

- 1 -

Description

AGENT FOR IMPROVING EARLY INSULIN SECRETORY CAPACITY

Field of the Invention

[0001]

The present invention relates to an agent for improving early insulin secretory capacity.

Background of the Invention

[0002]

Diabetes is a metabolic disease in which a hyperglycemic condition is a cardinal symptom. Continued chronic hyperglycemia is known not only to be complicated by microangiopathy such as nephropathy, retinopathy, and neuropathy but also to facilitate development of arteriosclerotic diseases such as myocardial infarction and cerebral infarction. In other words, it can be said that diabetes is a disease which dramatically influences the life expectancy in addition to QOL. Diabetes includes a plurality of disease types, among which major disease types are type 1 diabetes and type 2 diabetes.

[0003]

Of these, in type 2 diabetes, a reduction in the insulin secretory capacity of pancreatic β cells and a reduction in the insulin sensitivity (insulin resistance) in target tissue such as the liver, skeletal muscles, and adipose tissue result in relative insufficiency in the

effect of insulin to thereby cause hyperglycemia. Diabetes as a lifestyle related disease rapidly increasing now is type 2 diabetes, and the development thereof is strongly influenced by the Western lifestyle such as overeating, high-fat diets, and inadequate exercise as environmental factors.

Type 2 diabetes in Japanese people is often mainly caused by a reduction in the insulin secretory capacity, particularly in the early insulin secretory capacity, which is necessary for inhibiting a rapid increase in the blood glucose level after carbohydrate ingestion, rather than by the insulin resistance. Accordingly, a method for improving the early insulin secretory capacity is desired.

[0004]

In contrast, as reports with respect to lactic acid bacteria, it has been reported that the polysaccharide-glycan complex of the cell wall of a gram-positive bacterium inhibits an increase in the blood glucose level and urine glucose (Patent Literature 1) and that bacterial cells (dead cells) of *Lactobacillus casei* have an action of inhibiting an increase in blood glucose (Non Patent Literature 1).

Citation List

Patent Literature

[0005]

Patent Literature 1: JP-A-H8-59492

Non Patent Literature

[0006]

Non Patent Literature 1: Endocrine Journal 1997, 44(3),
357-365

Summary of the Invention

Problems to be Solved by the Invention

[0007]

However, conventional hypoglycemic agents, which focus extremely on improvements in fasting hyperglycemia, have a risk of causing hypoglycemia. Of these, insulin secretagogues including sulfonylurea agents and glinide agents increase not only postprandial early secretion (first-phase additional secretion) but also late secretion (second-phase additional secretion) and fasting basal secretion. Thus, there has been a problem in that exhaustion of pancreatic β cells is brought about, in addition to a hypoglycemia risk, to thereby result in a further reduction in the insulin secretory capacity and hyperglycemia. To solve such a problem, and in view of the fact that type 2 diabetes in Japanese people is mainly caused by a reduction in the early insulin secretory capacity, as described above, development of agents for improving only the early secretory capacity is desired.

Accordingly, an object of the present invention is to provide a novel agent for improving early insulin secretory capacity.

Means for Solving the Problems

[0008]

Accordingly, the present inventors studied to solve the problem described above and found that viable cells, not dead cells, of a lactic acid bacterium have specifically excellent action of improving the early insulin secretory capacity, to thereby complete the present invention.

[0009]

That is, the present invention provides the following [1] to [9].

[0010]

[1] An agent for improving early insulin secretory capacity, comprising, as an active ingredient, a composition comprising 1×10^{10} or more viable cells of a lactic acid bacterium.

[2] The agent for improving early insulin secretory capacity according to [1], wherein the agent for improving early insulin secretory capacity is configured such that 1×10^{10} or more viable cells of a lactic acid bacterium are ingested per day.

[3] The agent for improving early insulin secretory capacity according to [1] or [2], wherein the lactic acid bacterium is a lactic acid bacterium belonging to the genus *Lactobacillus*.

