

## Prediction of Metabolized Sugar Levels from the IAUC (Incremental Area Under the Curve) of Rats

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**Abstract.** Modern people's nutrient intakes in life are much higher than levels typically required. The excessive sugars in processed foods are being recognized as serious social problems and it has become important to predict the metabolized sugar levels in blood. In this study, the modified measurement methods of ISO 26642 international standard (Determination of glycemic index and recommendation for food classification) using animal testing were conducted. The levels of blood glucose and IAUC (incremental area under the curve) from animal testing compared with the results from those of Sydney University. The results showed the possibility of overcoming the problems of high costs and a relatively long experimental period in traditional GI (glycemic index) experiments.

**Keywords:** prediction, metabolized sugar level, IAUC, ISO 26642

### 1. Introduction

In recent years, modern people's nutrient intakes in everyday life are much higher than levels typically required. Especially, the processed food diet containing excessive sugars are being recognized as increasingly serious social problems.[1]-[3] As a result, it has become important to measure the level of blood sugar contents and to predict the GI(glycemic index) of some processed foods.[4]-[7] In this study, the modified measurement methods of ISO 26642 international standard (Determination of glycemic index and recommendation for food classification) [8] using animal testing were conducted. ISO 26642 needs many essential prerequisites. The most important parts are subjects and test conditions. In case of subjects, a selection of a minimum of 10 healthy subjects shall be made on the basis of a) no known food allergies or intolerances, b) no medications known to affect glucose tolerance. In case of conditions, the subjects shall take no: a) food or drink other than water for 10 hours or more prior to the test; b) alcohol on the previous evening; c) vigorous exercise on the morning of the test. Australia Standard, as in ISO standard, also requires many prerequisites.[9]

These complex and demanding prerequisites made the industrial application of glycemic index very difficult. To ensure the level of glycemic index in some processed foods, we used rat subjects instead of human subjects and modified the measurement methods of ISO 26642.

The results of rat subjects from within the modified measurement methods of ISO 26642 indicated a similar GI compared with results from human subjects (the Sydney University's GI). This study showed the possibility of overcoming the problems of high costs and the relatively long experimental periods in traditional GI experiments.

### 2. Materials and Methods

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## 2.1. Biological Materials

(1) Male SPF Sprague-Dawley rats (6 weeks) were used.

(2) Male SPF Sprague-Dawley rats were placed in individual stainless steel wire-mesh cages in a room with 12:12 h light-dark cycle, temperature of 22-24°C and a relative humidity of 45 ± 5 %. The rats were fed with a normal diet for the first seven days of adaptation.

## 2.2. Reference Food

(1) Anhydrous glucose powder (Sigma Aldrich, purity 96%)(Table I)

(2) The reference food were tested in each subject at three times on separate days within the immediate 1 month period surrounding the testing of the products.

(3) The blood glucose response to the reference food shall be expressed as the IAUC(incremental area under the curve).

(4) The mean within-subject CV for the reference food for the group of subjects tested shall be ≤ 30%.

## 2.3. Test Food

(1) Vitasoy® Soymilky (Australia). (Table I)

(2) Ocean Spray® Cranberry juice (USA). (Table I)

(3) Vitasoy® Ricemilk (Australia). (Table I)

(4) Campbell® V8 Vegetable juice (USA). (Table I)

Table I: Experimental design of the study

Group	No. of animal	Reference food (g/kg body weight)	Test food (g/kg body weight)
G1	10	Glucose	Soymilky
G2			Cranberry juice
G3			Ricemilk
G4			V8 Vegetable juice

## 2.4. Methods

(1) After allowing the rats to abstain from food for 12 hours, each experimental substance was orally administered.

(2) Two blood samples were taken in the fasting state and the average result were taken as the baseline blood glucose concentration, expressed in millimoles per liter. Taking two samples within 5 min is acceptable.

(3) Blood samples shall be taken at 15 min, 30 min, 45 min, 60 min, 90 min, and 120 min intervals, and assayed for glucose.

(4) The blood glucose response to the reference food shall be expressed as the IAUC(incremental area under the curve).[7]

(5) Calculation of GI shall be as follows. For an individual subject, the GI of the test food( $I_{G,t}$ )is given by:

$$I_{G,t} = \text{IAUC of the test food} / \text{average IAUC of the reference food} \times 100$$

## 2.5. Statistical analysis

(1) All the test results were analyzed by SPSS program (version 20.0, SPSS Inc., Chicago, USA) to obtain average and standard deviation.

