Rapid Rehydration and Moderate Plasma Glucose Elevation by Fluid Containing Enzymatically Synthesized Glycogen

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Summary Enzymatically synthesized glycogen (ESG) has high solubility and its solution has low osmotic pressure. Therefore ESG solution could be rapidly absorbed and could be adequate for water rehydration and carbohydrate supplementation during exercise. The object of this study was to evaluate the gastric emptying time and plasma glucose elevation after an administration of ESG solution in comparison with another carbohydrate solution by using a laboratory animal. Male BALB/c mice were administered 10% w/v solution of glucose, maltodextrin, starch, naturally synthesized glycogen (NSG) and ESG at a dose of 20 μL/g body weight for the measurement of gastric emptying rate (Experiment 1) and 10 μL/g body weight for the measurement of plasma glucose elevation (Experiment 2). The osmolarity of gastric content was lower in the ESG and maltodextrin group than the other carbohydrate group. Weight of gastric fluid was significantly lower in the ESG and water group than the glucose group ($p<0.01$). Plasma glucose level was significantly lower in the ESG group than the glucose group from 0 to 60 min after administration ($p<0.01$), whereas plasma glucose level was same from 60 to 120 min for the ESG and glucose group ($p=0.948$). In Experiment 3, BALB/c mice ran on a treadmill for 2 h and were administered 8% of ESG or glucose solution (1.75, 3.5 or 7.0 μL/g body weight) every 20 min during running. There was no difference in post-exercise muscle glycogen level. These data suggest that 1) ESG beverage does not disturb water absorption because of its short gastric emptying time and 2) ESG slowly elevates plasma glucose level and maintains it for a prolonged time compared to the glucose solution.
Key Words gastric emptying time, exercise, osmolarity, carbohydrate

The primary reason to ingest a sports drink is to replace body fluids lost in sweat and to provide an exogenous form of energy, typically carbohydrates (*1, 2*). Once a sports drink is ingested, the efficiency of rehydration depends on the rates of gastric emptying and intestinal absorption (*3, 4*). To maximize the rates of gastric emptying and intestinal absorption during exercise, the proper osmolarity (*5*), sodium concentration, and form and amount of carbohydrate required have been subjects of debate and experimental investigation for more than two decades (*6, 7*).

A high concentration of a simple sugar beverage, such as glucose and fructose, has high osmolarity and delays gastric emptying time of the solution and water rehydration. On the other hand, a glucose polymer solution has an advantage in its low osmotic pressure compared to a glucose monomer solution (*8–10*). From the viewpoint of energy supplementation, an isotonic maltodextrin beverage can contain 5 times more calories than an isotonic glucose monomer beverage. However the gastric emptying time of another dextrin whose molecular weight is much higher than that of maltodextrin in unclear.

Glycogen, the major storage polysaccharide in animals and microorganisms, has a higher molecular weight and lower osmotic pressure compared to maltodextrin. Glycogen is a highly branched (1→4)(1→6)-linked alpha-D-glucan with a molecular weight ranging from 1,000 to 1,000,000 kDa (*11*).

Recently, a novel enzymatic process for glycogen synthesis was developed, in which starch is used as a starting material (*12*). Enzymatically-synthesized glycogen derived by this process, herein referred to as ESG, has a molecular weight in the rage of 3,000–30,000 kDa, and is soluble in water, giving an opalescent solution resembling glycogen derived from natural sources such as animal tissues or shellfish (NSG).

In this study, three experiments were performed independently to clarify the effect of ESG on water absorption and carbohydrate metabolism. In Experiments 1

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Abbreviations: AUC, area under the curve; BW, body weight; ESG, enzymatically synthesized glycogen; FFA, free fatty acid; NSG, naturally synthesized glycogen.

Table 1. Molecular weight and fluid osmolarity of administered beverages.

	Average molecular weight (g/mol)	Osmolarity (mOsmol/L)
Water		0
Glucose	180	636
Maltodextrin1	4,400	111
Maltodextrin2	35,000	18
ESG	7,000,000	9
NSG	6,500,000	196

Fluid osmolarity is the value of its 10% w/v solution. ESG, enzymatically synthesized glycogen; NSG, naturally synthesized glycogen.

and 2, gastric emptying time and plasma glucose elevation after an administration of an ESG beverage were evaluated by using a laboratory animal. In Experiment 3, ESG or glucose solutions were intermittently administered during 2 h of treadmill running and residual muscle glycogen and plasma energy substrate were measured.

