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Relationship between insulin sensitivity index and cognitive function in diet-induced insulin resistant rats

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Received September 6, 2008, accepted October 12, 2008

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Pharmazie 64: 410–414 (2009) *doi: 10.1691/ph.2009.8733*

Clinical and animal studies have revealed significant cognitive impairment in type II diabetic subjects. However, whether there is a relationship between insulin resistance and cognitive function is poorly understood. In the present study, we used a high fat diet to induce insulin resistance (IR) in rats, insulin sensitivity index (ISI) (= FINS × FPG/22.5) to assess the extent of insulin resistance and the Morris Water Maze Task to judge cognitive function. The relationship between insulin sensitivity index and cognitive function was determined by analysing the correlation between ISI and the time rat spent in targeted quadrant, as well as between ISI and the times the rat swam across the very point where a platform was previously placed, using Pearson's method. Perfect negative correlation between ISI and cognitive function existed when ISI fell within a certain range, which indicates that insulin resistance is associated with cognitive function impairment in some cases where ISI might be an indicator.

1. Introduction

Alzheimer's disease (AD) and type 2 diabetes mellitus (DM) are two of the most common and devastating health problems in the elderly. They share a number of common features (Halter 1996). Epidemiological studies on cognitive impairment in patients with DM found evidence of cross-sectional and prospective associations between type 2 DM and moderate cognitive impairment, involving memory and executive functions (Cosway et al. 2001; Strachan 1997; Miles et al. 1922). The Zutphen Study of community dwelling elderly people in the Netherlands found that diabetes and impaired glucose tolerance in non-diabetic subjects were associated significantly with poorer cognitive functions (Kalmijn et al. 1995). A 4-year prospective study also showed that older women with impaired fasting glucose levels performed more poorly on cognitive tests than those with normal glycaemia (Yaffe et al. 2004). Both prospective and cross-sectional studies suggest that type 2 DM is associated with an increased risk of both AD and vascular dementia (Pasquier et al. 2006).

Insulin resistance is the condition in which normal amounts of insulin are inadequate to produce a normal insulin response from fat, muscle and liver cells, while ISI is a key index indicating the degree of insulin resistance (Li et al. 1993), and is often expressed as FINS × FPG/22.5. Peripheral insulin resistance has been hypothesized as mediating the observed clustering of vascular risk factors such as type 2 DM, hypertension, and obesity (Reaven 1988). Findings from the Kuopio population study which found hyper-insulinaemia to be associated with recent onset AD in non-diabetic subjects (Kuusisto et al. 1997) support this hypothesis. Recently studies also suggest that long term consumption of sucrose-sweetened water (or consequent increase of caloric intake) causes more weight gain, induces insulin resistance, and exacerbates AD-like cognitive impairment and cerebral amyloid deposition independent of dietary fat intake in rats (Cao et al. 2007).

Even though insulin resistance has emerged as an enormous health care problem, involving the fields of obesity, diabetes, hypertension, and cardiovascular diseases (Olefsky 1990; Reaven 1998), as well as being one of the underlying mechanisms for the effects of high fat diets on AD (Ho et al. 2004), no existing study has shown the potential relationship between insulin resistance and cognitive function. Therefore, we used a high fat diet to induce insulin resistance (IR) in rats, insulin sensitivity index (ISI) (= FINS × FPG/22.5) to assess the degree of insulin resistance and the Morris Water Maze Task to judge their cognitive function. The relationship between insulin resistance and cognitive function was determined by analysing the correlation between ISI and the time the rat spent in the targeted quadrant, as well as between ISI and the times the rat swam across the exact point using Pearson's method.

2. Investigations and results

2.1. Intake of high fat diet led to increased body weight, glucose and insulin intolerance in rats

During the modeling process especially after the second week, the average body weight of the model group was significantly higher than that of the control group ($P < 0.01$) (Fig. 1a), showing potential insulin resistance appearing in the model rats.

