Evaluation of glycemic index, hypolipidemic and hypoglycemic activities of "*osu une*" on Alloxan induced diabetic rats.

Abstract

Background: The incidence of diabetes has been on the increase due to increase in sedentary lifestyle together with increase in life expectancy. "*Osu une*" is a native meal in Anambra State, Nigeria used in the management of diabetics. There is no scientific based study/data to ascertain the effect on blood glucose level. **Objective**: The study investigated the glycemic index, hypolipidemic and hypoglycemic activities of "*osu une*" on Alloxan induced rats. **Methods:** The unripe banana was purchased, processed into powder and prepared into crude extract with methanol. Twenty adult male Albino rats were purchased and grouped into four of five rats each based on their body weight. They received standard rat chow for 7 days of acclimatization period. The animals were fasted for 12hr after which Alloxan at a dose of 42mg/kg was induced intravenously through tails of group 2-4 rats. Only animals with clinical signs of severe diabetes and fasting glucose ≥ 126mg/dL in two successive determinations were considered diabetic. Group one were on rat chow alone throughout the experiment without Alloxan, Group two '*osu une*' extract at a concentration of 400 mg/kg body weight were orally administered with rat chow, Group three, glucophage tablets was orally administered with rat chow and Group four received rat chow alone. Clinical parameters such as body weight, blood glucose, total cholesterol, triglycerides, High and low density lipoprotein were analyzed using standard method. Blood was collected from the ocular vein and used for Laboratory analysis. Ten healthy subjects aged between 24 -40 participated in this study. They were fed with the standard food (50 g glucose) on day one and the test foods on day two, after an overnight fast. Blood samples were taken at 0, 30, 60, 120, and 180 min after the food had been eaten. All results were expressed as mean ± SD and statistical analysis were evaluated by one way ANOVA, mean was separated using Duncan New multiple range test. Significance was accepted at $P< 0.05$. **Results:** The result showed that rats that received '*osu une*' extract showed an increase in body weight from 130.70mg/kg to 146.20mg/kg, while rats that received glucophage tablets also showed an increase in body weight from 126.01mg/kg to 158.81mg/kg after inducing diabetes. The rats fed '*osu une*' extract had a decrease in fasting blood glucose level, total cholesterol, triglycerides, LDL and increase in HDL. The test diet had a low glycemic index of 9.59. **Conclusion**: The study shows that "*osu une*" can play a key role in the management of Diabetes Mellitus.

Keywords: glycemic index, hypolipidemia, hypoglycemia, '*osu une*', diabetes, Alloxan.

1.1 Introduction

Diabetes mellitus is a public health problem which affect all age groups but mostly common among adult from 50 years and above. Diabetes mellitus is a metabolic disorder which is

associated with excess sweet urine known as glycosuria. It is a dangerous illness which predisposes the body system to other forms of diseases (Nwankwo *et al.*, 2018). It is a chronic metabolic disease that occurs when fasting glucose level is greater than or equal to 126 mg/mL (ADA, 2010). Diabetes can occur as a result of the pancrease inability to produce insulin or defects in cellular glucose uptake (Sanofi, 2013).

The WHO (2016) had estimated that about 422 million people worldwide are suffering from diabetes mellitus in 2014 and 642 million by the year 2040. The incidence of diabetes has increased dramatically in recent years worldwide. Diabetes deaths were 1.5 million in 2012 and will reach 3.7 million in 2040 worldwide (IFD, 2013). The African continent with an estimate of 32.8 million patients in 2014 is estimated to increase to 41.4 million in 2035 (WHO, 2016).

Glycemic index (GI) is a value used to measure how much specific foods increase the blood sugar levels. Foods are generally classified based on low (55 or less), medium (56-69) or high (70 and above) and ranked on a scale of 0-100. The lower the GI of a food, the less likely it may affect the blood sugar levels (Racheal, 2020). However, foods with low GI would be very effective in the management of diabetic patients.

The treatment of diabetes with insulin injection is economic demanding and beyond the reach of many families in developing Countries of the World. People prefer functional foods in the management of diseases which has a lasting and little or no side effect than medicine. Generally, people easily accept their traditional foods with health benefits because they are used to them, they know how to produce them and enjoy the meals containing them. Plant foods with antidiabetic properties are available, less expensive and have few adverse side effects when properly processed (Guler *et al*., 2015). In addition, according to the World Health Organization (WHO), the investigation of hypoglycemic properties of medicinal plants has become more significant (Miaffo *et al*., 2019). Identification of different foods with low glycemic index for the management of diabetes is paramount to reduce monotony in their diet and create variety.