[4] The agent for improving early insulin secretory capacity according to any of [1] to [3], wherein the lactic acid bacterium is *Lactobacillus casei*.

[5] The agent for improving early insulin secretory capacity according to any of [1] to [4], wherein the lactic acid bacterium is *Lactobacillus casei* YIT9029.

[6] Use of a composition comprising 1×10^{10} or more viable cells of a lactic acid bacterium for producing an agent for improving early insulin secretory capacity.

[7] A composition comprising 1×10^{10} or more viable cells of a lactic acid bacterium for use in improving early insulin secretory capacity.

[8] A method for improving early insulin secretory capacity, comprising administering to a subject in need thereof or ingesting an effective amount of a composition comprising 1×10^{10} or more viable cells of a lactic acid bacterium.

[9] The method for improving early insulin secretory capacity according to [8], wherein 1×10^{10} or more viable cells of a lactic acid bacterium are ingested per day.

Effects of the Invention

[0011]

It was found that, in the agent for improving early insulin secretory capacity of the present invention, viable cells of a lactic acid bacterium have particularly potent action. The agent, which improves only the early

insulin secretory capacity, has a lower hypoglycemia risk and a lower risk of further inducing hyperglycemia caused by exhaustion of pancreatic β cells.

Modes for Carrying out the Invention

[0012]

The active ingredient of the agent for improving early insulin secretory capacity of the present invention is a composition comprising 1×10^{10} or more viable cells of a lactic acid bacterium. As shown in Examples described later, lactic acid bacteria containing a large amount of dead bacteria have no effect of improving the early insulin secretion, and thus, the effect of the present invention is specific to viable cells of the lactic acid bacterium. The viable bacterial cells as used herein can be measured, for example, by applying the composition diluted as appropriate with saline or Ringer's solution onto a medium suitable for measurement of lactic acid bacteria, such as a plate-count agar medium with BCP or a TOS propionate medium with mupirocin, and counting colonies of the lactic acid bacterium appeared after culturing at 37°C for three days. It should be noted that, in the case of an obligate anaerobe, the anaerobe may be maintained in an anaerobic jar degassed and filled with carbon dioxide and/or nitrogen.

[0013]

Examples of the lactic acid bacterium include, without a particular limitation, the genus *Lactobacillus*,

the genus *Lactococcus*, the genus *Enterococcus*, the genus *Pediococcus*, the genus *Leuconostoc*, and the genus *Bifidobacterium*. Of these, the genus *Lactobacillus* and the genus *Bifidobacterium* are preferable, and the genus *Lactobacillus* is more preferable.

Additionally, examples of the lactic acid bacteria of the genus *Lactobacillus* include *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus zeae*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus gasseri*. Of these, *Lactobacillus casei* is preferable, and among *Lactobacillus casei*, *Lactobacillus casei* YIT9029 (FERM BP-1366) is more preferable.

[0014]

The viable cells of the lactic acid bacterium have an effect of improving an early insulinogenic index, as shown in Examples described later. The early insulinogenic index as used herein refers to an early insulinogenic index calculated by the following expression from the fasting blood glucose and fasting blood insulin before glucose loading and the blood glucose and blood insulin 30 minutes after glucose loading in a 75-g oral glucose tolerance test.

[0015]

Early insulinogenic index

$$= \frac{\text{Insulin 30 minutes after loading} - \text{Fasting insulin } (\mu\text{U/mL})}{\text{Blood glucose 30 minutes after loading} - \text{Fasting blood glucose (mg/dL)}}$$

[0016]

The early insulinogenic index of a healthy individual is 0.4 or more, and the early insulinogenic index of an individual having an abnormal early insulin secretory capacity is less than 0.4. It is said that individuals having an early insulinogenic index of less than 0.4 have a high likelihood of developing diabetes.