METHODS

Animals. Male 5 to 15-wk-old BALB/c mice (SPF from Japan Shizuoka Laboratory Center, Hamamatsu, Japan) were used. They were housed in cages ($33 \times 23 \times 23$ cm; 5 mice per cage) under controlled conditions of temperature ($24.5 \pm 1°C$), humidity ($55 \pm 5\%$), and lighting (lights on from 8:00 to 20:00). They were provided with a stock diet (type MF; Oriental Yeast Co., Ltd., Tokyo, Japan) and water ad libitum. All procedures were performed in accordance with the Animal Experimentation Guidelines of Sugiyama Jogakuen University.

Experimental materials. Glucose (Wako Pure Chemical Industries, Ltd.), maltodextrin1 (TK-16, Matsutani Chemical Industry Co., Ltd.), maltodextrin2 (Pinedex 100, Matsutani Chemical Industry Co., Ltd.), ESG (ESG 7,000k, Ezaki Glico Co., Ltd.), NSG (mytilus glycogen, biosaccharide GY, Laboratories Serobiologiques) and starch (Wako Pure Chemical Industries, Ltd.) were used. Their molecular weight and the osmolarity of the 10% solution are shown in Table 1.

Experiment 1: Gastric emptying rate after carbohydrate ingestion during resting. Mice were fasted for 20 h (17:00–13:00) and prohibited to drink for 3 h (10:00–13:00) before administration. Each mouse was orally administered with 10% w/v carbohydrate or glucose solution at a dose of 20 μL body weight via a stomach sonde. Mice were subjected to a laparotomy and both cardia and pylorus were tied with surgical threads and the stomach was removed under diethyl-ether anesthesia. Gastric content was collected by a syringe with 21G needle, and residual content was aspirated with a paper towel. The stomach was weighed twice, before and after the collection of gastric content. The difference in stomach weight was regarded as the weight of gastric content.

Osmolarity of gastric content was measured cryo-scopically with an osmometer (Osmomat 030; Gonotec, Berlin, Germany).

Experiment 2: Blood glucose response after carbohydrate beverage ingestion during resting. The experiment was performed in a crossover design. Mice were randomized to carbohydrate or glucose (control) groups. After being fasted for 18 h (17:00–11:00), each mouse was orally administered with 10% w/v carbohydrate or glucose solution at a dose of 10 μL/g body weight via a stomach sonde. Blood samples were collected under diethyl-ether anaesthesia, from the retro-orbital plexus with a surgical plastic cannula tube ($\phi 0.8$ mm $\times 8$ mm) before and 15, 30, 60 and 120 min after oral administration. Experiments were repeated twice on each carbohydrate beverage on the second. That is, the group administered the carbohydrate solution on the first day was administered the glucose beverage and the other group administered the carbohydrate beverage.

Experiment 3: Post exercise muscle glycogen and plasma prameters. Mice were randomized to ESG (3,000k) and glucose groups. Mice were fasted for 2 h before the start of exercise and they ran on a treadmill (KN-73, Natsume Seisakusho Co. Ltd., Tokyo, Japan) for 2 h at the constant speed of 9 m/min. They were orally administered 8% of ESG or glucose solution at a dose of 1.75 μL/g bw every 20 min during running exercise (0, 20, 40, 60, 80 and 100 min from the start of running). Immediately after 2 h of running, they were killed by decapitation and the gastrocnemius muscle and blood samples were collected.

In experiment 3, three doses (1.75, 3.5 and 7 μL/g bw) were tested in the same experimental design. Each administered dose corresponded to 0.42, 0.84 or 1.68 mg/g body weight/h.

Plasma parameters. Plasma samples were obtained by centrifugation and stored at $-80°C$ until assayed. Plasma glucose, triglyceride, FFA, glycerol levels were assayed using a commercial available kit (Glucose Test Wako, Wako Pure Chemical Industries, Ltd.) and insulin levels were assayed using an ELISA kit (Levis insulin mouse U type, Shibayagi co. jp, Japan). Lactic acid levels were assayed using a commercial kit (Determinar LA, Kyowa Medex Co., Ltd., Japan). The area under the curve (AUC) values for plasma glucose levels were calculated for 0 to 60 min and 60 to 120 min for each carbohydrate solution.

