Insulin and Glucose Transporter Gene Expression in Obesity and Diabetes (43420)

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Abstract. **In order to determine the role of insulin and glucose transporter gene expression in the development of diabetes in obesity, we examined insulin and GLUT2-liver type and GLUT4-muscle-fat type glucose transporter mRNA levels in obese and diabetic rats. Ventromedial hypothalamus-lesioned (VMH), Zucker fatty (ZF), and Wistar fatty (WF) rats were used as models. VMH and ZF rats are most frequently used as models for simple obesity. In contrast, WF rats, which have been established by transferring the *fa* gene of ZF rats to Wistar Kyoto rats, develop both obesity and diabetes. Pancreatic insulin content of VMH rats at 10 weeks after the operation and of ZF rats at 5 and 14 weeks of age was significantly higher than that of controls. On the other hand, insulin content of WF rats at 5 and 14 weeks of age was not significantly different from that of lean littermates. The insulin mRNA levels of VMH rats were increased progressively and were significantly higher than those in sham-operated animals at 4 and 10 weeks after the operation. In ZF rats, the insulin mRNA levels at 5 and 14 weeks of age were significantly higher than those of their lean littermates. In WF rats, by contrast, the insulin mRNA levels were similar to those of lean littermates at 5 and 14 weeks of age. The insulin mRNA levels of WF rats were about 40% of that of ZF rats at 14 weeks of age. On the other hand, at 14 weeks of age, the GLUT2 mRNA levels of liver were significantly higher in ZF and WF rats than those in their respective littermates, but not at 5 weeks of age. The GLUT4 mRNA levels of skeletal muscle in both ZF and WF rats were not significantly different from those of controls. It is suggested that the inability of WF rats to augment insulin gene expression in response to a large demand for insulin is associated with the occurrence of diabetes, and that the activation of GLUT2 mRNA without the activation of GLUT4 mRNA is common to obesity with and without diabetes.** [P.S.E.B.M. 1992, Vol 200]

The maintenance of normal glucose homeostasis depends on three physiological processes that must be coodinated; insulin secretion, stimulation of glucose uptake by splanchnic and peripheral tissues, and suppression of hepatic glucose production. Thus, defects at the level of the β cell, muscle, or liver can lead to the development of diabetes mellitus (1).

Obesity acts as a diabetogenic factor by increasing the insulin requirement, due to insulin resistance of peripheral tissues, but the primary factors in provoking diabetes in obesity have not yet been clarified. In order to determine the role of insulin and glucose transporter gene expression in the development of diabetes in obesity, we have examined insulin, GLUT2, and GLUT4 mRNA levels in obese and diabetic rats.

Ventromedial hypothalamus-lesioned (VMH), Zucker fatty (ZF), and Wistar fatty (WF) rats were used as models. VMH and ZF rats are most frequently used as models for simple obesity (2, 3). In contrast, WF rats, which have been established by transferring the *fa* gene of ZF rats to Wistar Kyoto rats, develop both obesity and diabetes (4).

Methods

Animals. *VMH rats.* Female Wistar rats aged 8–9 weeks were used. Electrolytic lesions of the bilateral ventromedial hypothalamus nuclei were performed by the method of Ishikawa and Shimazu (5) with slight modifications, as reported previously (6). Sham-operated animals were used as controls.

ZF and WF rats. Male ZF rats, male WF rats, and their lean littermates at 5 and 14 weeks of age were used.

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Procedure. All animals were given laboratory chow *ad libitum*. Under ethyl ether anesthesia, blood samples were obtained from the inferior vena cava, and the pancreas, liver, and gastrocnemius muscle were removed. Total RNA was extracted from these tissues by the method of Chirgwin *et al.* (7). Insulin, GLUT2, and GLUT4 mRNA levels were examined by RNA blotting analysis. The probes utilized were rat preproinsulin I, rat GLUT2, and human GLUT4 cDNA (8–10). Extraction of pancreatic insulin was performed by the method of Grodsky and Forsham (11).

