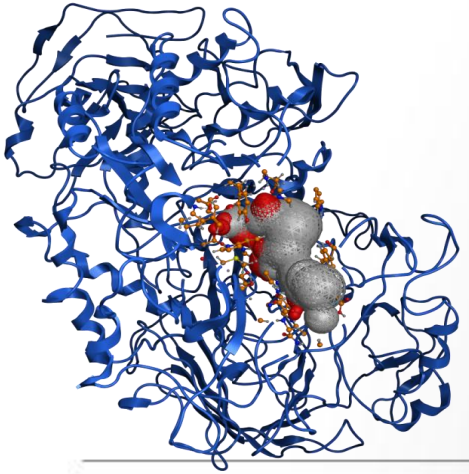
New concept of prebiotics :Prebiotic Enzyme Transglucosidase

Satoshi Koikeda



9th American Biotechnology Congress

What we are



Amano Enzyme **Europe**



Amano Enzyme
China



Amano Enzyme
Japan



Amano Enzyme
Asia Pacific Office



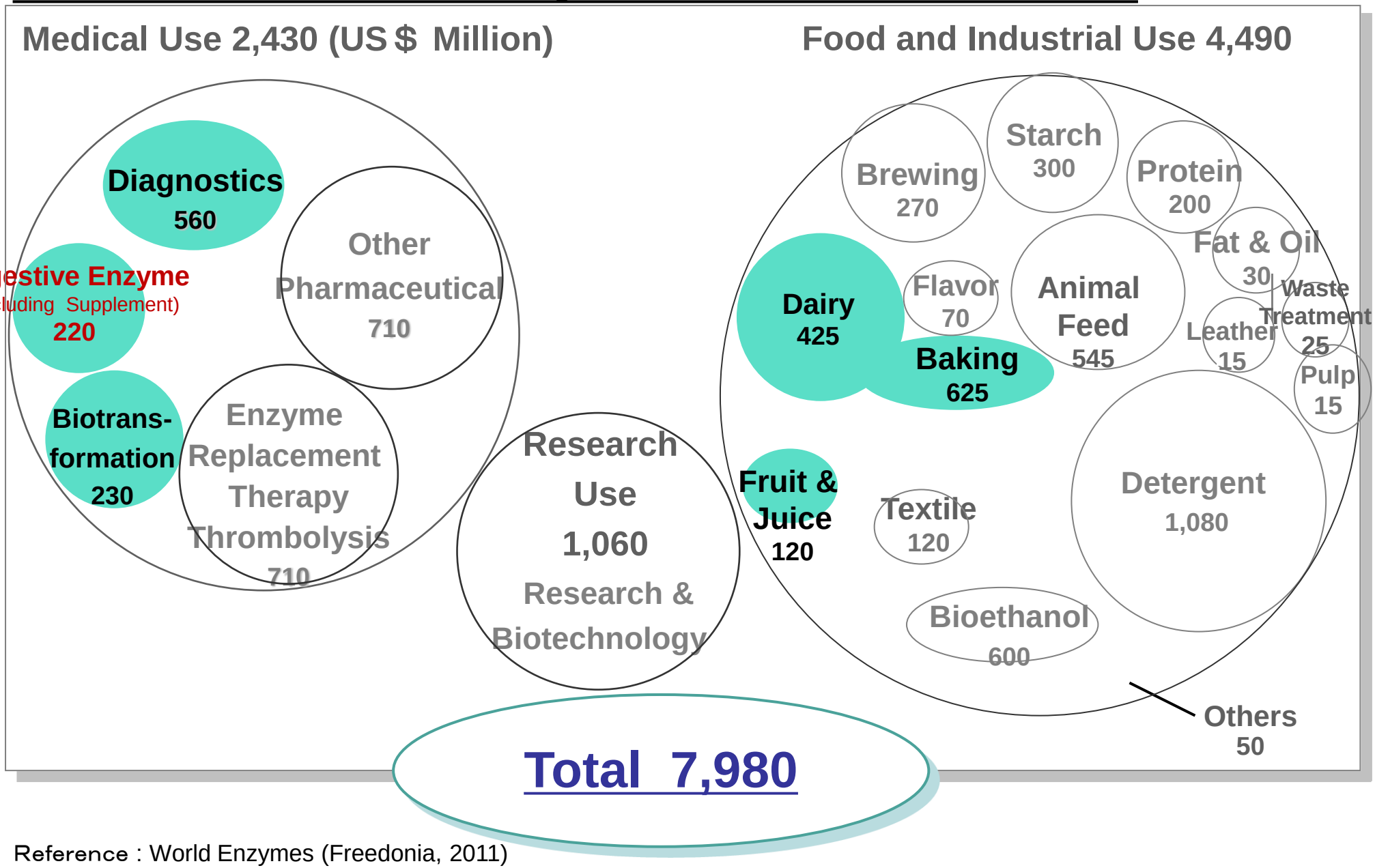
Amano Enzyme
USA



Amano Enzyme
Mexico



World Markets for Enzymes (Estimated) 2015



Reference : World Enzymes (Freedonia, 2011)

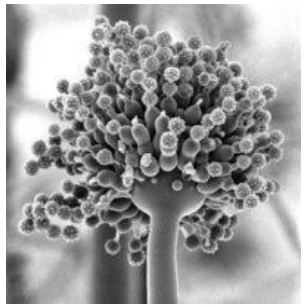
Digestive aid enzyme has a long history

Aspergillus oryzae

Invented by Jōkichi Takamine (1894)



Jōkichi Takamine, 1854 – 1922





Wheat bran

Water extraction
Powderization



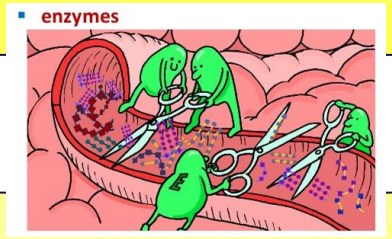
Produced by Parke-Davis (1895)





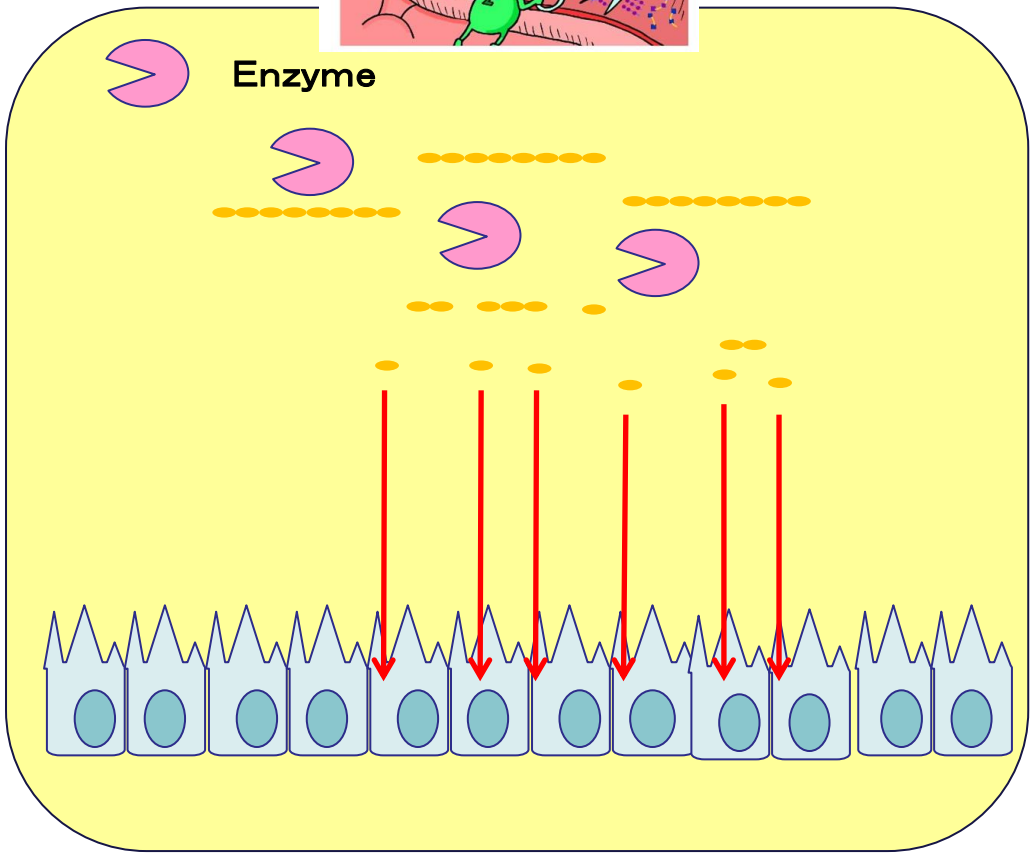
Expected function of digestive aid enzyme