"*Osu une"* is a traditional dish peculiar to the people of Anambra State, Nigeria. The diet is prepared for the management of diabetes mellitus according to the folktale. "*Osu une*" is a pudding made from unripe banana, oil, crayfish, salt, spices and water. The essence of the study is to scientifically ascertain the effect of the diet in the management of diabetes. There is no previous anti-diabetes study performed on the extract of "*osu une*". The objective of this study

was to evaluate the glycemic index, hypoglycemic and hypolipidemic activities of "*osu une*" in alloxan-induced diabetic rats.

1.1 Materials and method

1.2.1 Test food and processing

The plant material consisted of fruit of unripe banana purchased from main market Enugu and authenticated at herbarium of Department of Biotechnology and Botany, University of Nigeria Nsukka. The unriped bananas were washed with tap water, the bark peeled, sliced into smaller sizes and crushed using an attrition mill until a soft paste was obtained. The milled unriped banana were mixed with red oil, crayfish, spices, onions and water. The mixed paste were tied in small quantity in a folded foil paper and allow to boil for 30 mins in a pressure cooker.

1.2.2 Chemicals and drugs

Alloxan and other chemicals used for the study was purchased from a local chemical store at Enugu. Glucophage tablets was purchased from a local pharmacy store at Enugu.

1.2.3 Preparation of methanolic extract

Methanolic extraction was done using a modified method of Bhandari & Kawabata (2004). Dried ground "*Osu une*" diet weighing two hundred grams (200 g) were soaked in 80 ml of methanol and kept over night. The suspension was filtered through Whatman No.1 filter paper, and the filtrate was diluted to make up to 100ml with methanol. Sample solutions were stored at 4°C in amber bottles and served as the stock solution for the study.

1.2.4. Animal housing

Twenty adult male Albino rats, from the same colony, weighing between 150-153g were purchased from the Faculty of Veterinary Medicine, University of Nigeria Nsukka, Nigeria. The rats were housed in individual metabolic cages equipped to separate feaces and urine. The rats had exactly 12 h of light and 12 h of darkness in a day. The experiment was carried out in strict compliance with the National Research council guidelines on the care and use of laboratory animals (National Research Council, 2010). The Animal Experimentation Ethics Committee of University of Nigeria Nsukka, approved the use of laboratory animals.

1.2.5 Induction of diabetes

The rats were divided into 4 groups of 5 rats each. They received standard rat chow for 7 days of acclimatization. After a 7-day acclimatization period, the rats were weighed prior to grouping. Animals were fasted for 12 h and diabetes was induced using intravenous Alloxan at a dose of 42 mg/kg of body weight into the tail veins to groups 2-4. Group one was on rat chow alone throughout the experiment without administering Alloxan. Group two received rat chow and 500mg/kg extract of "*osu une*", group three received rat chow and Glucophage tablets and group four received rat chow alone. Only animals with clinical signs of severe diabetes and fasting glucose ≥ 126 mg/dL in two successive determinations were considered diabetic and used for the study. Clinical (body weight, water intake, food intake, and urine output) and laboratory (blood glucose, hemoglobin, total cholesterol, triglycerides, and high and low lipoproteins) parameters were analyzed. Clinical parameters were obtained using individual metabolic cages. Blood glucose tests were performed using evolve glucometer. Fasting blood glucose levels of the animals were evaluated on days 7, 14 and 28 of the experiment.

1.2.6 Blood collection

Blood was collected from the ocular vein and used for laboratory analysis. Total cholesterol assay was performed following a colorimetric enzymatic method described by Trinder using Dialab kit (Trinder, 1969). The HDL-c assay was described by Wiebe *et al*. (1997) using Inmesco kit. The triglycerides level was determined by enzymatic colorimetric method described by Cole *et al*. (1997) using Dialab kit. The LDL-c level was deduced from the other lipids previously obtained using the formula described by Richmond (Richmond, 1973).

1.2.7 Proximate analysis

The proximate analysis for moisture, crude protein, crude fibre, fat and ash was carried out using standard methods (AOAC, 2015).