Muscle glycogen analysis. Muscle samples were stored at $-80°C$ until assayed. The muscle glycogen content was measured spectrophotometrically by a method using enzymatic techniques as described elsewhere (*13*). Briefly, after hydrolysis of the muscle sample in 0.6 mol/L HCl at 100°C for 2 h, the glucose residues were determined with the commercial kit described above.

Statistics. Each value is expressed as the means$\pm$ SE. Comparisons of more than three groups were performed by one-way ANOVA followed by Tukey's post hoc test (Experiment 1). Comparisons of two groups in the crossover design were performed by paired t-tests (Experiment 2). Comparisons of two groups were per-
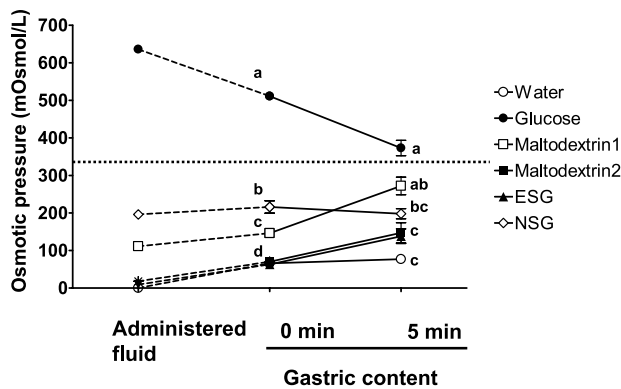
Fig. 1. Osmotic pressure of gastric contents of mice after administration of carbohydrate beverages. The dotted line shows the average plasma osmotic pressure of mice which were fasted for 18 h ($n=3$). Gastric fluid was collected 0 and 5 min after administration of each fluid. Values are means±SE ($n=13$). Values without common superscripts are significantly different, $p<0.05$.
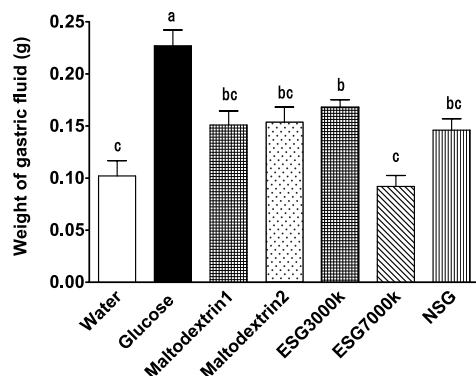

Fig. 2. Weight of gastric contents of mice after administration of carbohydrate beverages. Gastric fluid was collected 0 and 5 min after administration of each fluid. Values are means±SE ($n=13$). Values without common superscripts are significantly different, $p<0.05$.

Fig. 3. Plasma glucose level after administration of carbohydrate beverages. Mice were orally administered 10 μL/g body weight of a beverage after 18 h of fasting. Plasma was collected before and 0, 15, 30, 60, 90, 120 and 180 min after the administration of carbohydrate beverages. Values are means±SE ($n=8$–22). *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs glucose, paired t-test.

formed by Student's t-test (Experiment 3). Significance was set at the 0.05 level.

RESULTS

Osmolarity of each carbohydrate beverage (Experiment 1)