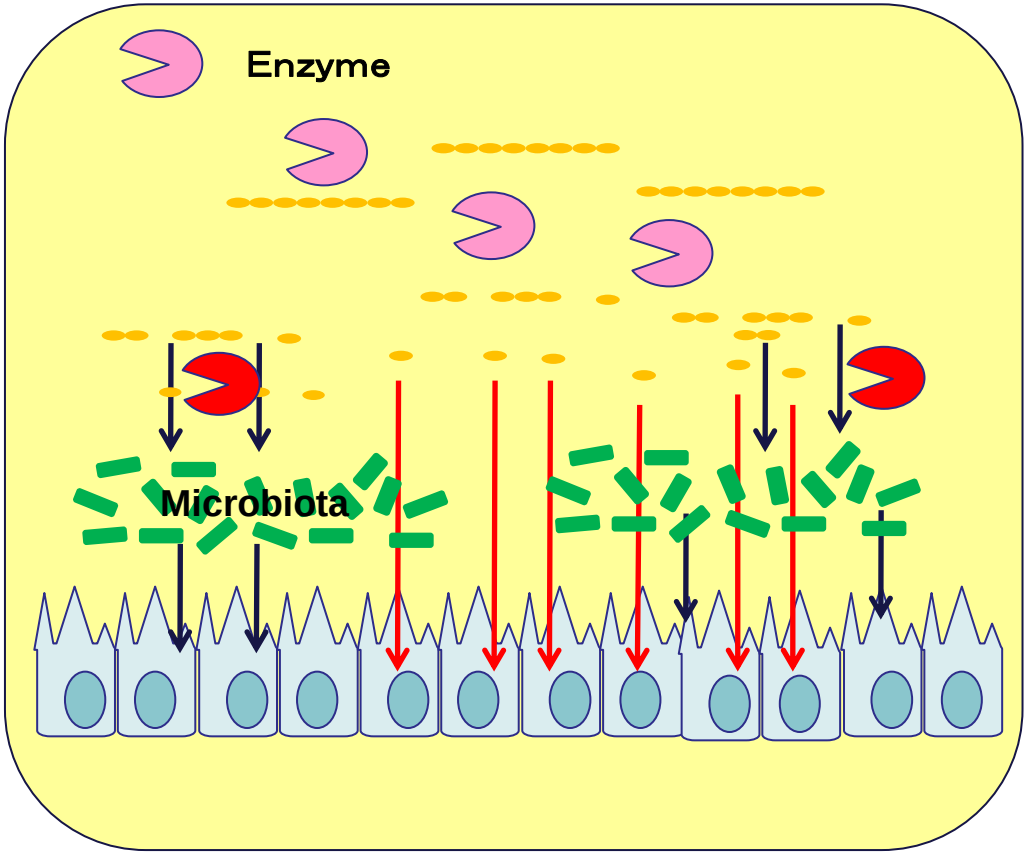
Regulating Nutrition Absorption



Regulating Nutrition absorption and Microbiota System

Novel digestive enzyme





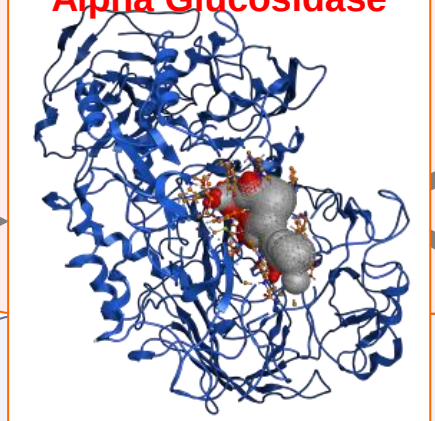
Transglucosidase is bifunctional enzyme

Starch

Maltose

Carbohydrate degradation

Alpha Glucosidase



Glucose

Nutrition

Transglucosidase reaction

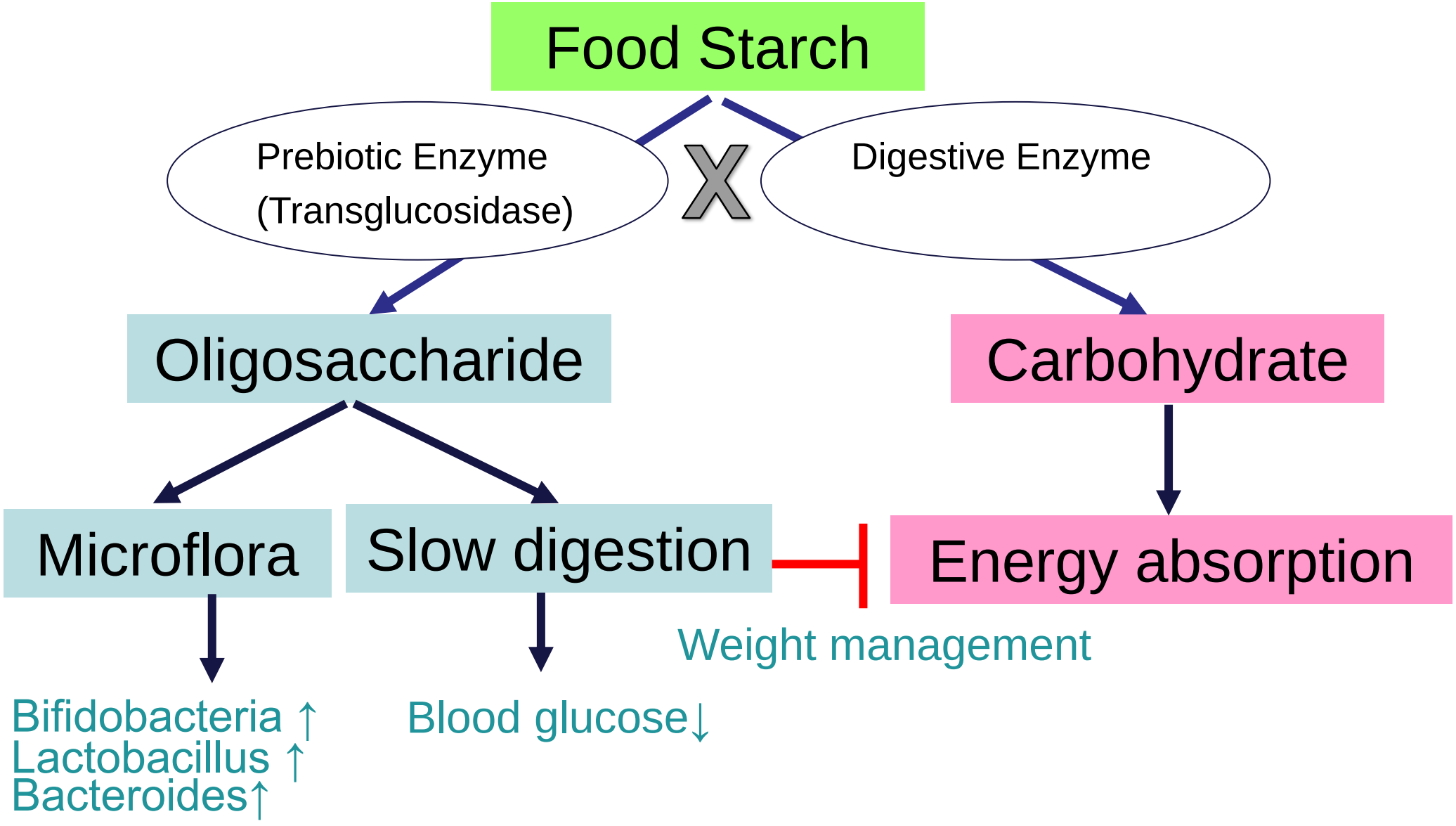
Isomaltose

Prebiotics

panose

Isomaltotriose

Prebiotic enzyme concept on Transglucosiadase



Transformed Oligosaccharides by Transglucosidase

In vitro analysis

Stir and Dissolution with amylase

↓

Adjust pH 5.5

↓

Add TG 90ku

↓

37 ℃, up to 90 min

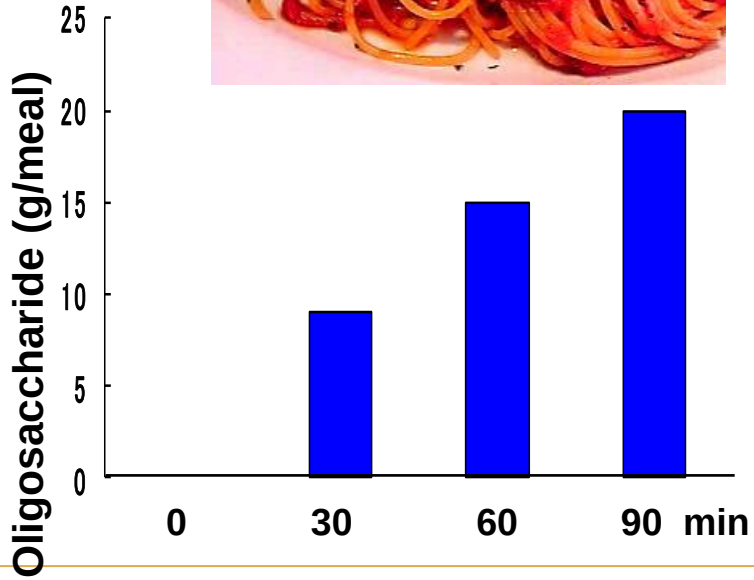
↓

HPLC Analysis

Pasta (100g)