[0017]

In the present invention, an improvement in the early insulin secretory capacity means that such an early insulinogenic index is brought to the level of a healthy individual, for example, the early insulinogenic index is brought to a level of 0.4 or more, preferably 0.4 to 5.0. Additionally, the improvement also means that the additional insulin secretion of the second phase is not allowed to increase, preferably, the blood insulin concentration 60 minutes after glucose loading or the blood insulin concentration 120 minutes after glucose loading is not allowed to increase.

[0018]

When an individual having an early insulinogenic index of less than 0.4 was allowed to ingest 1×10^{11} cells of the lactic acid bacterium per day, the early insulinogenic index was improved. In contrast, in the case where the amount of the viable cells of the lactic acid bacterium ingested was small, no effect of improving the early insulinogenic index was achieved.

Accordingly, a composition comprising 1×10^{10} or more viable cells of the lactic acid bacterium is preferably ingested per day, a composition comprising 1×10^{11} or more viable cells of the lactic acid bacterium is more preferably ingested per day, and a composition comprising 1×10^{11} to 1×10^{12} viable cells of the lactic acid bacterium is even more preferably ingested per day.

[0019]

Target subjects for the agent for improving early insulin secretory capacity of the present invention are not particularly limited, but they are preferably human subjects having an early insulinogenic index of less than 0.4, in other words, patients with type 2 diabetes or individuals likely to develop type 2 diabetes, and, in particular, more preferably those likely to develop type 2 diabetes. Those likely to develop type 2 diabetes as used herein mean individuals having an early insulinogenic index of less than 0.4 as well as a fasting blood glucose level of 100 mg/dL or more and/or a blood glucose level 120 minutes after glucose loading of 130 mg/dL or more, and refer to individuals on the borderline between diabetic patients and healthy persons.

[0020]

It is preferred that the agent for improving early insulin secretory capacity of the present invention be orally ingested in one to three portions per day. Additionally, as for the ingestion period, the agent is ingested preferably consecutively for one week or more,

is ingested more preferably consecutively for four weeks or more, and is ingested even more preferably consecutively for eight weeks or more.

[0021]

The formulation forms of the agent for improving early insulin secretory capacity of the present invention are not limited as long as the agent comprises a composition comprising 1×10^{10} or more viable cells of the lactic acid bacterium. For example, the agent can be made into a commonly-used pharmaceutical formulation. Examples of such a formulation include solid formulations such as tablets, granules, powders, and capsules, liquid formulations such as solutions, suspensions, and emulsions, and lyophilized formulations. These formulations can be prepared by being mixed with a non-toxic pharmaceutical carrier as appropriate according to conventional means for formulation. Examples of such a non-toxic pharmaceutical carrier include glucose, lactose, sucrose, starch, mannitol, dextrin, fatty acid glyceride, polyethylene glycol, hydroxyethyl starch, ethylene glycol, polyoxyethylene sorbitan fatty acid ester, amino acid, gelatin, albumin, water, and saline. Additionally, commonly-used additives such as a stabilizer, a humectant, an emulsifier, a binder, a tonicity agent, and an excipient are appropriately added as required.

[0022]

The viable cells of the lactic acid bacterium are preferably used in the form of a fermented product from

the viewpoints of ease of ingestion, continuity of ingestion, and the like.

[0023]

As fermented products as used herein, fermented milk, fermented soy milk, fermented fruit juice, fermented plant liquid and the like prepared by fermentation by means of the viable cells of the lactic acid bacterium, which are the active ingredient, are suitably used. These fermented products can be produced in accordance with routine methods. For example, as for fermented milk, the viable cells of the lactic acid bacterium are inoculated and cultured in a sterilized milk medium. The resultant culture is homogenized to obtain a fermented milk base. Meanwhile, a syrup solution separately prepared is then added thereto and mixed, and the mixture is homogenized by a homogenizer or the like. A flavor is further added thereto and thus a final product can be obtained.