The osmolarity of a beverage is directly related to the solute composition of the beverage. Research indicates that sports drinks should be hypotonic or isotonic to ensure rapid gastric emptying (*14*). The osmolarity of each administered carbohydrate beverage was plotted in Fig. 1. The osmolarity of the glucose beverage was about twice as high as the plasma osmolarity of fasted mice, shown as a dotted line. The other beverage showed lower osmolarity compared to the plasma osmolarity, and the osmolarity of NSG and maltodextrin1 were over 100 mOsmol/L and that of ESG and maltodextrin2 were below 20 mOsmol/L. Thus, our results indicate that all the administered carbohydrate beverages were hypotonic and only the glucose beverage was hypertonic.
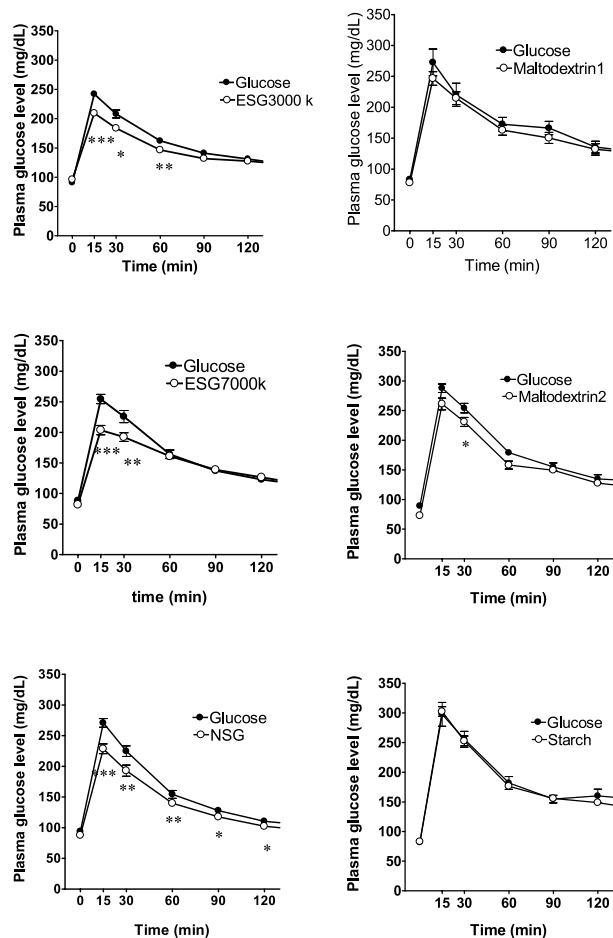
Osmolarity of gastric contents after carbohydrate ingestion (Experiment 1)

We orally administered carbohydrate beverages to mice and gastric contents were collected immediately and 5 min after administration. In all groups of mice, fluid osmolarity was changed in the stomach. ESG and the other carbohydrate beverage raised their osmolarity, whereas only glucose beverage decreased its osmolarity within 1 min after administration. The osmolarity of gastric contents in ESG and maltodextrin2 were lower than for the other carbohydrate beverage 5 min after administration, but the differences were not significant. The water group showed no change in osmolarity within 5 min after administration.

Gastric emptying time after carbohydrate ingestion (Experiment 1)

It is thought that the rate of gastric emptying is the main limiting factor in the assimilation of ingested beverage (*3, 4*). Any delay in gastric emptying is detrimental to the effectiveness of a beverage in situations where the constituents of the beverage need to be rapidly utilized. We measured the stomach weight twice, before
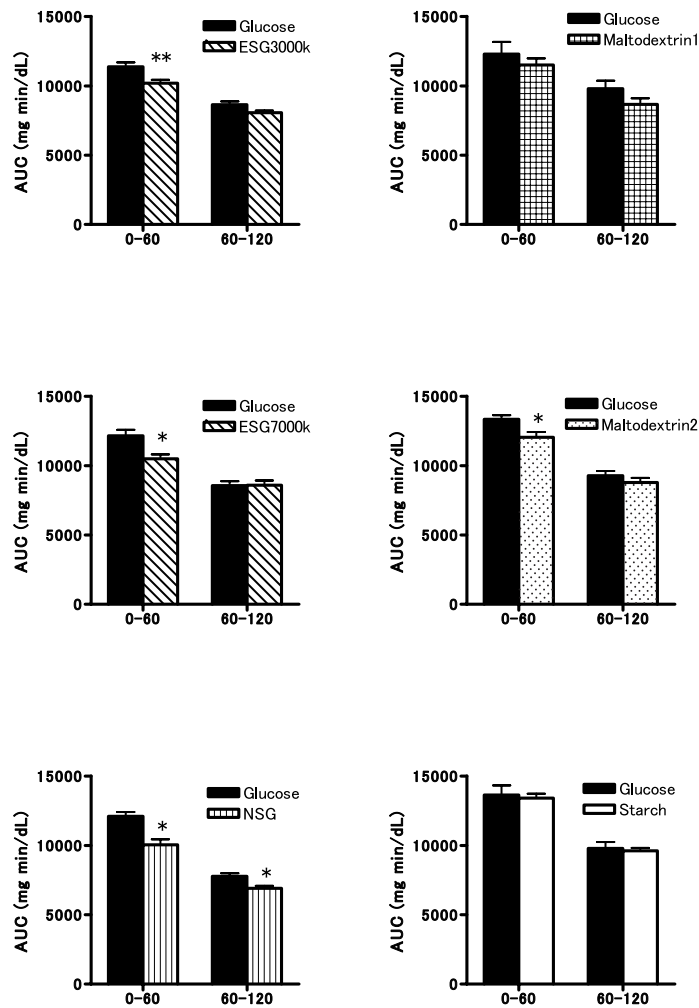
Fig. 4.   Area under the curve of plasma glucose level for the first 60 min (A) and 120 min (B) after an administration of carbohydrate beverages. Mice were orally administered 10 μL/g body weight of a beverage after 18 h of fasting. Plasma was collected before and 0, 15, 30, 60, 90, 120 and 180 min after the administration of carbohydrate beverages. Values are means$\pm$SE ($n=8$–22). $*p<0.05$, $**p<0.01$ vs glucose, paired t-test.