Fifty grammes (50g) of available carbohydrate for the test food sample was calculated from the results of the proximate analysis and the measured portion of the food was served to the subjects. The control diet were administered (50g glucose) in 200ml of distilled water.

1.2.8 Subject

Ten (10) healthy human subjects, aged between 24-40 (5 males and 5 females) were selected from the students and staff of the Enugu State University of Science and Technonolgy, Nigeria. They were clinically normal, non diabetic and non-smokers. The subjects were appraised verbally and they gave their informed consent. The protocol and procedures employed were reviewed and approved by the Ethics Committee of the Enugu State University Teaching Hospital, Parklane Enugu. The procedures followed were also in accordance with the ethical standards of the responsible committee on human experimentation of the Helsinki Declaration of 1975, as revised in 2008.

1.2.9 Determination of blood glucose

Volunteers for the investigation fasted overnight. They were asked not to perform any strenuous activities or take long walks. They were requested to remain seated for the duration of the test.

Capillary pricked-finger blood samples were taken at baseline (0 min), 30, 60, 90, 120 and 180 mins after consumption of the food. The blood sample was placed immediately on a test strip which was inserted into a calibrated Glucometer (Evolve^{R}) which gave direct readings after few seconds.

Day 1

The study started in the morning after an overnight fast by the individuals. A fasting blood sample was taken at 0 min; then after this, the subjects consumed 50 g standard food (50 g of glucose powder dissolved in water) in a comfortable place. The standard food was constituted with 200 ml of water. Blood samples were taken at 30, 60, 120, and 180 min. The blood glucose concentrations were determined immediately using the glucometer.

Day 2

After an overnight fast, the test foods were consumed by the same group of subjects. Blood samples were taken at 0, 30, 60, 90, and 120 min. The blood glucose concentrations were determined immediately using the glucometer.

The incremental areas under the glycemic response curve were calculated geometrically (Wolever & Jenkins, 1986). The GI was calculated by expressing the glycemic response area for the test drink as a percentage of the mean response area of the glucose drink taken by the same subjects. The following formula was applied:

$$GI = \frac{\text{Area under the curve for 50g carbohydrate from test food X 100}}{\text{Area under the curve for 50g carbohydrate from glucose}}$$

The GI for the food and control was calculated as a mean from the respective average GI of the individuals.

1.2.10 Statistical analysis

All results were expressed as mean ± SD (Standard Deviation). Statistical analyses were evaluated by one-way ANOVA mean separated using New Multiple Range Test. Statistical significance was accepted at $p < 0.05$.

1.2 Result

Table 1 Effect of osu-une extract 500 mg/kg **on the relative body weight in alloxan-induced diabetic rats g.**

Day	Rat chow without diabetic	Rat chow with glucophage	Rat chow with osu-une extract	Rat chow alone
7	150.72^{a} ±1.16	151.24^{a} ±0.48	153.10^{a} ±0.35	152.98^{a} ±0.81
14	155.24^{a} ±0.53	116.01^{b} ±1.23	110.70^{b} ±0.56	124.60^{b} ±2.09
28	152.10^{b} ±0.78	158.81^{a} ±0.31	146.20^{c} ±1.10	110.20^{d}±0.96

Each value is expressed as mean ± SD (n = 5).

Table 2 Effect of osu-une extract 500 mg/kg **on fasting blood glucose in alloxan-induced diabetic rats mg/dl .**

Day	Rat chow without diabetic	Rat chow with glucophage	Rat chow with osu-une extract	Rat chow alone
7	118.72^{a} ±1.54	119.24^{a} ±0.38	119.10^{a} ±0.31	118.98^{a} ±0.18
14	119.24^{b} ±0.84	233.01^{a} ±1.26	233.70^{a} ±0.62	234.60^{a} ±0.82
28	118.10^{a} ±0.43	121.81^{b}±0.17	126.20^{c} ±1.66	240.20^{d} ±0.06

Each value is expressed as mean ± SD (n = 5).

