Plasma Glucose and Insulin Concentration in Tilapia after Oral Administration of Glucose and Starch

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Oral carbohydrate administration tests were performed in tilapia. Either glucose or starch in gelatin capsules were administered orally after the tilapia were put on a fast for 24 h. Blood was sampled at selected time intervals from 1 to 12 h thereafter. The concentration of plasma glucose of tilapia peaked at 3 h after oral ingestion of both carbohydrates. Plasma insulin concentration in tilapia ingested with glucose was higher ($p < 0.05$) at 1 h after oral administration than those ingested with starch and the control group. Plasma insulin dilution curve of tilapia was parallel to the insulin standard curve of human beings.

Key words: tilapia, glucose tolerance, insulin

Fish in general utilize dietary carbohydrate poorly. For instance, Furuichi and Yone¹⁾ noted depressed growth and feed efficiency in red sea bream, yellowtail and carp fed diets high in carbohydrate content. The optimum level of dietary dextrin in these fishes was lower than that in domestic animals.

Oral glucose tolerance tests have been conducted in brook trout,²⁾ rainbow trout,³⁾ common carp,^{4,5)} red sea bream,^{5,6)} yellowtail,^{4,5)} and channel catfish.⁷⁾ In each case, the oral administration of glucose resulted in a persistent hyperglycemia. A similar persistent hyperglycemia was observed when rainbow trout were fed diets containing 15 and 30% glucose.⁸⁾

Different types of carbohydrate may not be equally utilized by fish. For example, the utilization of starch has been observed to be higher than that of glucose and dextrin for carp, red sea bream,⁹⁾ and tilapia.¹⁰⁻¹³⁾ Wilson and Poe⁷⁾ indicated that channel catfish were unable to utilize dietary mono- and disaccharides as an energy source.

The purpose of the study was to compare the changes of plasma glucose and insulin concentrations in tilapia after oral administration of glucose and starch.

Materials and Methods

Experimental Animal

Tilapia (*Oreochromis niloticus* × *O. aureus*), weight 471.4 ± 19.7 g, were obtained from local commercial market and acclimated to our laboratory conditions for two weeks in a plastic tank (74W × 95L × 45H cm). During this period they received a commercial feed (Luck Star, Hung Kuo Industrial Co. Taipei, Taiwan). The proximal composition of the commercial feed is: moisture, 11.75%; crude

protein, 24.35%; crude lipid, 5.69%; ash, 6.03%; crude fiber, 3.01%; N-free extract, 49.17%.

Experimental Procedure

At the beginning of the experiment, fish were weighed and divided into three groups with 8 fish per group representing the starch, glucose, and the control group. They were then transferred to small tanks (30.5W × 61.0L × 55.5H cm) with one fish per tank and were put on a fast for 24 h. One fish from each group was sampled without carbohydrate administration as the 0 h sample. Each of the carbohydrates was administered orally at a dosage of 167 mg/100 g body weight¹⁴⁾ in gelatin capsules with one capsule per fish. The control group received an empty capsule. The oral administration procedure was performed as described by Hung.¹⁴⁾

After the oral administration of carbohydrate for each fish, blood was sampled from the caudal vein at 1, 2, 3, 4, 6, 8, and 12 h and each fish was only sampled once. Blood samples were immediately centrifuged at $8,500 \times g$ for 5 min (HBI microcentrifuge, Saddle Brook, NJ). The supernatant was removed for glucose analysis using a blood sugar determination kit based on a glucose oxidase method (Kyokuto Pharmaceutical Industrial, Tokyo, Japan). Plasma insulin was determined by RIA (radioimmunoassay) using a guinea pig anti-porcine-insulin antiserum as an antibody, ¹²⁵I-labelled insulin as a tracer, and PEG (polyethylene glycol) as separation method.^{15,16)} The standard curve was calculated using the logit-log method and was linearized through five cycles of weighted least-squares regression by an on-line Hewlett-Packard 9815A calculator. The sensitivity, recovery, and parallelism of insulin assay were checked as described by Yalow and Berson.¹⁷⁾

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Statistical Analysis

The concentration of plasma glucose and plasma insulin in fish oral ingested with glucose and starch were compared by Student's *t* test.

Results and Discussion

Plasma glucose concentrations of tilapia after oral ingestion of glucose and starch are illustrated in Fig. 1. Generally speaking, plasma glucose concentrations within 3 h were higher in fish ingested with glucose, followed by starch and then the control group. There appeared a rather smaller peak at 1 h of the glucose group which may be caused by a time-releasing property of the gelatin capsule with an individual variation of the fish. In fish, which ingested both glucose and starch, the plasma glucose concentrations peaked at approximately 3 h after the oral ingestion.