[0024]

On culturing, fermentation may be carried out by using, in combination with the lactic acid bacterium, other microorganism, for example, yeasts such as the genus *Saccharomyces*, the genus *Candida*, the genus *Rhodotorula*, the genus *Pichia*, the genus *Schizosaccharomyces*, the genus *Torula*, and the genus *Zygosaccharomyces*, and filamentous fungi such as the genus *Aspergillus*, the genus *Penicillium*, the genus *Eurotium*, the genus *Monascus*, the genus *Mucor*, the genus

Neurospora, and the genus Rhizopus, but fermentation is preferably carried out only by using the lactic acid bacterium.

Examples

[0025]

The present invention is described hereinbelow in further detail with reference to Examples.

[0026]

Production Example 1 (Production of a composition of the present invention)

To a powdered skim milk solution in which *Lactobacillus casei* YIT9029 had been inoculated and which then had been fermented at 37°C, syrup was mixed. After addition of a fragrance, the mixture was homogenized and charged into a vessel, so that the composition of the present invention was obtained. The viable bacterial count of the *Lactobacillus casei* in 100 ml of this fermented milk product was 1×10^{11} cells.

Additionally, a composition which contained no *Lactobacillus casei* and had a flavor adjusted by addition of lactic acid equivalent to the fermented milk product was used as a placebo. It should be noted that the placebo had the same composition as the composition of the present invention except the above.

[0027]

Test 1 (Example)

(Experimental method)

Nineteen males (having an average early insulinogenic index, an average fasting blood glucose level, and an average blood glucose level 120 minutes after glucose loading of 0.28, 107 mg/dL, and 172 mg/dL, respectively) having an early insulinogenic index of less than 0.4 were allowed to ingest a bottle (100 ml) of the composition containing 1×10^{11} viable cells of the *Lactobacillus casei* of Production Example 1 (hereinbelow, the composition is referred to as the composition of the present invention) per day for eight weeks, and seventeen males (having an average early insulinogenic index, an average fasting blood glucose level, and an average blood glucose level 120 minutes after glucose loading of 0.29, 111 mg/dL, and 178 mg/dL, respectively) having an early insulinogenic index of less than 0.4 were allowed to ingest a bottle (100 mL) of the placebo containing no *Lactobacillus casei* per day for eight weeks. It should be noted that the test subjects were instructed, during the test, not to change their lifestyles for meals, drinking, sleep and others as much as possible from before participation in the test, and to avoid excess exercise, abstemious diets, and overeating significantly deviating from the usual range.

[0028]

(Results)

The early insulinogenic indices before, four weeks after, and eight weeks after ingestion are shown in Table 1. As shown in Table 1, it was confirmed that ingestion

of 1×10^{11} viable cells of the *Lactobacillus casei* per day improved the early insulin secretion compared with the placebo. Additionally, the group in which 1×10^{11} viable cells of the *Lactobacillus casei* per day were ingested showed substantially no change in the fasting blood insulin concentration, which is an index of the amount of basal insulin secretion, although the results are not shown. Furthermore, since the blood insulin concentration 60 minutes after 75 g glucose loading (Table 2) and the blood insulin concentration 120 minutes after 75 g glucose loading (Table 3), which are indices of the amount of the additional secretion of the second phase, were reduced, it was confirmed that the composition of the present invention did not increase the basal insulin secretion and the additional secretion of the second phase. From this, it was confirmed that ingestion of 1×10^{11} viable cells of the *Lactobacillus casei* per day specifically improved the early insulin secretory capacity.

[0029]

[Table 1]

(Unit (μ U/ml) / (mg/dl))

	Measured value			Change rate		
	0w	4w	8w	0w	4w	8w
Composition of the present invention	0.28	0.40	0.41	-	43.68%	50.39%
Placebo	0.29	0.30	0.33	-	8.21%	17.43%

*The values are average values.