Table 3 Effect of osu-une extract 500 mg/kg **on Total cholesterol in alloxan-induced diabetic rats** (mg/dL)**.**

Day	Rat chow without diabetic	Rat chow with glucophage	Rat chow with osu-une extract	Rat chow alone
7	31.84[a] ±0.18	30.20[a] ±0.15	34.00[a] ±0.04	32.50[a] ±0.72
14	33.00[b] ±0.65	45.10[a] ±0.62	44.50[a] ±0.36	43.90[a] ±0.54
28	30.05[a] ±0.23	34.60[b] ±0.85	37.20[c] ±0.26	44.00[d] ±0.01

Each value is expressed as mean ± SD (n = 5).

Table 4 Effect of osu-une extract 500 mg/kg **on Triglyceride in alloxan-induced diabetic rats** (mg/dL)**.**

Day	Rat chow without diabetic	Rat chow with glucophage	Rat chow with osu-une extract	Rat chow alone
7	22.10[a] ±0.23	21.00[a] ±0.16	23.00[a] ±0.32	20.90[a] ±0.17
14	23.00[b] ±0.47	35.30[a] ±0.52	34.60[a] ±0.51	33.80[a] ±1.33
28	23.05[b] ±0.08	20.70[c] ±0.63	22.60[c] ±0.91	35.00[a] ±1.66

Each value is expressed as mean ± SD (n = 5).

Table 5 Effect of osu-une extract 500 mg/kg **on HDL in alloxan-induced diabetic rats** (mg/dL)**.**

Day	Rat chow without diabetic	Rat chow with glucophage	Rat chow with osu-une extract	Rat chow alone
7	12.76a ±1.12	12.95a ±0.45	13.12a ±0.57	13.50a ±0.07
14	13.00 a ±0.08	10.20a ±0.21	10.70a ±0.68	9.90a ±1.22
28	13.05c ±0.01	18.30a ±0.32	13.60b ±1.31	6.07b ±0.41

Each value is expressed as mean ± SD (n = 5).

Table 6: Effect of osu-une extract 500 mg/kg **on LDL in alloxan-induced diabetic rats** (mg/dL)**.**

Day	Rat chow without diabetic	Rat chow with glucophage	Rat chow with osu-une extract	Rat chow alone
7	16.40[a] ±0.13	17.35[a] ±0.67	15.90[a] ±0.03	16.20[a] ±0.50
14	16.10[b] ±0.09	26.70[a] ±0.45	28.30[a] ±0.42	26.90[a] ±0.18
28	16.00[a] ±0.14	14.10[a] ±0.11	15.80[a] ±0.07	27.45[b] ±0.68

Each value is expressed as mean ± SD (n = 5).

Table 7: Proximate composition of *"osu une"*

Sample	Protein	Ash	Fat	Moisture	Fibre	CHO
Osu une	37.13±0.23	1.27±0.16	2.86±0.32	35.50±43	5.38±0.14	17.86 ±0.39

Mean±SD

Table 8: The calculated carbohydrate in 100g of prepared food and serving size used for the determination of GI

Sample	Calculated CHO in 100g Of prepared food	portion size (g)
Osu une	17.86	280

Mean±SD

Table 9: Blood glucose concentration (mg/dl) of subjects

Sample	**0 mins**	**30 mins**	**60 mins**	**120 mins**	**180 mins**
Glucose	69.75	125.29	106.64	98.42	94.18
Osu une	72.34	75.71	79.22	81.88	76.30

Mean±SD

Table 10: Glycemic index of "*osu une*"

