990; 259: R842-8.*
28. Rappaport EB, Young JB, Landsberg L. *Effects of 2-deoxy-D-glucose on the cardiac sympathetic nerves and the adrenal medulla in the rat: further evidence for a dissociation of sympathetic nervous system and adrenal medullary responses. Endocrinology 1982; 110: 650-6.*
29. Vollenweider P, Tappy L, Randin D, Schneiter P, Jequier E, Nicod P, Scherrer U. *Differential effects of hyperinsulinemia and carbohydrate metabolism on sympathetic nerve activity and muscle blood flow in humans. J Clin Invest 1993; 92: 147-54.*
30. Satoh N, Ogawa Y, Katsuura G, Numata Y, Tsuji T, Hayase M, Ebihara K, Masuzaki H, Hosoda K, Yoshimasa Y, Nakao K. *Sympathetic activation of leptin via the ventromedial hypothalamus: leptin-induced increase in catecholamine secretion. Diabetes 1999; 48: 1787-93.*
31. Lu H, Duanmu Z, Houck C, Jen KL, Buison A, Dunbar JC. *Obesity due to high fat diet decreases the sympathetic nervous and cardiovascular responses to intracerebroventricular leptin in rats. Brain Res Bull 1998; 47: 331-5.*