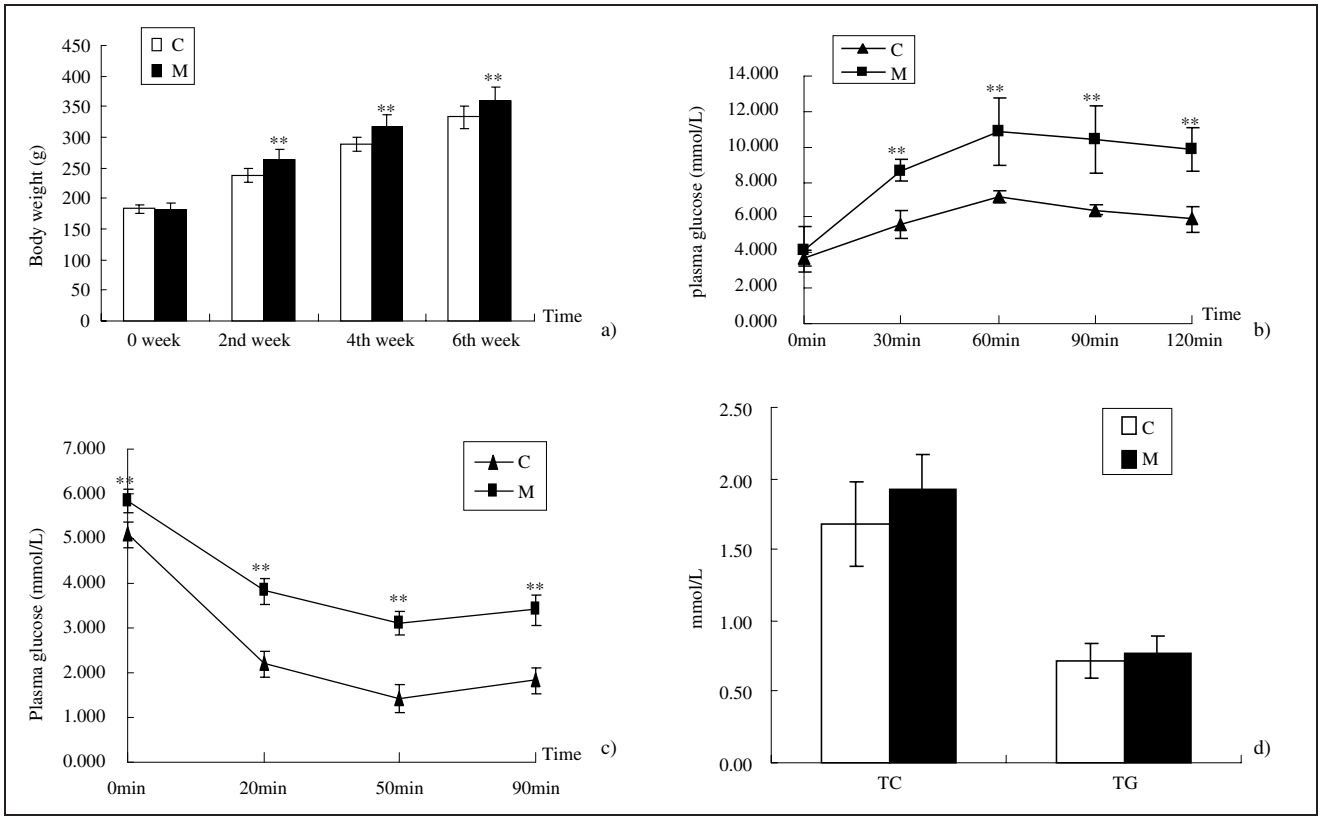

Fig. 1: a) Changes of body weight in insulin resistant (IR) rats. Each rat was weighed every two weeks from the beginning of the experiment. The model group was fed with high fat food, while the control group was fed with basic foodstuff. Data represent means ± SD; n = 15 per group; **, $p < 0.01$ vs. control group. b) Oral glucose tolerance response. Plasma glucose was monitored over time following oral administration of glucose (2 mg/kg body weight) to fasted rats. The high fat group showed impaired glucose tolerance compared with the control group. Data represent means ± SD; n = 6 per group; **, $p < 0.01$ vs. control group. c) Insulin tolerance response. Plasma glucose was monitored over time following intraperitoneal injection of Insulin (0.75 U/kg body weight) (2 mg/kg body weight) to fasted rats. Data represent means ± SD; n = 7 per group. **, $p < 0.01$ vs. control group. d) Fasting plasma lipid levels. Blood lipid in rats was measured in the 6th week of the experiment, and evaluated in terms of total cholesterol and total triglyceride. Data represent means ± SD; n = 6 per group

For OGTT, there were no differences between groups in the means of plasma glucose recorded before testing ($P > 0.05$). During the process, the model rats' plasma glucose recorded at 30 min, 60 min, 90 min, and 120 min, was significantly higher than that of the control group ($P < 0.01$) (Fig. 1b). The area under the curve (AUC) in model group (1106.74 ± 47.27 units, $n = 6$) was larger than that of control group (720.20 ± 125.99 units, $n = 6$).
For ITT, during the process, the model rats' plasma glucose recorded at 0 min, 20 min, 50 min, and 90 min was significantly higher than that of the control group ($P < 0.01$) (Fig. 1c). Combined with the result drawn from Fig. 1c that the AUC of the model group (330.41 ± 16.08 units, $n = 7$) was larger than that of the control group (191.43 ± 12.70 units, $n = 7$), both showed the appearance of insulin resistance in model rats.
Blood lipid analysis showed that there were no differences between the groups in terms of total cholesterol (TC) and total triglycerides (TG) ($P > 0.05$) (Fig. 1d).

2.2. Insulin sensitivity index (ISI)