Sample	Glycemic index	Classification
Osu une	9.59	low

Mean±SD

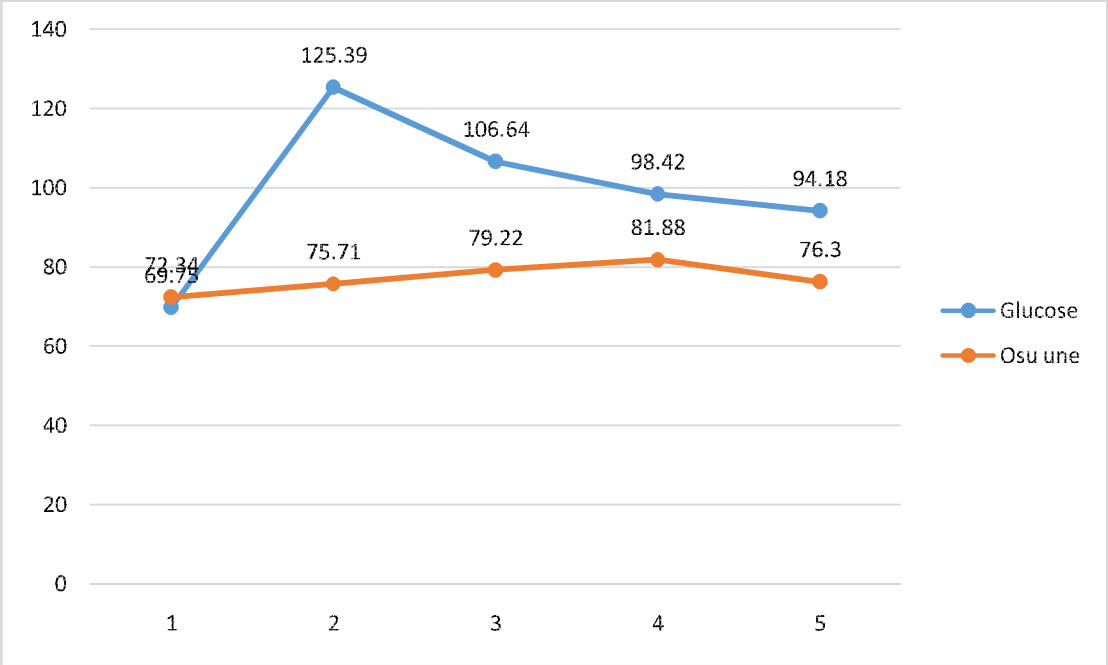

Fig. 1. Blood glucose concentration of the subjects

1.3 Results and Discussion

There is a tremendous increase in the incidence of diabetes mellitus worldwide. It is necessary to discover other foods with a low glycemic index that will help in the dietary management of diabetes mellitus. The aim of this study is to evaluate the glycemic index and determine the effect of "*osu une* " on the hypoglycemic and hypolipidemic activities of diabetic-induced rats.

Body weight: The mean body weight of the rats were shown on Table 1. On day 7 after acclimatization, the results were between 150.72- 152.10g. There is no significant difference in the mean body weight of the rats on day 7. This is because all the rats were healthy and pooled from the same colony. On day 14 after induction of diabetes, the mean weight of the rats was

between 110.70- 155.24g. Groups 2-4 were significantly different from group 1 that alloxan was not administered to $P< 0.05$. There is a significant decrease in the body weight of the group 2-4 which were administered with alloxan. The decrease in body weight observed in the rats induced with diabetes is due to the hydrolysis of protein (protein turnover) lipid reserves in muscle tissue to produce energy, because of the inability of these tissues to metabolize blood glucose (Pari & Maheswari, 1999). Another characteristic peculiar to type 1 diabetes is severe loss of body weight, probably due to muscle atrophy (Farida & Shoukry, 1988). Alloxan causes a depletion in the muscle and liver thereby mimicking a case of an animal deprived of a high protein diet. Sequel of this a diabetic patient is advised to consume a high protein diet. On day 28, the mean body weight ranged between 110.20-158.81g. There is a significant increase of $P< 0.05$ in the body weight of the rats of groups 2 and 3 who received Glucophage drug and *osu une* extract. The result of this study is in line with the findings of (Miaffo *et al*., 2019) that observed a decrease in the relative body weight of rats administered with alloxan and an increase in the relative body weight administered with Glucophage drug and plant extract to diabetic induced rats. The significant increase in the weight observed in the group that received extract indicates its effect on the control of muscle atrophy. Whitton & Hems (1975) observed a significant increase in the relative body weight of the rats after administering the extract, which was attributed to its effect on the control of muscle atrophy.

Fasting blood glucose: Table 2 shows the mean fasting blood glucose of rats. On day 7, which was the first day after acclimatization the mean fasting glucose level was between 118.72-119.24mg/dl. On day 14, which was the day diabetes was confirmed, the fasting blood glucose was between 119.24-234.60mg/dl. On day 28, which was the last day of treatment/ experiment, the mean fasting blood glucose level was 118.10- 240.20mg/dl. There is a significant increase ($p< 0.05$) in the fasting blood glucose level of rats in groups 2-4 after administering alloxan to the rats to induce diabetes compared to group 1 the normal control. On day 28, there is a decrease in the fasting blood glucose level of rats in groups 2 and 3 that received Glucophage and *osu une* extract. The findings of this study are in line with other studies that observed a decrease in the fasting blood glucose level of rats after receiving antidiabetic drugs and plant extract (Madhuri & Mohanvelu, 2017; Miaffo *et al*., 2019). The decrease could be attributed to the ability of the extract to regenerate β cells of the islets of Langerhans, transport blood glucose

in peripheral tissue, stimulate glucose uptake by peripheral tissues, inhibit endogenous glucose production, and activate gluconeogenesis in the liver and muscles (Burcelain *et al*., 1995).