and after the collection of gastric content, and regarded the difference as the weight of gastric content. Gastric content weight was significantly smaller in the ESG group than in the glucose group ($p<0.01$). Gastric content weights of the glucose group were about 50% higher than for the three carbohydrate beverages (NSG, maltodextrin1, and maltodextrin2, $p<0.05$), whereas the gastric content weights of the ESG and water groups were about 40% lower than those of the three carbohydrate beverages (Fig. 2). Accordingly, all the carbohydrate solutions except for ESG delayed gastric emptying time compared to water; in contrast, the average gastric emptying time of ESG solution was not different compared to water.

Plasma glucose, insulin and FFA level after carbohydrate ingestion (Experiment 2)

During exercise, working skeletal muscles consume glucose as an energy, so the carbohydrate source in sports drink should elevate the plasma glucose level rapidly or slowly but sustainably. We investigated whether plasma glucose elevation after administration of ESG beverage is the rapid type or the sustainable type. Plasma glucose elevation in the ESG group was smaller

than in glucose, whereas plasma glucose decline was slower in the ESG group than in the glucose group. The peak plasma glucose levels of the maltodextrin1, maltodextrin2 and NSG group were also lower than for the glucose group. These data revealed that ESG did not elevate plasma glucose rapidly compared to glucose.

We, therefore, focused on the sustainability of the plasma glucose level after ESG administration. The fall of the plasma glucose level occurred in 15 min after administration in all groups and the differences in plasma glucose level between the glucose and carbohydrate group gradually decreased over time. The plasma glucose value of the ESG group reached the same value as that of the glucose group 60 min after administration. On the other hand, the plasma glucose level of maltodextrin1, maltodextrin2 and NSG did not reach the same value as glucose group 60 min after administration. Therefore, we judged that ESG solution slowly elevated the plasma glucose level and maintained it for a prolonged time compared to glucose solution (Fig. 3).

The AUC of the plasma glucose level also shows the sustainability of plasma glucose after ESG administration. The AUC of the plasma glucose level from 0 to

60 min was significantly lower in ESG, as well as NSG and maltodextrin2, compared to the glucose group. However the AUC of the plasma glucose level of the ESG group from 60 to 120 min was the same as for the glucose group ($p=0.95$). On the other hand, the other carbohydrate solutions such as NSG ($p<0.0001$), maltodextrin1 ($p<0.10$) and maltodextrin2 ($p=0.25$) tended to display lower AUCs for plasma glucose from 60 to 120 min. These data could indicate that plasma glucose gradually declines after ESG administration compared to glucose solution administration (Fig. 4).