A significant difference in ISI between the two groups existed at the 7th week following induction of high fat food in rats, indicating the high level of insulin resistance of the rats in the model group (Table 1).

2.3. Cognitive function impairment by Morris Water Maze Task

A significant difference in the time rats spent in the targeted quadrant between the two groups ($P < 0.05$), indicated possible cognitive function impairment in the model rats, though a comparison between the two groups in terms of the times that rats crossed the point where the platform was originally placed showed no significant difference ($P > 0.05$).

2.4. Correlation between ISI and results obtained from Morris Water Maze Task

Perfect negative correlations between ISI and the time the rat spent in the targeted quadrant, as well as between ISI and the times rat swam across the exact point where a platform was previously placed were found in the model group (Fig. 2c, Fig. 2d) but not in the control group (Fig. 2a, Fig. 2b), which indicated that a negative correlation between ISI and cognitive function existed when the ISI fell within a certain range.

3. Discussion

Though previous research has suggested that insulin resistance in skeletal muscle can promote atherogenic dyslipidemia by changing the pattern of ingested carbohydrate away from skeletal muscle glycogen synthesis into hepatic *de novo* lipogenesis, resulting in an increase in plasma triglyceride concentrations (Petersen et al. 2007), in our experiment total triglyceride levels showed no significant difference between the control and model groups (Fig. 1d). This may be because ours was a short term experiment which lasted only around 7 weeks compared to that mentioned

Table 1: Levels of serum glucose, serum insulin and insulin sensitivity index (ISI) in insulin resistant rats

Items	Group	n	7th week	9th week	11th week
FINS (μIU/ml)	Control	6	12.66 ± 2.66	11.23 ± 3.32	11.50±3.60
	Model	6	26.62 ± 4.56	25.42 ± 4.01	24.53 ± 4.15
FPG (mmol/L)	Control	6	4.35 ± 0.35	5.28 ± 0.20	5.41 ± 0.24
	Model	6	4.84 ± 0.35	5.46 ± 0.21	5.64 ± 0.23
ISI	Control	6	2.41 ± 0.33	2.65 ± 0.85	2.77 ± 0.92
	Model	6	5.71 ± 1.02**	6.17 ± 1.01**	6.15 ± 1.13**