In previous studies, we reported that the plasma glucose concentration in tilapia peaked at 2 h and 3–4 h after fed glucose and starch, respectively.^{11,12} This is somewhat different from the values observed in the present study. It has been reported that body size influenced the carbohydrate utilization in fish.¹³ Large fish gained significantly more body weight, had a better feed conversion ratio, protein and energy deposition than small fish when fed a glucose diet. However, these growth parameters were similar in both size fish when the starch was fed. The poor utilization of glucose has been attributed to fast absorption.¹⁸ The size of the fish used in the present study was 471 g, whereas, in previous studies, 1–2 g of fish were used. Large fish were considered to have better glucose tolerance than small fish. This may explain the delay in reaching the peak of the plasma glucose concentration of the glucose ingested fish. There may be another cause for the delay which need to be investigated in the future.

In the insulin RIA system of the present study, the non-specific binding (NSB) of the buffer was 3%, and that of serum 5%. The sensitivity of the assay was within 2.5 to 5 $\mu\text{U}/\text{ml}$. The averaged recovery of serum immunoreactive insulin (IRI) was $101 \pm 3.96\%$. Figure 2 illustrated that the plasma insulin level (approximately 7.5 $\mu\text{U}/\text{ml}$) in the fasting state drastically raised up at approximately 1 h after

the glucose administration and the level (41.6 $\mu\text{U}/\text{ml}$) was much higher ($p < 0.05$) than those of a starch administration fish and the control group. At 2 h after oral administration, the plasma insulin level was similar to 0 h and was steadily sustained until 12 h in glucose, starch, and also in the control group. Furuichi and Yone⁹ have indicated that carp, red sea bream and yellowtail exhibited a similar pattern of changes in plasma insulin levels after the administration of glucose. The plasma insulin levels which were similar values (approximately 20 $\mu\text{U}/\text{ml}$) in the fasting state increased to the maximum nearly 2 h after glucose administration. The maximum insulin level was compared between the fish-fed diets containing dextrin at 40% level, however, it was highest in carp (54.1 $\mu\text{U}/\text{ml}$), followed by red sea bream (43.8 $\mu\text{U}/\text{ml}$), and yellowtail (38.2 $\mu\text{U}/\text{ml}$) in decreasing order. The plasma insulin level of a normal human being is 10 to 20 $\mu\text{U}/\text{ml}$ in the fasting state similarly to that of fish, but it increased to the maximum (approximately 80 to 130 $\mu\text{U}/\text{ml}$) within a 60-minute period after the administration of 100 g glucose. In the insulin RIA system of Furuichi and Yone,⁹ a single antibody technique was used, however, a double antibody technique was used in the present study. Therefore, it was difficult to compare the measured values between the two studies.

In the present study, the pattern of plasma insulin concentrations in the glucose ingested fish and in the starch ingested fish were different. This may result from the difference in absorption speed between glucose and starch. To our knowledge no plasma insulin pattern of the starch oral administrated fish has been reported. Future investigation is needed regarding the plasma insulin pattern of the oral starch administration in smaller fish.

In comparing the plasma insulin pattern following the oral glucose administration in fish with the pattern of human beings, it has been hypothesized that fish in nature to be diabetic.^{5,7,19} The question remains is that whether the plasma insulin pattern of human beings is comparable to that of fish per se. In the present study, the plasma insulin level was determined by heterogenous RIA method. By using pure human insulin as standard and ¹²⁵I-labelled human insulin as tracer, the fish plasma insulin was determined after incubating with guinea pig anti-porcine-insu-

Fig. 1. The plasma glucose profile of tilapia after oral carbohydrate administration.

Fig. 2. The plasma insulin profile of tilapia after oral carbohydrate administration.

Fig. 3. The percent bound versus human insulin concentration or fish plasma diluate plot by logit-log transformation showed a good parallelism.

lin antiserum. Tilapia plasma was sampled as 1/8, 1/4, 1/2, and 1 folds of reactive volume and boric acid buffer compensated to the same final volume. As shown in Fig. 3, a good parallelism of a human being insulin standard curve and fish plasma insulin dilution curve was observed.

Recently it has been reported that fish plasma insulin concentrations were to be in the same range as mammalian insulin concentration or even higher.^{20,21)} Thus, an alternative hypothesis of impaired hormone-receptor activity in fish has been formulated.^{22,23)} Our results of the present study were unable to verify this hypothesis. Nevertheless, a comparable plasma insulin pattern in tilapia to that in human beings found in this study along with the very high plasma insulin concentration in the glucose ingested fish compared to that of starch ingested fish may indirectly support the hypothesis.

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