The change in the glucose level in the starch group was different from that of the other group of mice. There were no differences between the starch and glucose group from 0 to 90 min after administration. The difference between starch and glucose was observed from 90 to 180 min, though there were no significant differences. The starch group also showed a lower AUC for plasma glucose level compared to the glucose group from 120 to 180 min (data not shown).
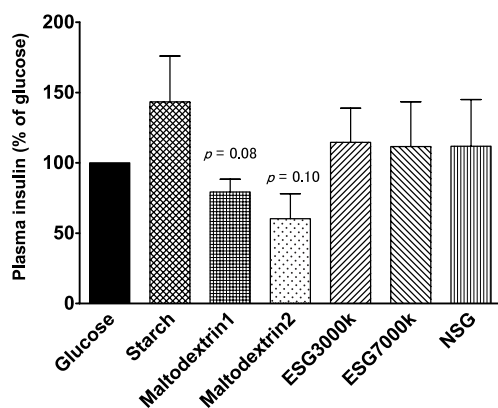

Fig. 5. Plasma insulin level at 15 min after an administration of carbohydrate beverages. Mice were orally administered 10 μL/g body weight of a beverage after 18 h of fasting. Values are means±SE ($n=8$–22).

Plasma insulin levels in the ESG 3,000k, ESG 7,000k and NSG groups were not lower than in the glucose group, whereas plasma glucose levels of these groups were lower than for the glucose group. Plasma insulin levels in both maltodextrin groups tended to be lower than in the glucose group ($p=0.08$ and 0.10, Fig. 5).

Plasma FFA levels were at the highest values 30 min after administration in all the groups except for the starch group. Plasma FFA levels in maltodextrin2, ESG 3,000k and ESG 7,000k were higher than in glucose at the highest value, but the differences were not significant. On the other hand, plasma FFA levels in maltodextrin1 and NSG were lower than in glucose at the highest value and the differences were not significant (Fig. 6).

Post exercise muscle glycogen and plasma prameters (Experiment 3)

In the groups given low and middle doses (0.42 and 0.86 mg/g body weight/h), plasma insulin levels were lower than in the corresponding glucose groups and there was a significant difference in the dose of 0.86 mg/g body weight/h but there were no differences in plasma glucose level in either dose group. Though there were no significant differences in the other parameters, all the doses of ESG group showed a higher average FFA level and lower average lactic acid level.

In the ESG group given the highest doses (1.68 mg/g body weight/h), the plasma glucose level was significantly lower than in the corresponding glucose group ($p<0.05$) and the plasma insulin level in the ESG group was higher than in the glucose group but the differences were not significant (Table 2).

DISCUSSION

In this study we found that a newly developed ESG solution does not delay gastric emptying time compared to other carbohydrate solutions, such as glucose or maltodextrins. We also observed that ESG slowly elevated plasma glucose level and maintained it for a prolonged
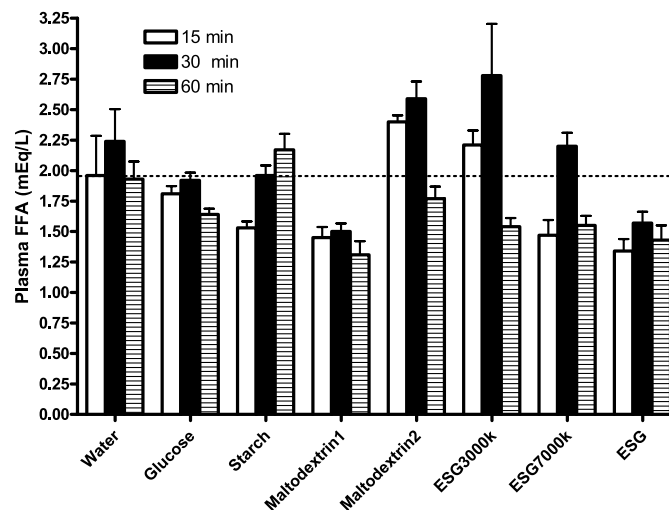

Fig. 6. Plasma FFA level at 15, 30 and 60 min after an administration of carbohydrate beverages. Mice were orally administered 10 μL/g body weight of a beverage after 18 h of fasting. The dotted line indicates the FFA level of the glucose group at 30 min after administration. Values are means±SE ($n=8$–22).

Table 2.   Post-exercise muscle glycogen and plasma parameters.