The ISI of each rat in the two groups was calculated according to the formula ISI = FINS × FPG/22.5 in the 7th, 9th, and 11th week during the experiment. Data represent means ± SD; n = 6 per group; **, $p < 0.01$ vs. control group

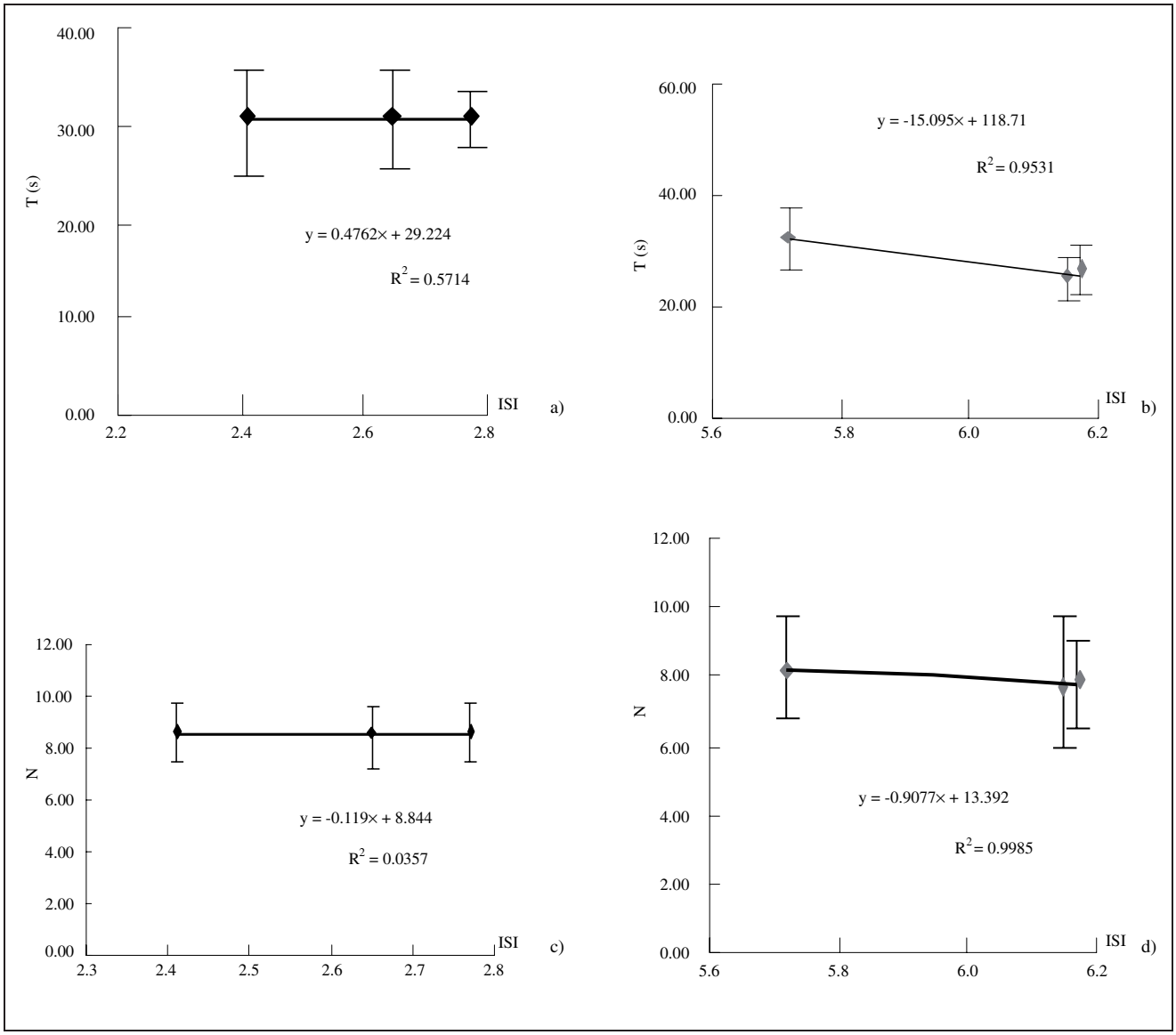

Fig. 2: Correlation between T and ISI in control group.
a) $Y = 0.4762x + 29.224$, $R^2 = 0.5714$ b) $Y = -15.095x + 118.71$, $R^2 = 0.9531$.
Correlation between N and ISI in control group.
c) $Y = -0.119x + 8.844$, $R^2 = 0.0357$ d) $Y = -0.9077x + 13.392$, $R^2 = 0.9985$

above whose duration was as long as two years, and, at this point, we conclude that the effect of insulin resistance on blood lipids may be delayed for a while. But this would not influence the study in our experiment, as the insulin sensitivity index clearly showed the existence of insulin resistance.

Granted that possible interference factors within the Morris Water Maze task, such as people being around during testing, may exert a small effect on the credibility of the results, parameters such as the time the rat spent within the targeted quadrant and the number of times it crossed the point where platform was originally placed may be considered essential in this study to evaluate the rats' cognitive function, since they indicate the rat's memory and cognitive ability indirectly, in that the longer it spends in the targeted quadrant and the more often it crosses the virtual point where the platform previously was, the better will be the rat's memory

Table 2: Comparison of results between groups in Morris Water Maze Task

Items	Group	n	7th week	9th week	11th week
Time spent in targeted quadrant (T)(s)	Model	10	$32.40 \pm 5.52^*$	$26.40 \pm 4.40^*$	$25.00 \pm 4.06^*$
	Control	10	30.40 ± 5.06	30.40 ± 4.74	30.60 ± 2.80
Times that rats crossed point	Model	10	8.20 ± 1.40	7.80 ± 1.23	7.80 ± 1.81
platform where originally placed (N)	Control	10	8.60 ± 1.07	8.40 ± 1.07	8.60 ± 1.07