(2) We used Student's *t*-test to compare the control group and test group. When compared with the control group, the results were deemed significant if  $p < 0.05$

## 3. Results and Discussion

### 3.1. Serum Response

(1) Serum glucose response to Soymilky. \*  $p < 0.05$

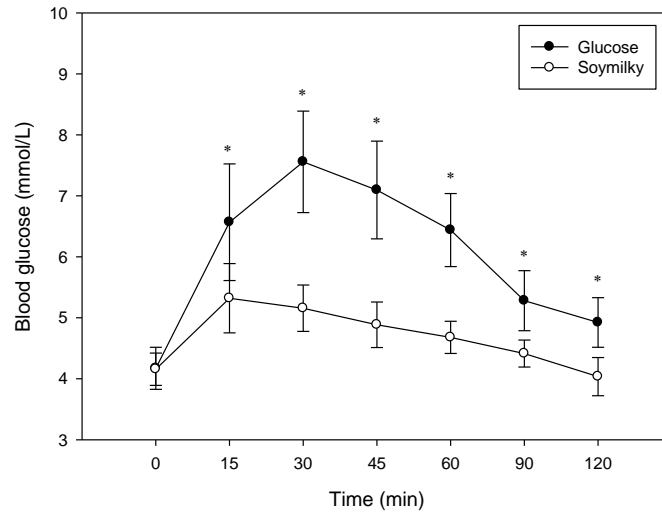


Fig. 1: Serum glucose response to the glucose and Soymilky.

(2) Serum glucose response to Cranberry juice. \*  $p < 0.05$

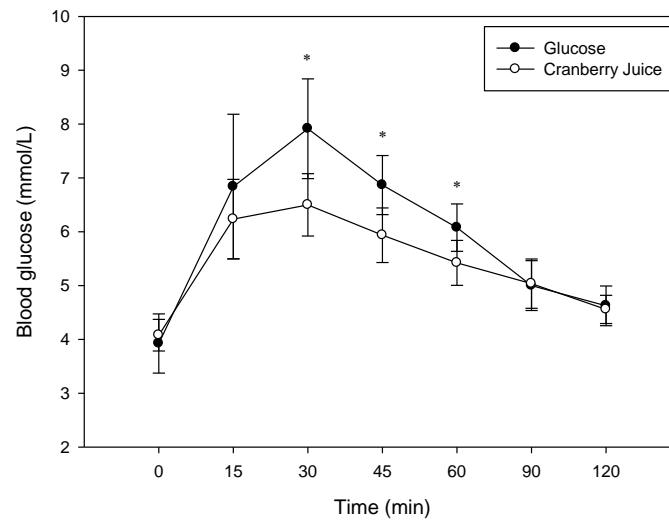


Fig. 2: Serum glucose response to the glucose and Cranberry juice.

(3) Serum glucose response to Ricemilk. \*  $p < 0.05$

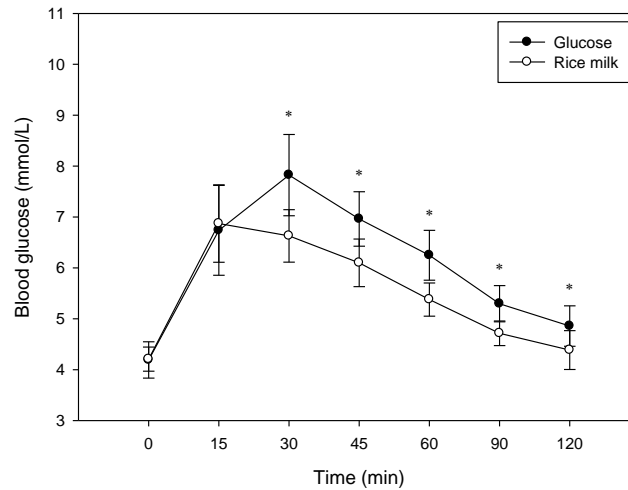


Fig. 3: Serum glucose response to the glucose and Ricemilk.