Carbohydrate 70g

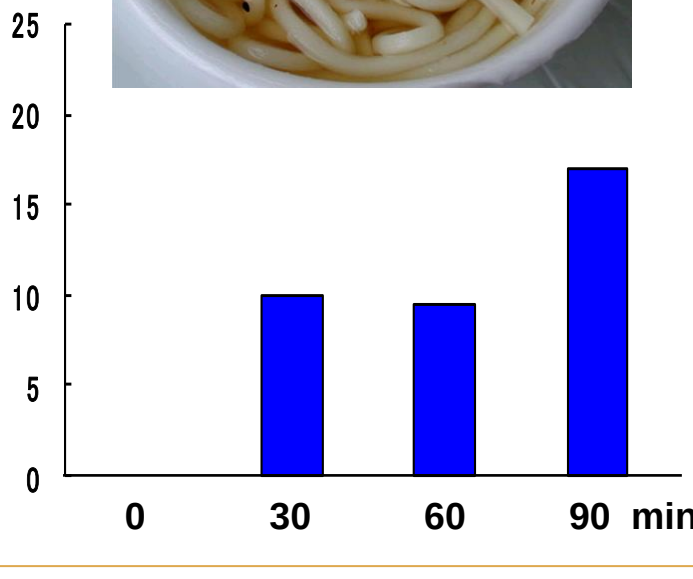




Noodle (100g)

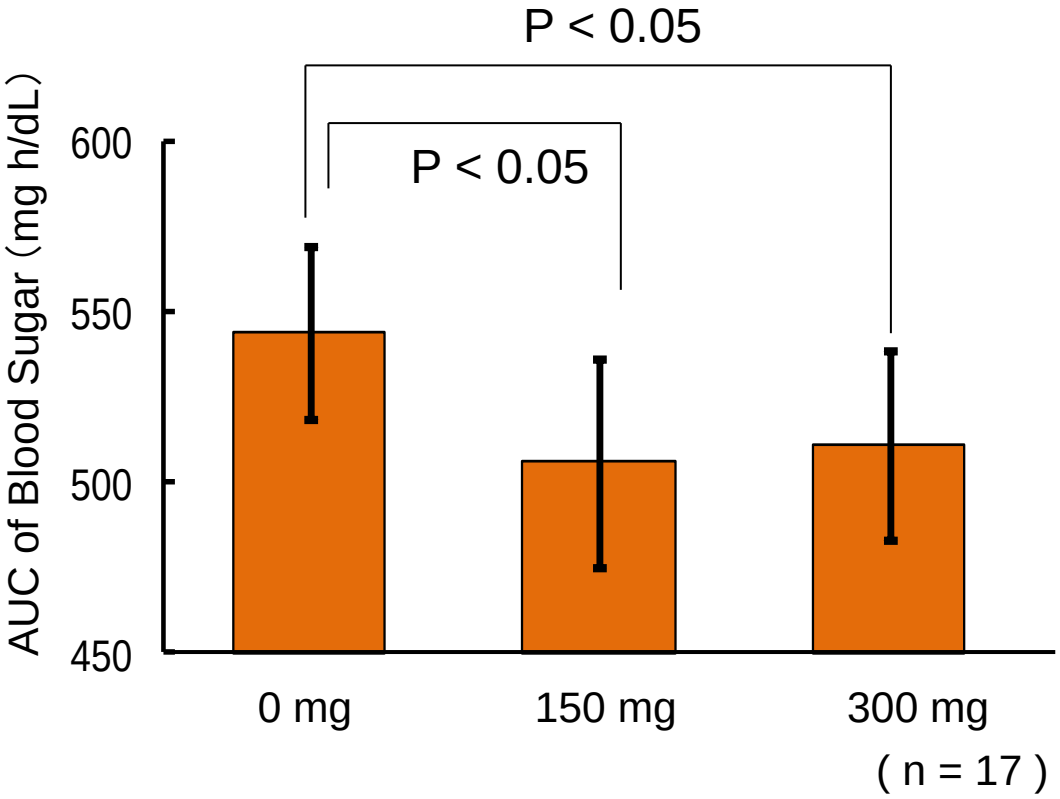
Carbohydrate 70g

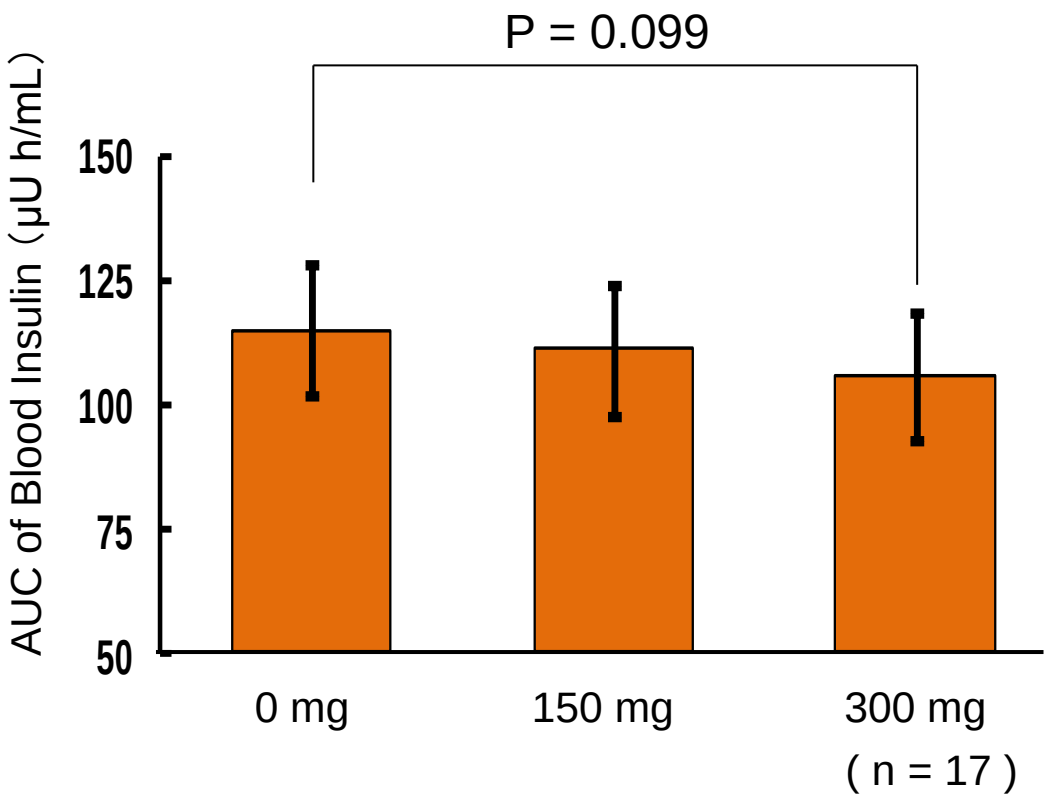




Prevention of Postprandial Hyperglycemia and Hyperinsulinemia in Healthy Volunteer

- 522Kcal meal: Protein (14.4g), Fat (2.1g), Carbohydrate (111g)
  + Transglucosidase
- Blood sampling: every 30 min for 2 h





Sasaki M et al. J. Clin. Biochem. Netr., 41, 191-196, 2007

Efficacy in diabetes patients

Trial Design:

•3 months' randomized, double-blind, placebo-controlled trial

Inclusion criteria:

•Type 2 diabetes outpatient

•HbA1c is stable from 5.8% to 7.5% (JDS)

6.2% to 7.9% (NGSP)

•Stable dosage of medication for at least 1 month

•Stable diabetes condition for at least 3 months

(change in HbA1c less than 1.0%)

Exclusion criterion:

•Gut resection history

Sasaki M et al. J. Clin. Biochem. Netr., 41, 191-196, 2007

Study End Points

Primary outcome:

•The change in HbA1c level

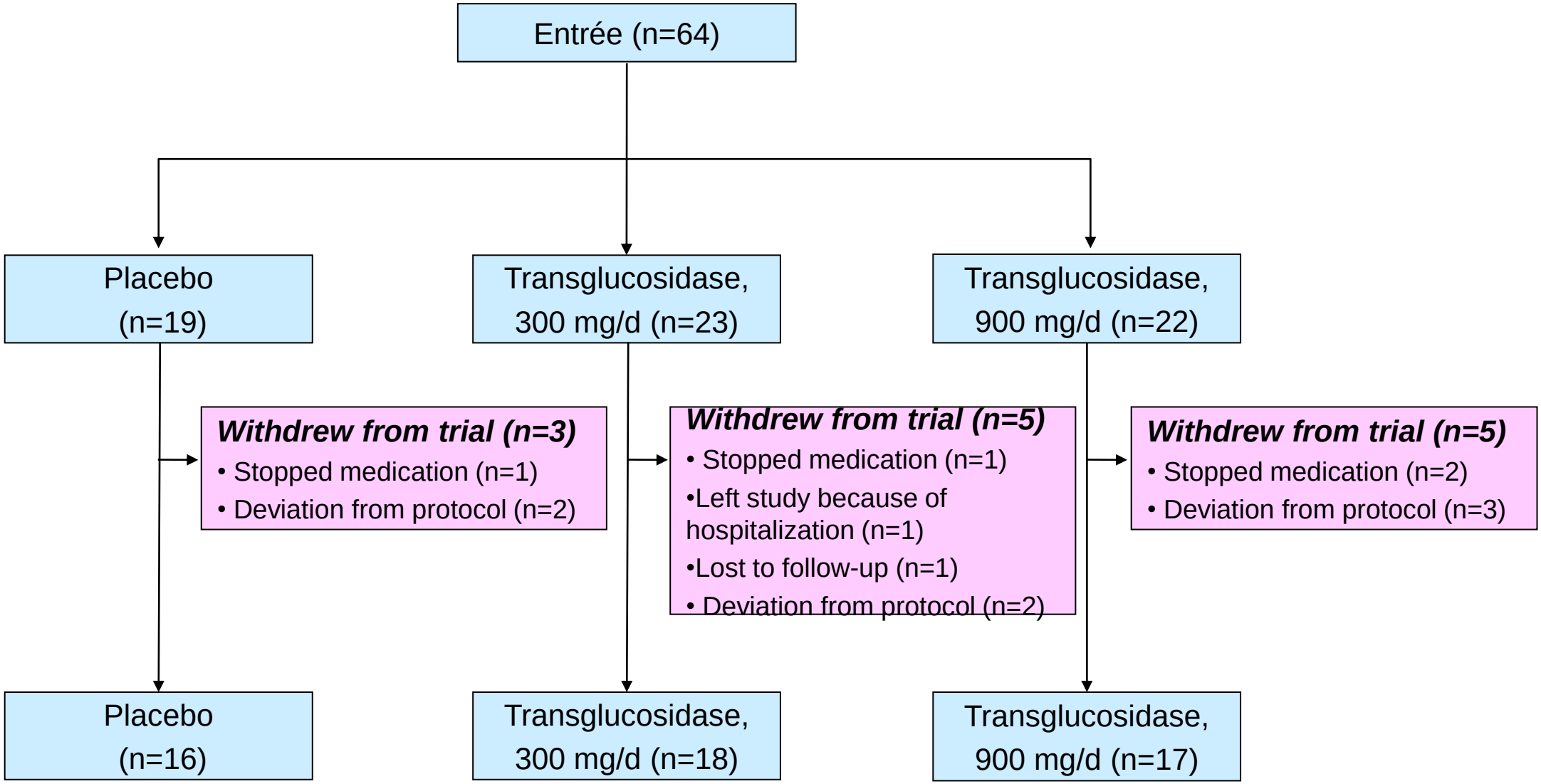
Secondary outcomes:

•The change in various other metabolic parameters

Fasting blood glucose (FBG), Glycosylated albumin (GA),
Plasma insulin level, Total cholesterol (T-CHO),
High-density lipoprotein cholesterol (HDL-C),
Low-density lipoprotein cholesterol (LDL-C),
Triglyceride (TG), Free fatty acid (FFA),
High-molecular-weight (HMW) adiponectin

M. Sasaki et al. Diabetes, Obesity and Metabolism, 4, 379–382, 2012

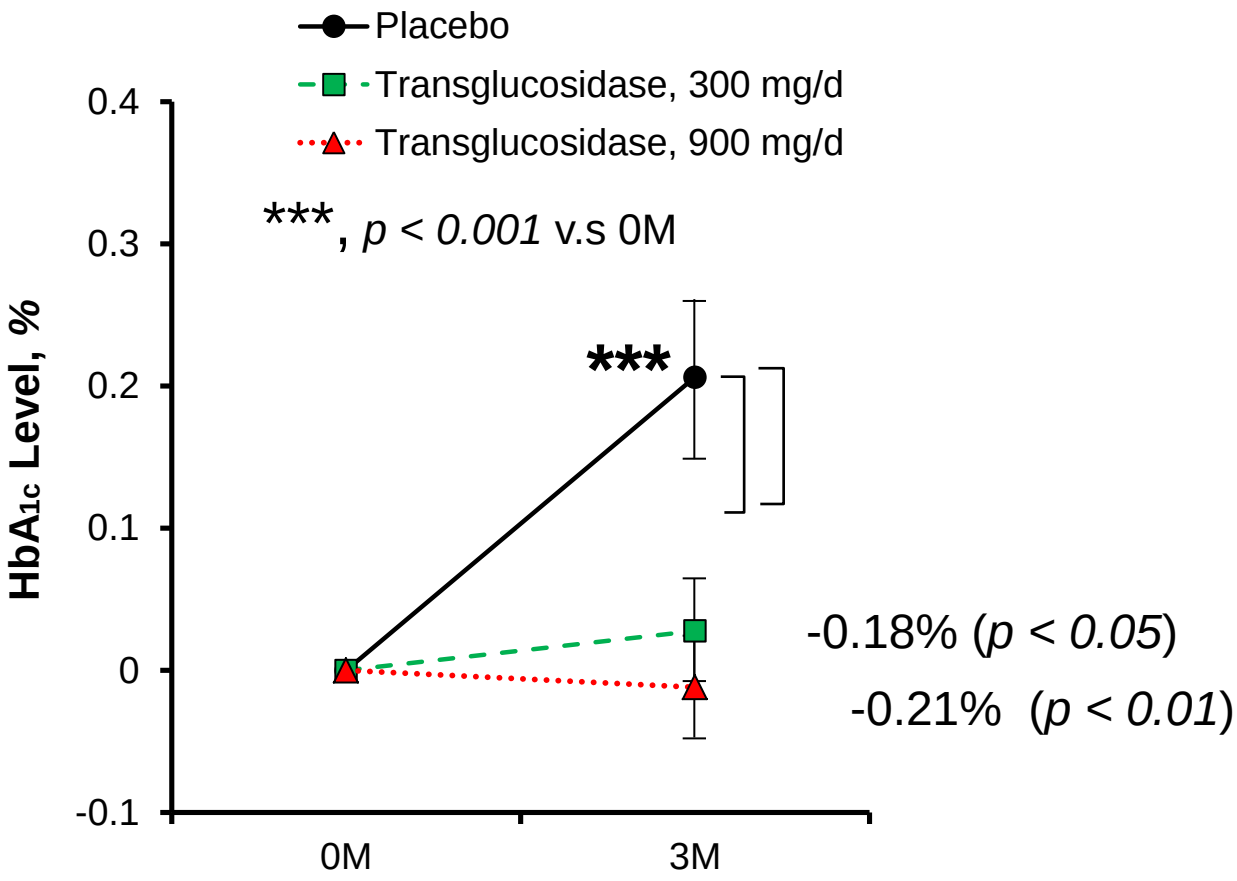
Study flow diagram



Presentation No.124

Primary outcome

Change of HbA1c



Mean ±SE

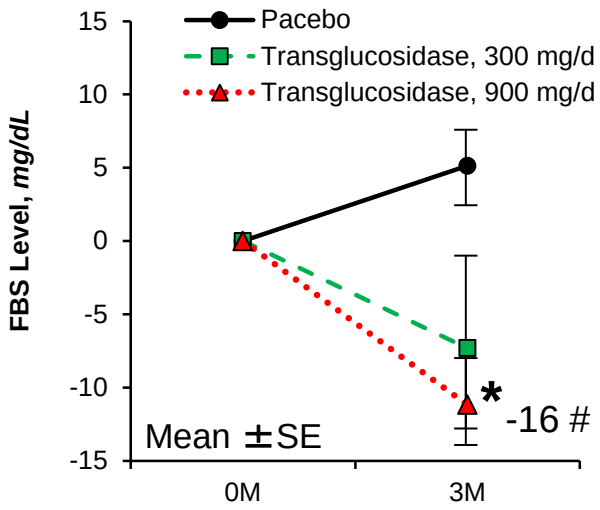
Calculated by using a two-way ANOVA and Benferroni post-hoc test