The test was performed in both groups in the 7th, 9th, and 11th weeks. The time rats spent in the targeted quadrant and the times that rats crossed the point where the platform was originally placed were recorded to evaluate the rats' cognitive function. Data represent means ± SD; n = 10 per group; *, $P < 0.05$ vs. control group

and the stronger its cognitive ability. And according to our definition: ISI==FINS × FPG/22.5, the increase of ISI will parallel the increasing extent of insulin resistance. As we found a negative correlation between ISI and T ($R^2 = 0.9531$) for the model group in Fig. 2b, this suggests that the larger the ISI, the smaller is T, and therefore the better the rat's memory and the stronger its cognitive ability. Similar conclusions may also be drawn from Fig. 2d, which shows a negative correlation between the ISI and N ($R^2 = 0.9985$) for the model group. On the other hand, such relationships seem not to apply with regard to data obtained from the control group. According to Fig. 2a, no obvious correlation was found between ISI and T ($R^2 = 0.5714$) for the control group and neither was one found in Fig. 2c between ISI and N ($R^2 = 0.0357$). Consequently, we could safely conclude that a positive correlation exists between ISI and IR and rats' overall memory and cognitive ability. And we also hypothesise that such a correlation appears only when the ISI falls within a certain range, given that healthy rats in the control group showed no such correlation. Of course, the rats in the control group could be described as physically healthy and mentally unimpaired, regarding their ISI (<2.80) calculated during the study, so their behavior in the Morris Water Maze task purely reflected their own natural intelligence, which was beyond our scope of determining the relationship of ISI and cognitive function under conditions of insulin resistance. Nevertheless, data obtained from the control group helped us in the further related study by convincing us that the possible correlation has its own inherent limitation.