(4) Serum glucose response to V8 Vegetable juice. \* p<0.05

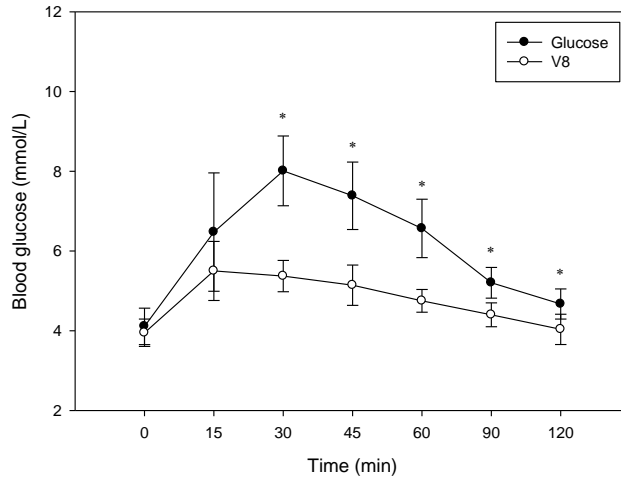


Fig. 4: Serum glucose response to the glucose and V8 Vegetable juice.

### 3.2. Discussion

After calculating the IAUC (incremental area under the curve) of four commercially manufactured goods – Soymilky(Figure 1), Cranberry juice(Figure 2), Ricemilk(Figure 3), V8 Vegetable juice(Figure 4) – the glycemic index(GI) of each test food were estimated.(Figure 5) The existing method (Sydney University)/the modified method (Animal test) ratio of GI in reference food (glucose) was 100/100. The ratio of Soymilky was 21/28. Cranberry juice was 68/66 and Ricemilk was 79/69 and V8 Vegitable was 43/41.(Figure 5) These results mean that the modified method (Animal test) can be well used as an alternative method. Through 2 phases of experiment results that were carried out under the same conditions (Figure 6) it was judged that the experiment methods of the existing experiment methods could be thoroughly carried out by replacing the human subjects with animals (rats).

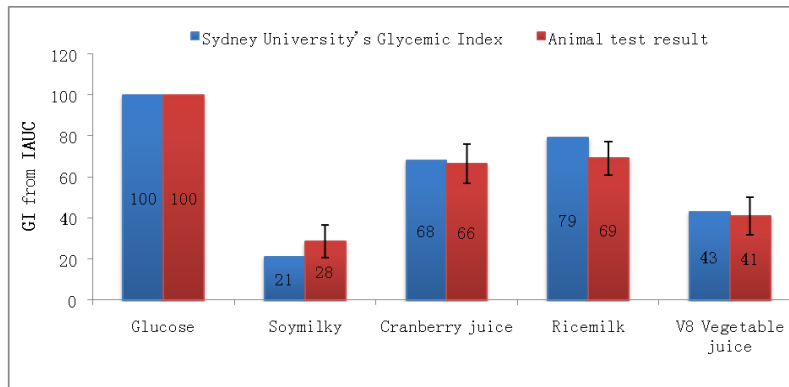


Fig. 5: GI of the existing method(blue) and the modified method(red) in test food.

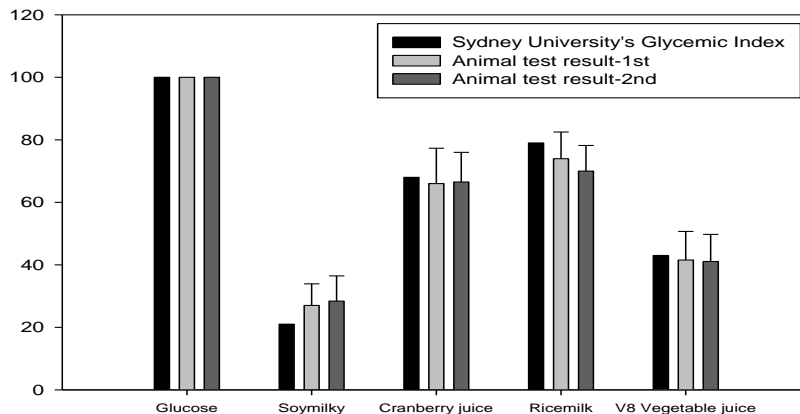


Fig. 6: Two phages of experiment under the same conditions.

### 3.3. Conclusions

The existing measurement method of the glycemic index requires high costs and a relatively long experimental period.[10], [11] To overcome these problems, rapid *in vitro* analysis methods have been evaluated.[12] But, the rapid *in vitro* analysis methods were criticized for not providing the numerical GI values.[12] Generally, the experimental period of the existing measurement method of the glycemic index takes about 16 weeks.[8]-[11] On the other hand, the modified measurement methods can reduce the experiment time from 16 weeks to 3 weeks. Also, it was deemed that the experiment costs could be reduced to about 1 tenth the cost. Therefore, the modified measurement method of ISO 26642 international standard is considered a good choice.

### 4. References

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