M. Sasaki et al. Diabetes, Obesity and Metabolism, 4, 379–382, 2012

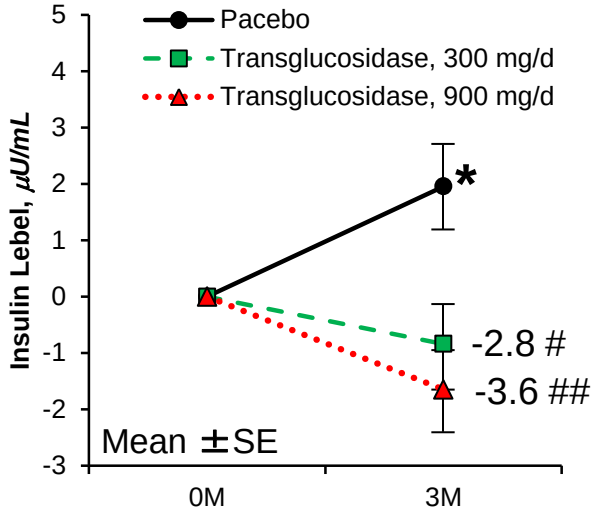
Presentation No.124

Secondary outcomes (1)

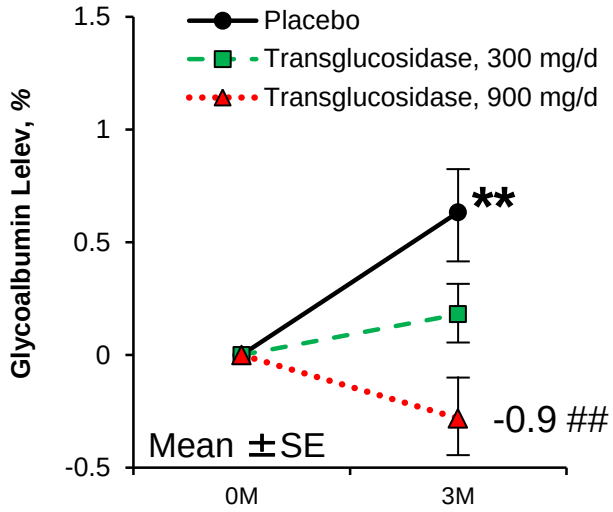
Fasting Blood Glucose



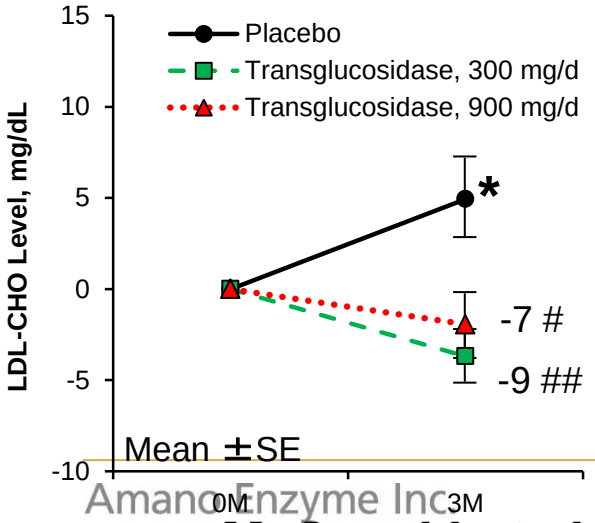
Insulin



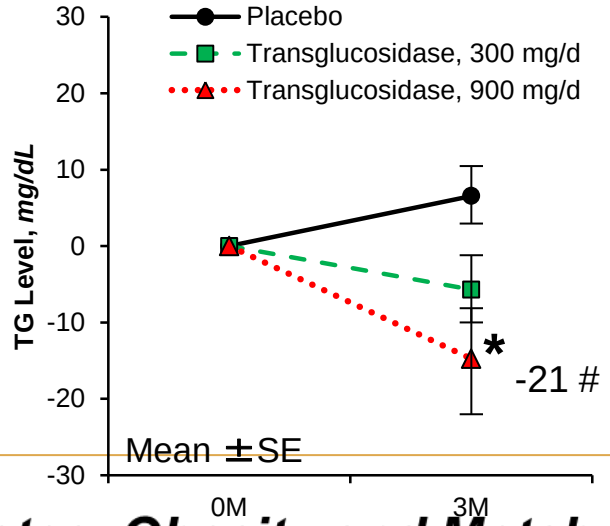
Glycoalbumin



LDL-Cholesterol



Triglyceride



#, $p < 0.05$ v.s placebo

##, $p < 0.01$ v.s placebo

*, $p < 0.05$ v.s 0M

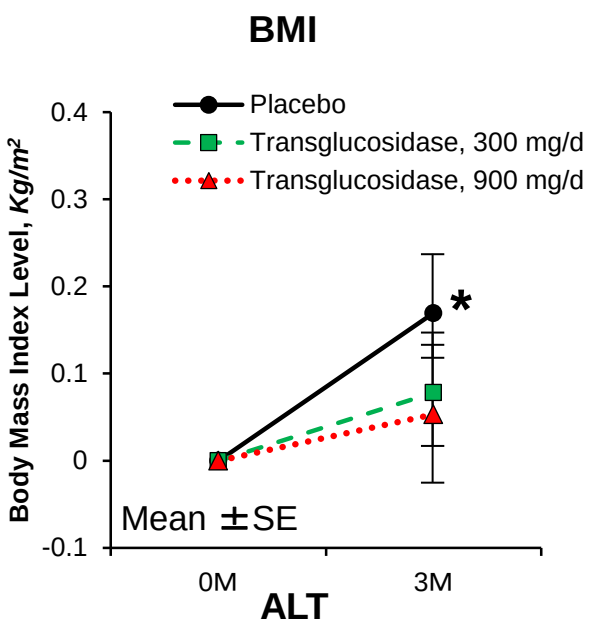
**, $p < 0.01$ v.s 0M

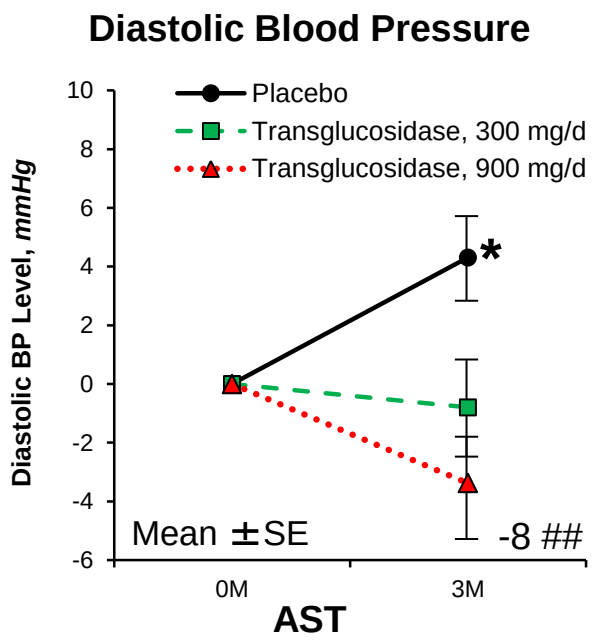
Calculated by using a two-way ANOVA and Benferroni post-hoc test

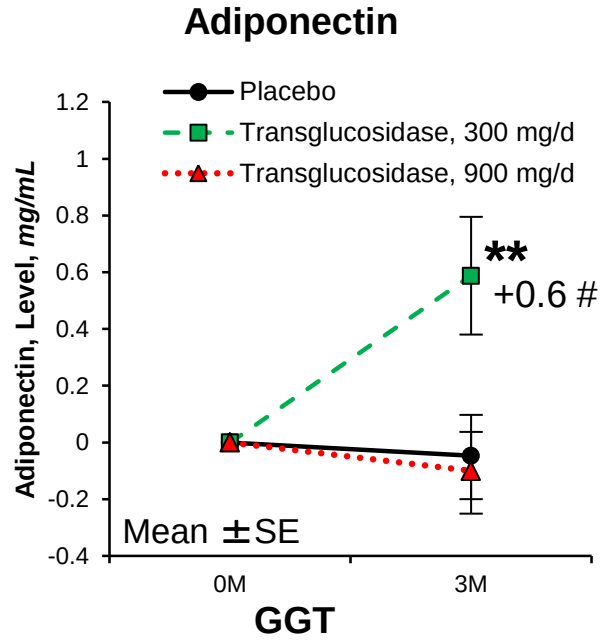
M. Sasaki et al. Diabetes, Obesity and Metabolism, 4, 379–382, 2012