Furthermore, as in our study we chose only three time points (7, 9, and 11 weeks) with an interval of two weeks, the ISI between two time points did not vary significantly ($P = 0.455$, $P = 0.978$ respectively), so this could allow some possible changes beyond our experimental periods or within the intervals to be ignored, thus preventing us from showing a possible correlation or changes in correlation between ISI and cognitive function. As a result, a further study with a longer period or shorter intervals and with a larger sample is warranted to find out more about the correlation and its practical range if it exists.

However, since cognitive function encompasses various concepts within a wide boundary that, psychologically, refer to an information processing view of an individual's psychological functions, and since other interpretations of the meaning of cognition link it to the development of concepts – individual minds, groups, organizations, and even larger coalitions of entities, can be modeled as societies which cooperate to form concepts, – in our primary study, we used the Morris Water Maze simply to evaluate the cognitive function as a whole, instead of focusing on one factor, for example memory, by ruling out others. As a result, the newly-found correlation between ISI and cognitive function in our study may vary or even be reversed when encountering a specific condition.

Moreover, what we found in this study is based on 60 model rats with insulin resistance induced by a high fat diet, supporting the notion that type 2 diabetes mellitus is associated with cognitive impairment (Haan 2006; Craft and Watson 2004). Nevertheless, we can not convincingly predict that the same results will also appear in a similar study conducted on humans, although there have been previous studies on type II diabetic seniors revealing those patients' cognitive impairment. According to earlier population studies, in a cohort of more than 1500 community-dwelling older adults tested with a comprehensive battery of memory and executive function tests, no significant differences were found between subjects with type 2 DM or impaired glucose tolerance and controls after adjustment for age, education, obesity, depression, blood pressure, and current oxygen use (Scott et al. 1998). In the Kupio study, DM did not affect memory significantly while, in contrast, hyperinsulinaemia was associated with impaired verbal memorys independently of the presence of dementia or diabetes (Vanhanen et al. 1999). DM was not associated with "cognitive impairment, or dementia" according to the definition of the Canadian Study of Health and Aging (Ebly et al. 1995), or "age-related cognitive decline" according to the definition of the DSM-IV (American Psychiatric Association A. 1994) in the Italian Longitudinal Study on Aging (Di Carlo et al. 2000). Consequently, inconsistent results might appear in a study on humans compared with those on an animal model in terms of our research. And since ISI is merely one way of expressing insulin resistance numerically, functioning as a bridge linking insulin resistance and cognitive function in this study, and as some ISI deficits remain unresolved (Ji 1998), additional more detailed study is needed to demonstrate and confirm a possible correlation in human beings.

Undoubtedly, finding such a correlation between ISI and cognitive function, especially one that is applicable to humans, is of overwhelming importance in clinical practice concerning diabetes treatment. Insulin has been shown to inhibit synaptic activity at excessively high or low levels (Palovcik et al. 1984), and to down regulate choline acetyltransferase in vitro (Brass et al. 1992), in vitro. And in their critical review of published studies on cognitive function in diabetic patients, Strachan et al. concluded that the etiology of any cognitive decrease in type 2 DM is likely to result from an interaction between metabolic abnormalities intrinsic to diabetes, diabetes-specific complications, and other diabetes-related disorders (Strachan et al. 1997). So insulin plays an important role in the occurrence and development of cognitive function impairment. Given that ISI is an ideal index to show the properties of insulin in the body from a certain aspect, if the exact correlation between ISI and cognitive function were illuminated, it would then be possible to assess brain function by simple indexes. And since the study of brain function is complicated, simplifying numerous tests into a single numerical index which is easy to obtain presents a promising prospect.

4. Experimental

4.1. Subject Animals

SD male rats (Animal Breeding Company, Mount Qinglong, Jiangning), SCXK (Su) 2003-0002

4.2. Feedstuff

Basic: consisted of wheat powder, corn powder, wheat bran, bean powder, fish powder, bone powder, various vitamins. Cal: 15 kJ/g.
High fat: 59% basic feedstuff, 20% lard, 10% egg yolk, 9.5% sucrose, 1.5% salt. Cal: 21kJ/g.