[0030]

[Table 2]

(Unit μ U/ml)

	Measured value			Change rate		
	0w	4w	8w	0w	4w	8w
Composition of the present invention	51.6	46.6	45.9	-	-2.55%	-4.34%
Placebo	50.7	54.8	55.5	-	5.83%	4.84%

[0031]

[Table 3]

(Unit μ U/ml)

	Measured value			Change rate		
	0w	4w	8w	0w	4w	8w
Composition of the present invention	61.0	51.1	55.4	-	-2.51%	-4.97%
Placebo	52.6	57.3	62.9	-	6.91%	10.32%

[0032]

Test 2 (Comparative experiment)

(Experimental method)

Eight males (having an average early insulinogenic index, an average fasting blood glucose level, and an average blood glucose level 120 minutes after glucose loading of 0.24, 117 mg/dL, and 173 mg/dL, respectively)

having an early insulinogenic index of less than 0.4 were allowed to ingest a packet (2 g) of powders containing 4×10^9 viable cells of and 2×10^{11} dead cells of the *Lactobacillus casei* per 2 g per day for eight weeks, and seven males (having an average early insulinogenic index, an average fasting blood glucose level, and an average blood glucose level 120 minutes after glucose loading of 0.16, 121 mg/dL, and 189 mg/dL, respectively) having an early insulinogenic index of less than 0.4 were allowed to ingest a packet (2 g) of a placebo containing no *Lactobacillus casei* per day for eight weeks. The early insulinogenic indices before and eight weeks after ingestion are shown in Table 4. It should be noted that the powders were prepared by adding powders having powdered skim milk and trehalose mixed at a ratio of 2:1 to lyophilized powders containing viable cells and dead cells of *Lactobacillus casei* YIT9029 to have an adjusted bacterial count as described above and that the placebo was prepared by mixing powdered skim milk and trehalose at a ratio of 2:1. It should be noted that the dead bacterial count in the powders was determined by measuring the total bacterial count of the *Lactobacillus casei* in the powders by the DAPI staining method and subtracting the viable count of the bacterium therefrom.

[0033]

(Results)

As shown in Table 4, in the case where 4×10^9 viable cells of the *Lactobacillus casei* were ingested per

day, it was confirmed that the early insulinogenic index was reduced at the 8th week and the early insulin secretory capacity was not improved.

[0034]

[Table 4]

(Unit (μ U/ml) / (mg/dl))

	Measured value		Change rate	
	0w	8w	0w	8w
Powder	0.24	0.22	-	-7.93%
Placebo	0.16	0.18	-	22.53%

*The values are average values.

- 18 -

Claims

[Claim 1]

An agent for improving early insulin secretory capacity, comprising, as an active ingredient, a composition comprising 1×10^{10} or more viable cells of a lactic acid bacterium.

[Claim 2]

The agent for improving early insulin secretory capacity according to claim 1, wherein the agent for improving early insulin secretory capacity is configured such that 1×10^{10} or more viable cells of a lactic acid bacterium are ingested per day.

[Claim 3]

The agent for improving early insulin secretory capacity according to claim 1 or 2, wherein the lactic acid bacterium is a lactic acid bacterium belonging to the genus *Lactobacillus*.

[Claim 4]

The agent for improving early insulin secretory capacity according to any one of claims 1 to 3, wherein the lactic acid bacterium is *Lactobacillus casei*.

[Claim 5]

The agent for improving early insulin secretory capacity according to any one of claims 1 to 4, wherein the lactic acid bacterium is *Lactobacillus casei* YIT9029.

[Claim 6]

Use of a composition comprising 1×10^{10} or more viable cells of a lactic acid bacterium for producing an agent for improving early insulin secretory capacity.

[Claim 7]

A composition comprising 1×10^{10} or more viable cells of a lactic acid bacterium for use in improving early insulin secretory capacity.

[Claim 8]

A method for improving early insulin secretory capacity, comprising administering to a subject in need thereof or ingesting an effective amount of a composition comprising 1×10^{10} or more viable cells of a lactic acid bacterium.

[Claim 9]

The method for improving early insulin secretory capacity according to claim 8, wherein 1×10^{10} or more viable cells of a lactic acid bacterium are ingested per day.