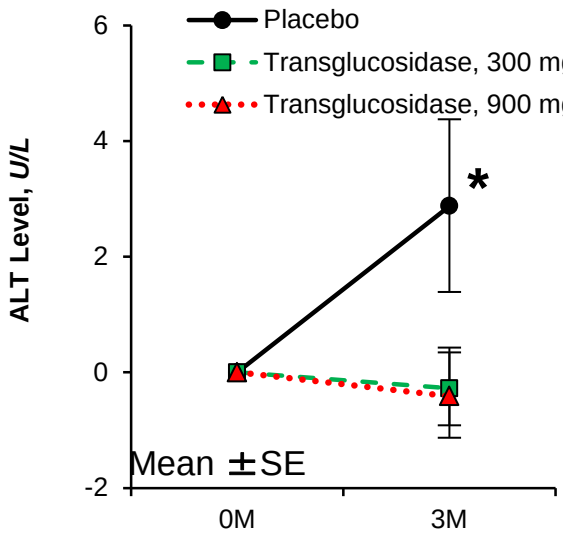
Presentation No.124

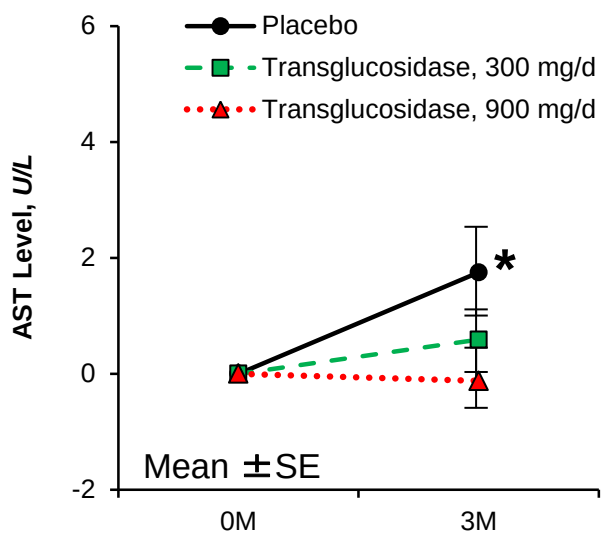
Secondary outcomes (2)

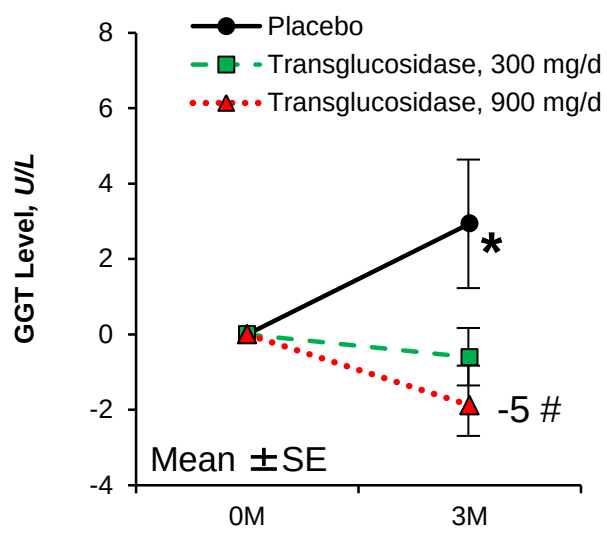












M. Sasaki et al. Diabetes, Obesity and Metabolism, 4, 379–382, 2012

Change of gut microbiota composition by transglucosidase administration

Placebo → TG 300mg/day, TG 900mg/day

Bacteroidete/Firmicutes

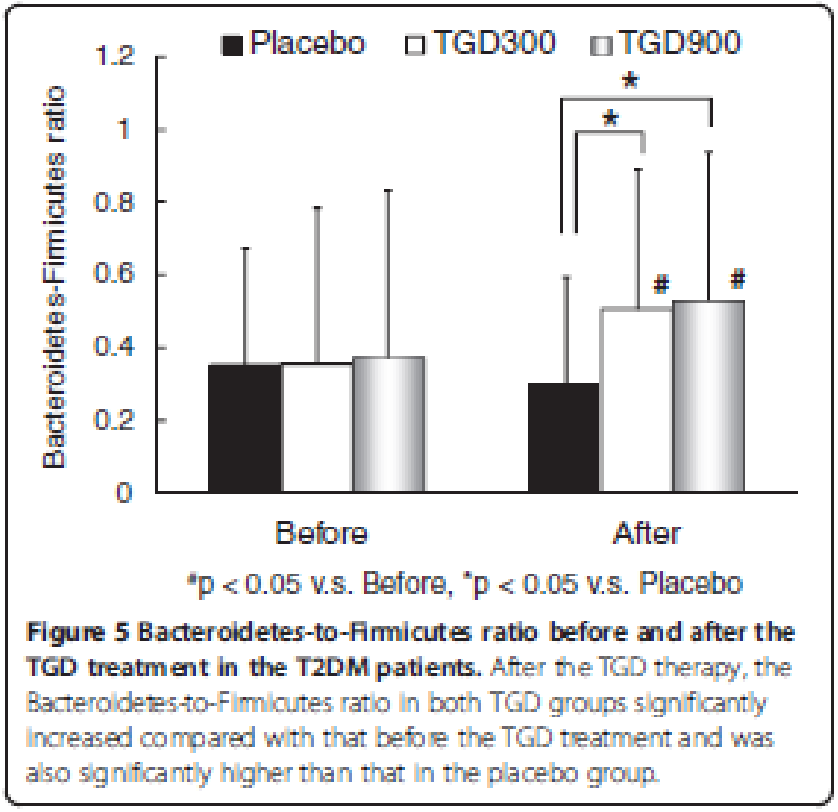
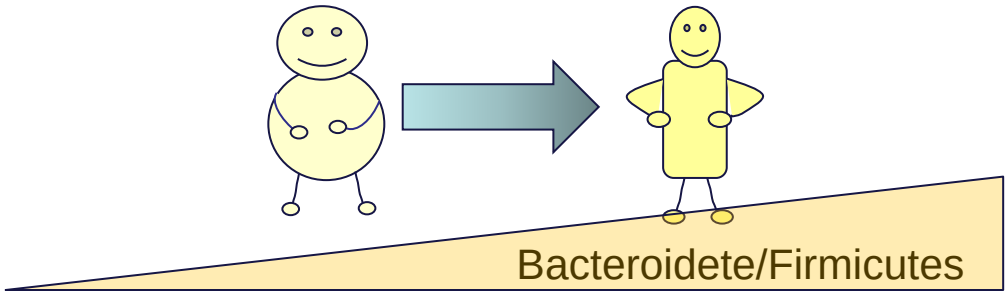
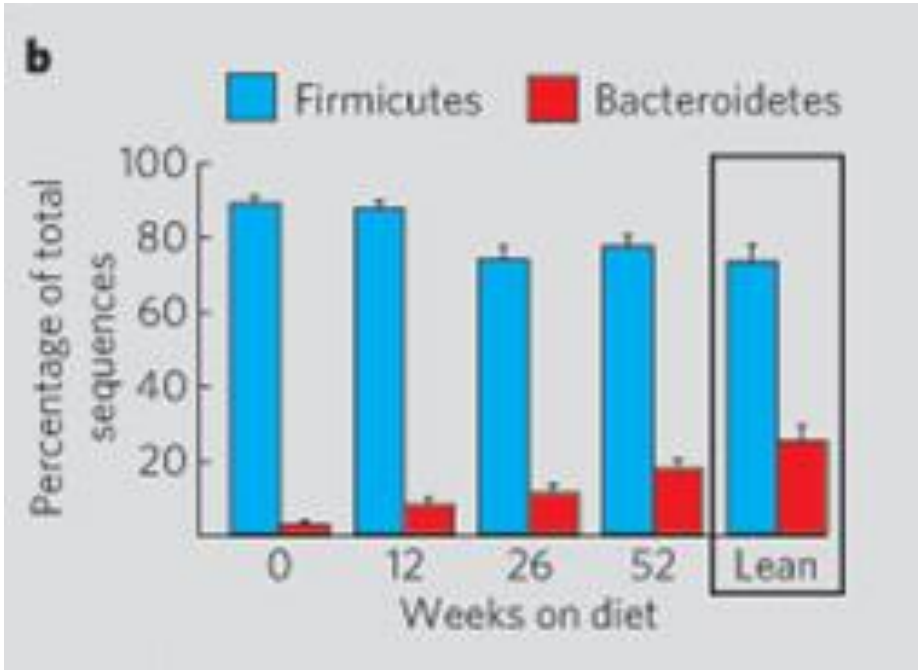


Figure 5 Bacteroidetes-to-Firmicutes ratio before and after the TGD treatment in the T2DM patients. After the TGD therapy, the Bacteroidetes-to-Firmicutes ratio in both TGD groups significantly increased compared with that before the TGD treatment and was also significantly higher than that in the placebo group.