		Dose (mg/g body weight/h)		
		0.42	0.84	1.68
Glucose	Glucose	169.8±14.5	206.3± 9.6	273.1±9.7
(mg/dL)	ESG	146.1±7.3	208.0±9.2	232.1±7.7*
Triglyceride	Glucose	97.7±7.4	74.9±8.0	63.2±4.8
(mg/dL)	ESG	114.4±12.4	78.3±4.5	55.9±2.9
FFA	Glucose	1.00±0.15	0.83±0.03	0.94±0.03
(mEq/dL)	ESG	1.29±0.09	0.89±0.09	0.95±0.04
Glycerol	Glucose	0.026±0.004	0.030±0.012	0.014±0.005
(g/L)	ESG	0.030±0.004	0.028±0.013	0.018±0.005
Insulin	Glucose	804±320	792±154	829±270
(ng/L)	ESG	520±185	398±52*	1,381±535
Gastrocnemius muscle	Glucose	1.73±0.16	1.14±0.20	2.59±0.36
glycogen (mg/g)	ESG	1.63±0.27	1.37±0.29	2.37±0.28

Values are means±SE ($n=8$–22). *$p<0.05$ vs corresponding glucose group.

time compared to glucose and other carbohydrate solutions. These observed properties of ESG solution could be applicable to carbohydrate source and water supply during exercise.

During exercise, an adequate supply of substrates is required for the continuing resynthesis of ATP in working muscles. Muscle fibers have limited stores of glycogen and so must also be able to extract glucose from the blood to meet the energy needs of the contracting fiber. An adequate supply of carbohydrate (in the form of blood glucose and muscle glycogen) is clearly related to exercise performance. Therefore an adequate ingestion of various forms of carbohydrate before, during, and after exercise has been systematically studied as a means of extending that fuel supply for a longer period of time (*15*).

Excessive increase of plasma glucose may inhibit lipid oxidation during exercise and reduce endurance exercise performance. Recent studies reported that the high glycemic index diet caused rapid elevation of plasma glucose and inhibited lipid oxidation during exercise in trained cyclists (*16*), and women (*17–19*). Pre-exercise insulin elevation by glucose ingestion could cause hypoglycemia during exercise because of enhanced glucose incorporation by insulin and muscle contraction (*20*) and could cause glycogen depletion (*21*). In the present study, ESG administration did not elevate plasma insulin level except for the doses of 1.68 mg/g body weight/h (Experiment 3). Observed higher plasma FFA level, lower lactic acid level and equal muscle glycogen level in the ESG group also indicated that ESG administration did not inhibit lipid oxidation during exercise (Experiment 3).

Maintaining a constant plasma glucose level is important for endurance athletes as well as diabetic subjects. As is well known, in insulin-dependent diabetic subjects, two types of insulin, namely continuous and rapid types, are used to maintain a constant plasma glucose level (*22*). Similarly in some kinds of sports drinks for endurance athletes, two types of carbohydrates are used and one, such as a monosaccharide or disaccharide, is rapidly absorbed while the other, such as maltodextrin, is slowly absorbed in the gastrointestinal tract. As shown in this study, ESG is absorbed as slowly as maltodextrin and furthermore, ESG is superior in maintainability of the plasma glucose level for a longer time compared to maltodextrin.

Thus the combination of ESG and a simple sugar could supply a carbohydrate source to working muscle fiber for an extended period of time at moderate speed. Combination of multiple food components could increase the osmolarity of the solution and delay gastric pressure and water absorption. On this point, the osmotic pressure of ESG is quite as low as water, and ESG does not block the maintenance of the osmotic pressure of the solution within hypotonic level.

Two observed properties of ESG solution, namely the slow elevation of plasma glucose and the fast gastric emptying time, may seem like a contradiction, but the slight amylase resistance of ESG could resolve the discrepancy. During the incubation with rat intestinal acetone powder, the digestibility of ESG was 15% less than that of dextrin (DE2-5). Partially degraded ESG (with alpha-amylase) activates murine macrophage cell line in the previous study (*23*).

In conclusion, a newly developed ESG solution does not delay gastric emptying time compared to other carbohydrate solutions, such as glucose or maltodextrins. ESG slowly elevated plasma glucose level and maintained it for a prolonged time compared to glucose. These observed properties of ESG solution could be applicable to carbohydrate source and water supply during exercise.

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