Statistics. Statistical analysis was performed using Duncan's multiple range test.

Results and Discussion

Body Weight, Plasma Glucose, and Insulin Levels. In VMH rats at 1, 4, and 10 weeks after operation and in ZF and WF rats at 5 and 14 weeks of age, body weight was significantly higher than that in the respective controls (Table I).

Plasma glucose levels of VMH and ZF rats were not significantly higher than those of respective controls throughout our experiment. On the other hand, plasma glucose levels of WF rats were significantly higher than those of lean littermates at 14 weeks of age, while the levels were not significantly different at 5 weeks of age.

Plasma insulin levels of VMH, ZF, and WF rats were significantly higher than those of the respective controls throughout our experiment, and showed a progressive increase with age.

Pancreatic Insulin Content and mRNA Levels. Pancreatic insulin content of VMH rats was not significantly different from that of sham-operated animals at 1 and 4 weeks after the operation, but became significantly higher at 10 weeks after the operation (Table II). In ZF rats at 5 and 14 weeks of age, pancreatic insulin content was significantly higher than that in lean littermates. On the other hand, pancreatic insulin content of WF rats at 5 and 14 weeks of age was not significantly different from that of lean littermates.

The insulin mRNA levels of VMH rats were not significantly different from those of sham-operated animals at 1 week after the operation, but increased progressively and significantly at 4 and 10 weeks after the operation. In both ZF rats and lean littermates, the insulin mRNA levels were increased with age, while the increase was more remarkable in the fatty animals. In contrast, the insulin mRNA levels were not significantly different between WF rats and lean littermates at 5 and 14 weeks of age. In addition, insulin mRNA levels of WF rats were significantly lower than those of ZF rats at 5 and 14 weeks of age.

Thus, the increased insulin mRNA levels associated with hyperinsulinemia in VMH and ZF rats suggests that activation of insulin gene expression to adapt to a large demand for insulin is common to simple obesity. In contrast, the inability to augment insulin mRNA levels to cope with increased insulin demand in WF rats seems to result in insufficient synthesis of

Table I. Body Weight, Plasma Glucose, and Insulin Levels of VMH, Zucker Fatty, and Wistar Fatty Rats and Their Controls in Fed State[a]

Group	Body wt (g)	Plasma glucose (mg/dl)	Plasma insulin (ng/ml)
VMH (wk)			
1	257 ± 7[b]	142 ± 16	2.9 ± 0.2[b]
4	358 ± 12[b]	146 ± 8	4.6 ± 0.5[b]
10	433 ± 22[b]	160 ± 13	12.2 ± 2.1[b]
Sham operated (wk)			
1	190 ± 4	141 ± 15	1.6 ± 0.2
4	202 ± 8	145 ± 9	2.3 ± 0.4
10	228 ± 8	148 ± 8	2.1 ± 0.1
Zucker fatty (wk)			
5	108 ± 7[c]	167 ± 12	3.2 ± 0.7[b]
14	497 ± 15[b]	175 ± 13	12.4 ± 1.7[b]
Zucker lean (wk)			
5	98 ± 6	158 ± 13	0.9 ± 0.2
14	322 ± 15	165 ± 14	1.3 ± 0.2
Wistar fatty (wk)			
5	114 ± 9[c]	149 ± 25	3.3 ± 0.6[b]
14	527 ± 15[b]	318 ± 25[b]	8.7 ± 1.0[b]
Wistar lean (wk)			
5	98 ± 7	144 ± 27	0.9 ± 0.4
14	360 ± 15	171 ± 16	2.2 ± 0.6

[a] Mean ± SD.
[b] $P < 0.001$, compared with the controls at corresponding weeks after the operation or age.
[c] $P < 0.05$, compared with the controls at corresponding weeks after the operation or age.