Sasaki M et al. BMC GastroenterologyJ, 41, 2013



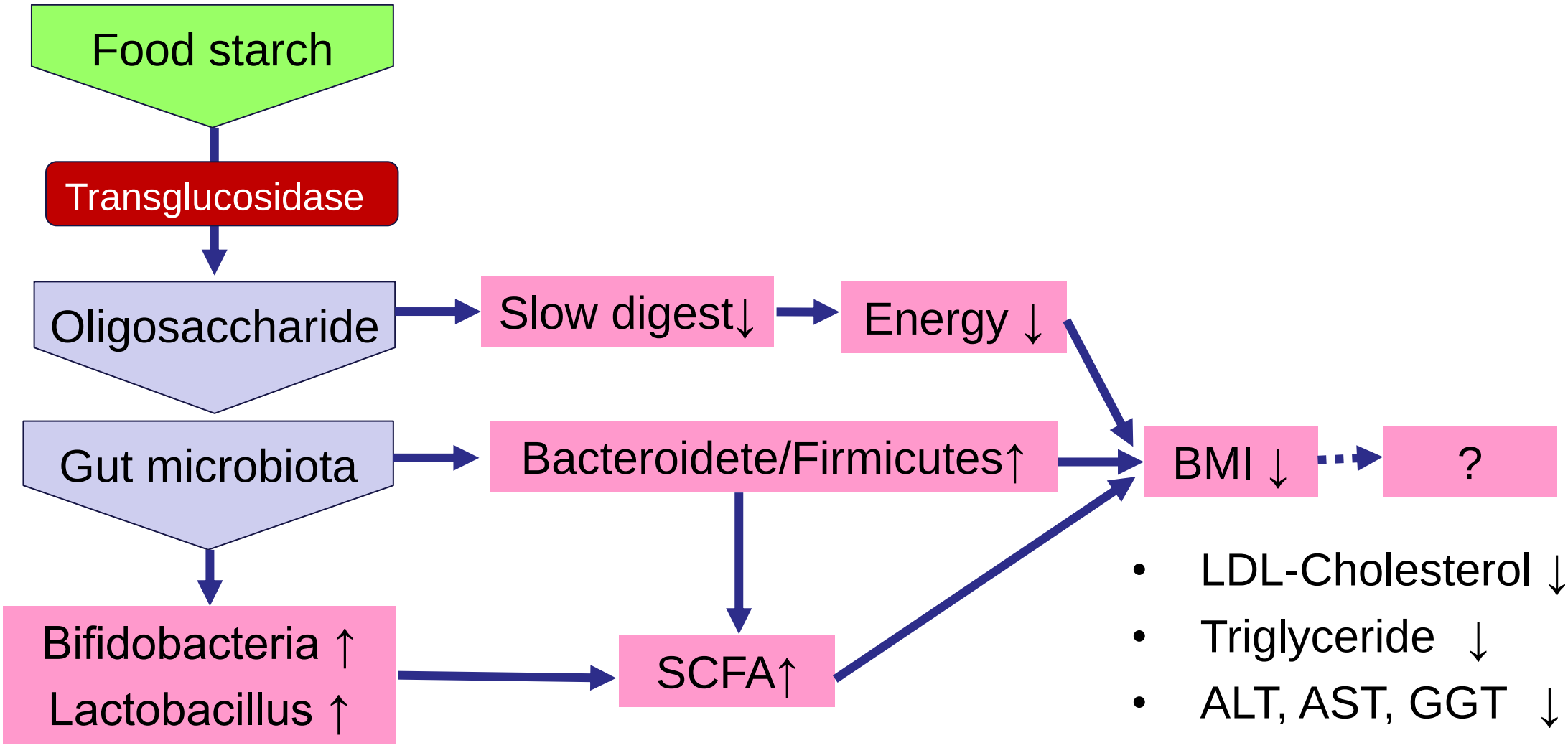
Bacteroidete/Firmicutes



→ **Weight loss**

Ley, R.E.et al. Nature, 444, 2006

Possible action of prebiotic enzyme



Comparison between prebiotics and probiotics

Prebiotics	Prebiotic Enzyme	Probiotics
✓Inconvenient intake ✓Unfamiliar food ingredient ✓Safety in the case of overdose	✓Convenient intake ✓Natural diet	✓Convenient intake ✓Safety of living bacteria

Conclusin

- **We proposed new enzyme concept: prebiotic enzyme.**
- **Developing Transglucosidase as prebiotic enzyme is on going.**
- **Clinical study resulted in lowering of HbA1c and blood insulin level, and improvements in metabolic and cardiovascular risk factors.**
- **Protein engineering makes Transglucosidase more effiently.**

Acknowlegements

Aichi Medical UniversitySchool of Medicine



Makoto Sasaki,

Naotaka Ogasawara,

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Akihito Iida,

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Takeshi Kamiya,

Hiromi Kataoka,

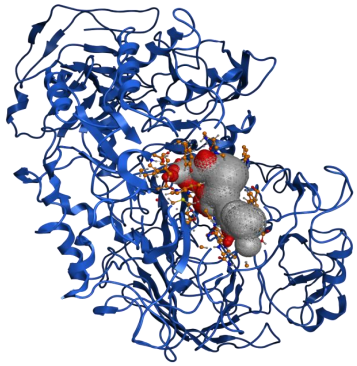
Takashi Joh



Thank you very much for your attention

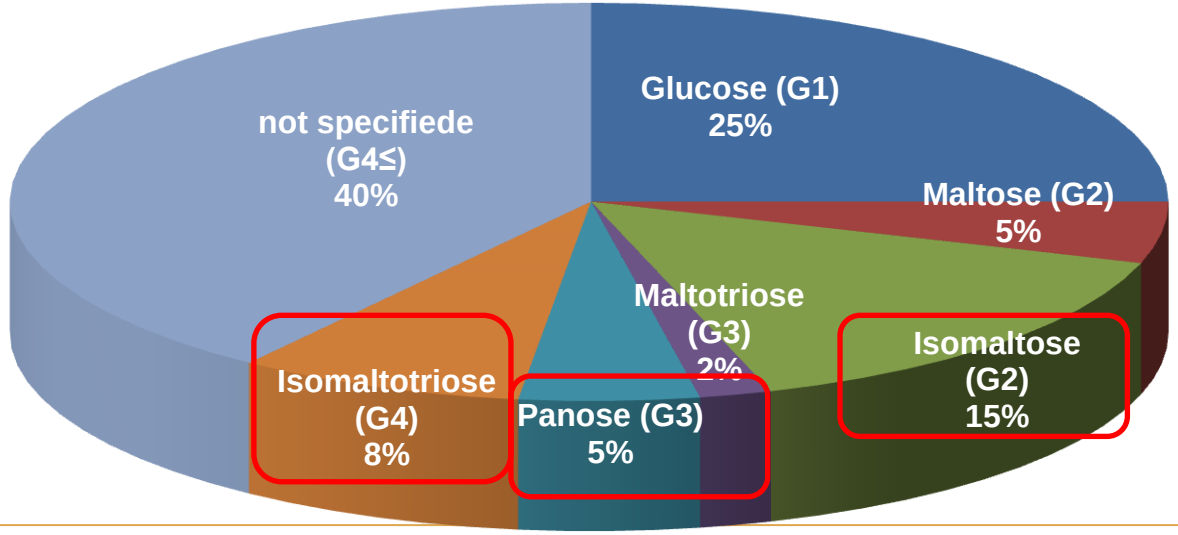
Production of Transformed Oligosaccharides by Transglucosidase

Corn Starch (DE 5～15) → IMOs (～30%)