4.3. Reagents

Glucose kit: Shanghai Rongsheng Biotechnology Company Limited; Insulin kit: Beijing Kemei Dongya Biotechnology Company Limited. Other reagents were of analytical purity bought from commercial suppliers.

4.4. Insulin resistant rat model

60 SD male rats were bought, which weighed 180 ± 10 g. After adaptation for 3 days in the lab, they were separated randomly by weight into a model group (MG) and control group (CG). Rats in control group were fed with basic feedstuff (25 g each) every day, while those in the model group were fed with high fat feedstuff (25 g each) every day. Water was allowed without limitation. The rats' weight was recorded every week, and the amounts of their food and water intake, as well as their fur condition, were also recorded. Experimentation started from the 7th week.

4.5. Evaluation of insulin resistant model

4.5.1. Oral Glucose Tolerance Test (OGTT)

In the 6th week of the experiment, after 12 h fasting (water was allowed), blood samples were obtained from the orbital vein using glass capillaries, which were then sealed at one end over a spirit lamp, and centrifuged (3000 rpm/min $\times$ 15 min). Blood serum was obtained. The FPG at 0 min was recorded by following the instructions with the glucose kit. Afterwards, each rat was fed with glucose solution (2 g/kg, i.g), and the FPG at 30 min, 60 min, 90 min, 120 min respectively after i.g. glucose solution were measured using the method referred to above.

4.5.2. Insulin Tolerance Test (ITT)

In the 6th week of the experiment, after 12 h fasting (water was allowed), the FPG was measured at 0 min. Then each rat was injected with insulin solution (0.75 U/kg, i.p.), and the FPG at 0 min, 20 min, 50 min and 90 min respectively after injection was measured using the same method as mentioned above.

4.5.3. Changes in rats' blood lipids

In the 6th week of the experiment, blood samples were obtained from the orbital vein, and centrifuged (2500 rpm/min, 10 min); plasma was obtained, which was used to determine the triglyceride and total cholesterol content.

4.6 Morris Water Maze Task

Insulin resistant rats were separated randomly into 3 groups, which then underwent the Morris Water Maze task at the 7th, 9th, and 11th week respectively. The same was done with the control rats. The experimental apparatus consisted of a circular water tank (120 cm in diameter and 50 cm in height), which was separated virtually into four same quadrants. A movable platform (10 cm in diameter and 25 cm in height) was set inside the tank, which was filled to a depth of 26 cm with water of temperature 25 ± 1 °C. The surface of the platform was 1 cm below the surface of the water. Black ink was added to the water to make it opaque. The pool was located in a large test room under a fixed overhead light. Rats were allowed to swim in the pool without the platform to familiarise them with the environment for 120 s one day before the experiment. The experiment consisted of two parts: navigation and the seeking task.

4.6.1. Navigation

The text intended to measure the rat's memory and its ability to learn was carried out at 3 d pre-surgery (training) and 1 d post-surgery (testing). The rats was gently placed in the water in one of the quadrants with its head facing the wall. The timer began recording then. If in any trial the animal did not find the platform in 120 s, the latent time would be 120 s, and the rat would then be removed from the water and placed on the platform for 30 s. Once the animal found the platform, it was allowed to sit for 5 s and was then removed from the maze. The time required to find the platform was recorded. Such training was repeated four times for each rat with an interval of 60 s both in the morning and afternoon.

4.6.2. Seeking task

The platform was moved on the fourth day of testing. The rat was gently placed in the water in one of the quadrants with its head facing the wall. The time the rat spent in the targeted quadrant and the number of times the rat swam across the exact point where a platform was previously placed were recorded and used to analyze the rat's cognitive function.

4.7. Statistic analysis

Data are expressed as means $\pm$ SD. The T-test was used in some cases if necessary.

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