Table II. Pancreatic Weight, Insulin Content, and mRNA Levels of VMH, Zucker Fatty, and Wistar Fatty Rats and Their Controls[a]

Group	Pancreatic wt (g)	Insulin content (μg/g)	mRNA Levels (%)
VMH (wk)			
1	0.76 ± 0.13	60.8 ± 5.2	103 ± 5
4	0.80 ± 0.17	85.4 ± 6.4	174 ± 16[b]
10	1.11 ± 0.16[c]	141.5 ± 13.7[b]	291 ± 25[b]
Sham operated (wk)			
1	0.76 ± 0.15	65.4 ± 7.5	100 ± 8
4	0.75 ± 0.08	75.5 ± 9.7	96 ± 6
10	0.78 ± 0.12	73.1 ± 7.8	91 ± 3
Zucker fatty (wk)			
5	0.44 ± 0.06	75.0 ± 4.4[b]	163 ± 21[b]
14	1.13 ± 0.06	141.3 ± 8.5[b]	430 ± 29[b]
Zucker lean (wk)			
5	0.43 ± 0.08	44.5 ± 3.5	100 ± 6
14	1.06 ± 0.08	66.0 ± 9.1	149 ± 9
Wistar fatty (wk)			
5	0.40 ± 0.06	46.0 ± 4.1	121 ± 13
14	0.98 ± 0.23	65.1 ± 8.1	176 ± 24
Wistar lean (wk)			
5	0.35 ± 0.06	40.7 ± 5.2	110 ± 10
14	1.22 ± 0.17	61.1 ± 5.0	160 ± 25

[a] Mean ± SD. The mRNA levels were expressed by relative abundance. The mean value of sham-operated animals at 1 week after the operation was used as a control in the VMH group, and the mean value of Zucker lean rats at 5 weeks of age was used as a control in the Zucker fatty and Wistar fatty groups; the control value was set at 100%.
[b] $P < 0.001$, compared with the controls at corresponding weeks postoperation or age.
[c] $P < 0.01$, compared with the controls at corresponding weeks after the operation or age.

Table III. Quantification of GLUT2 and GLUT4 mRNA Levels of Zucker Fatty and Wistar Fatty Rats[a]

Group	GLUT2 (%)	GLUT4 (%)
Zucker fatty (wk)		
5	90 ± 5	106 ± 12
14	182 ± 15[b]	125 ± 17
Wistar fatty (wk)		
5	124 ± 23	108 ± 12
14	263 ± 10[b]	90 ± 9

[a] Expression of GLUT2 and GLUT4 mRNA relative to the respective controls. Mean ± SD.
[b] $P < 0.001$, compared with the controls at corresponding age.

insulin and leads to the occurrence of diabetes (Table II).

GLUT2 and GLUT4 mRNA Levels. The GLUT2 mRNA levels of liver in ZF and WF rats were significantly higher than those in lean littermates at 14 weeks of age, while the levels were not significantly different at 5 weeks of age (Table III). On the other hand, the GLUT4 mRNA levels of skeletal muscle in ZF and WF rats were not significantly different from those in lean littermates throughout our experiment. These findings indicate that the increased GLUT2 mRNA levels in the liver are common to obese and obese diabetic animals, although the mechanism of the increase is still unclear at present. On the other hand, the GLUT4 mRNA levels in muscle in these animals showed no significant difference from those in their respective controls. It seems likely, therefore, that GLUT4 mRNA levels in some kinds of muscle (e.g., gastrocnemius) are not altered, although simple obesity and obesity with diabetes display peripheral insulin resistance, indicating that insulin may regulate glucose transport at a post-transcriptional stage, such as translation, translocation, or activation of this carrier protein in chronic obese and diabetic state.

Conclusion

A primary defect in the β cell may play an important role in the occurrence of diabetes in obesity, since an inability to activate insulin gene expression to meet a large insulin demand in WF rats is associated with the occurrence of diabetes.

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