Lipid profile: Table 3, 4, 5, and 6 shows the mean total cholesterol, triglyceride, HDL-c, and LDL-c level of rats. On day 7, which was the first day after acclimatization the mean total cholesterol, triglyceride, HDL-c, and LDL-c level were between 30.20- 34.00mg/dl, 20.90- 23.00 mg/dl, 12.76-13.50 mg/dl, and 15.90-17.35 mg/dl. On day 14, which was the day diabetes was confirmed, total cholesterol, triglyceride, HDL-c, and LDL-c level were between 33.00-45.10mg/dl, and 23.00- 25.30mg/dl, 9.90-13.00mg/dl, and 16.10-26.90mg/dl. On day 28, which was the last day of treatment/ experiment, the mean total cholesterol, triglyceride, HDL-c and LDL-c level were 30.05-44.00mg/dl, 20.70- 35.00mg/dl, 6.07- 18.30mg/dl and 14.10-27.45mg/dl. There is a significant increase ($p< 0.05$) in the total cholesterol, triglyceride, and LDL-c level but a significant decrease in the HDL-c level of rats in groups 2-4 after administering alloxan to the rats to induce diabetes compared to group 1 the normal control. Banda *et al*. (2018) observed an increase in TC, TG, VLDL, LDL, and a decrease in HDL in alloxan-induced diabetic rats. Hyperlipidemia occurs in diabetic-induced rats as a result of the excess mobilization of fat from the adipose tissue due to the underutilization of glucose (Akpan *et al.*, 2012). Studies show that the hormone lipase increases the ability to break down stored triacylglycerol into fatty acids thereby promoting the conversion of excess fatty acids to phospholipid and cholesterol in the liver (Rajaei *et al.*, 2015). The diabetic condition renders an enzyme lipoprotein lipase inactive thereby leading to hypertriglyceridemia and a reduction in HDL-c levels (Pushparaj *et al*., 2007). Krentz (2003) observed that hypertriglyceridemia, hypercholesterolemia and elevated LDL levels are the common factors that lead to the development of atherosclerosis and coronary heart disease in diabetes mellitus patients. On day 28, there is a significant decrease in the total cholesterol, triglyceride, LDL-c level as compared to group 4 but an increase in the total cholesterol level of rats ($p< 0.05$) in groups 2 and 3 that received Glucophage and *osu une* extract. The *L. edulis* administered to diabetic positive control groups had significant reductions in TC, TG, LDL, and VLDL as compared to the diabetic control whilst HDL levels were significantly increased (Banda *et al*. 2018). This could be attributed to increased utilization of glucose which led to the inhibition of lipid peroxidation and control of lipolytic hormones (Banda *et al*. 2018). Rajaei *et al*. (2015) observed that dietary

management and drug therapy that will lead to the lowering of serum lipid and elevation of HDL-c is associated with a decrease in the risk of cardiovascular disease and related complications.

Glycemic index: Studies show that different nutritional and physiological factors might have an effect on the blood glycemic response and the GI value of the foods (Omega and Omega, 2018). This may include but is not limited to the digestibility of the starch, interactions of starch with fiber, fat, and protein present, the proportion of the constituent nutrient, and the method of cooking. The low glycemic index of 9.59 of the meal could be attributed to the blood glycemic and the lipidemic response of the rats fed "*osu une*" after inducing diabetes.

1.4 Conclusion

In conclusion, "*osu une*" diet with low GI of 9.59 which caused a decrease in fasting blood glucose level, total cholesterol, triglycerides, LDL, and an increase in HDL is recommended for the dietary management of diabetes in a community where the diet is common.

Abbreviations: ANOVA: Analysis of variance; HDL-c: High-density lipoprotein cholesterol; LDLc: Low density lipoprotein cholesterol; GI: glycemic index

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