58 ℃
pH 5.5
48 hours
With amylase

Protein Engineering



Amano Enzyme Group History

1899 Pharmaceutical business started.

1948 Production of pharmaceutical enzyme (API) started.

1960 Enzyme R＆D Center inaugurated.

1961 Production of food-processing enzyme started.

1970 Production of diagnostic enzyme started.

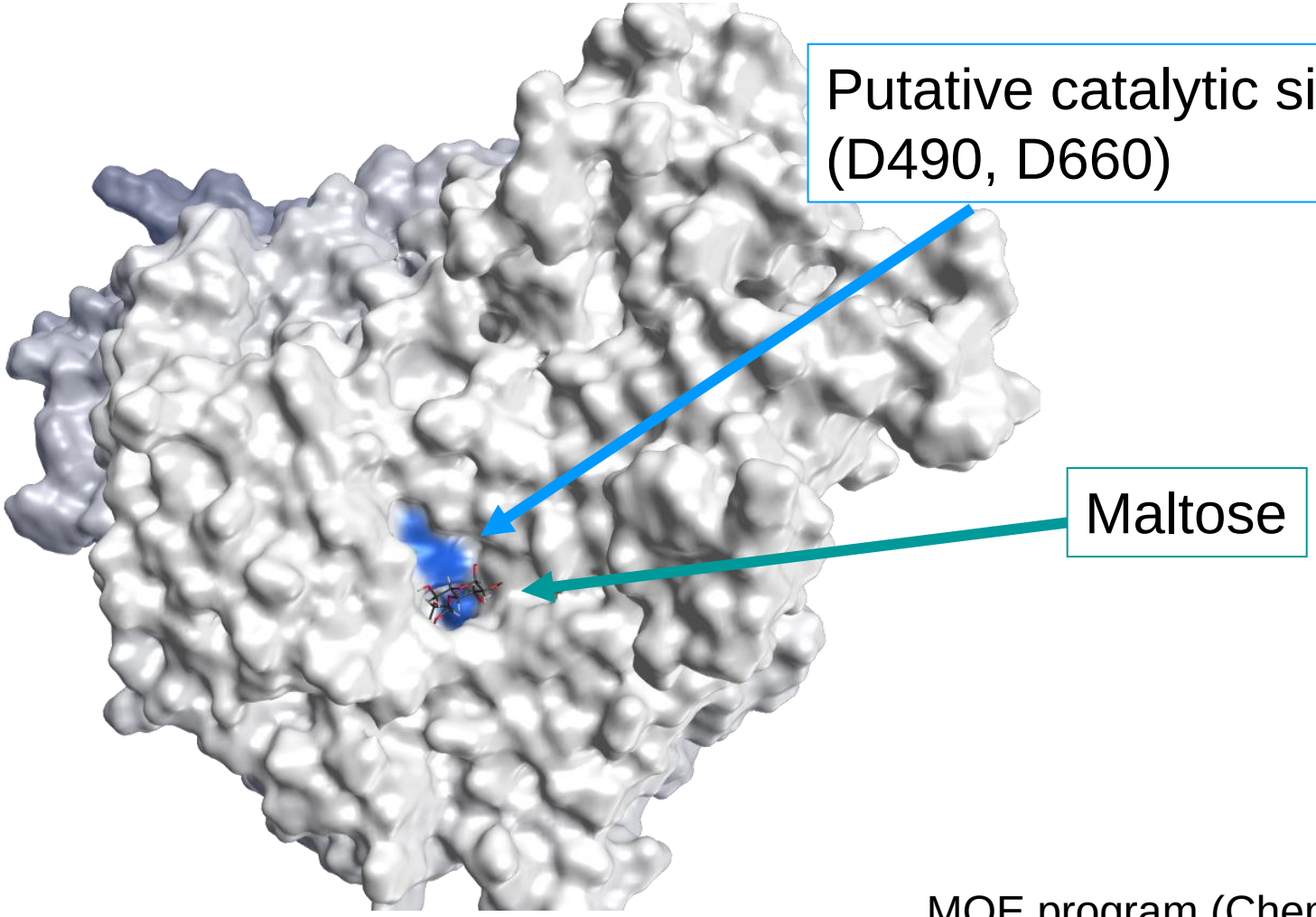
1981 Amano International Enzyme Co., Ltd. started in Virginia.
It was transformed to current Amano Enzyme USA Co.,Ltd. in Elgin IL.

1983 European office opened in Frankfurt, Germany. Now Amano Enzyme Europe Ltd. in Chipping Norton, OX, UK

2003 China office opened in Shanghai. Now Amano Enzyme China Ltd.

2012 Amano Enzyme Asia Pacific office opened in Kuala Lumpur, Malaysia.

Structuremodeling of Transglucosidase



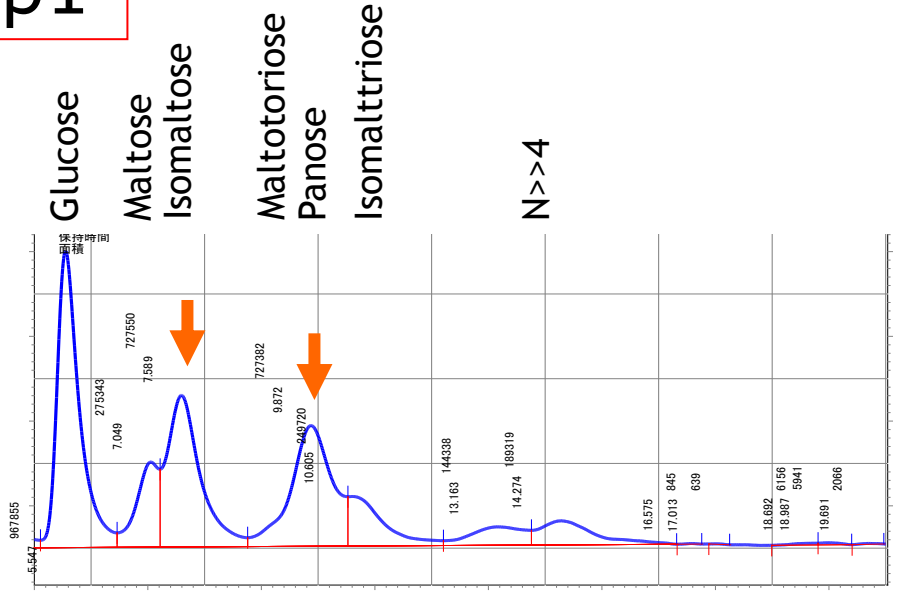
MOE program (Chemical Computing Group). The corrected aglA sequence from GenBank (accession number D45356) was aligned against Maltase-glucoamylase (Protein Data Bank: 2QLY).

Group 1 mutant

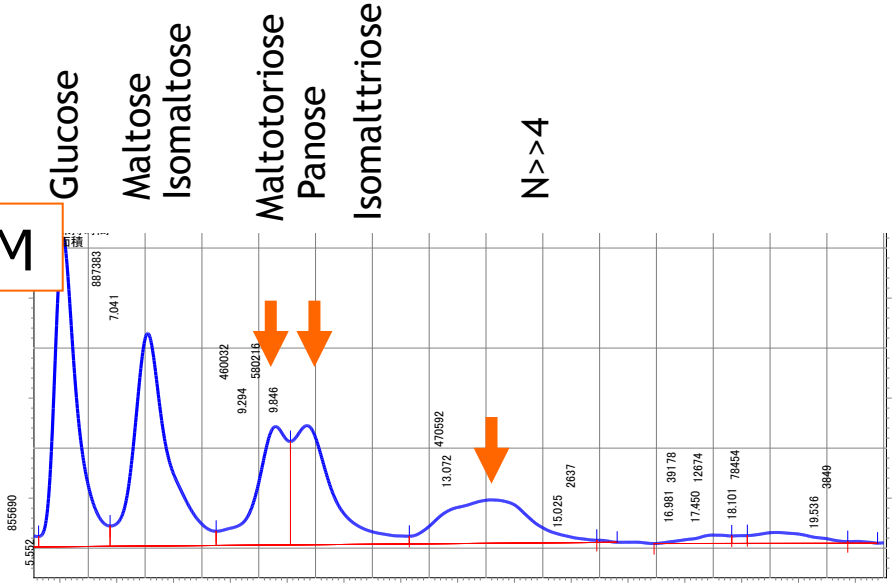
Substrate：25%maltose

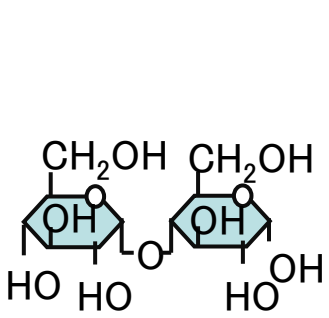
Group1

WT

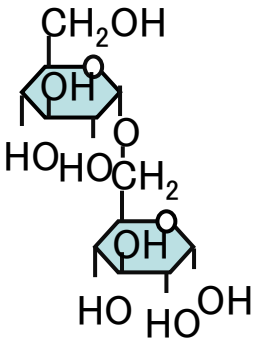


W343M

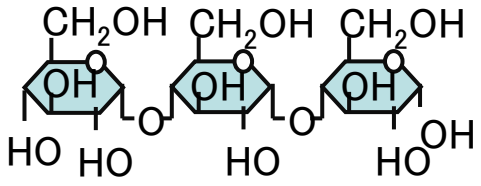




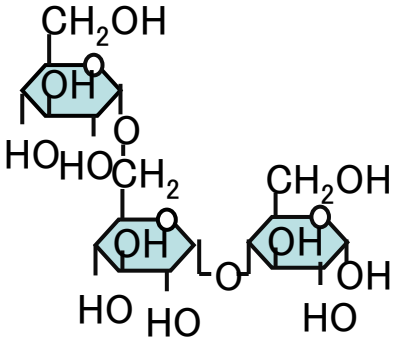
Maltose
(α-1,4)



Isomaltose
(α-1,6)



Maltotriose
(α-1,4, α-1,4)



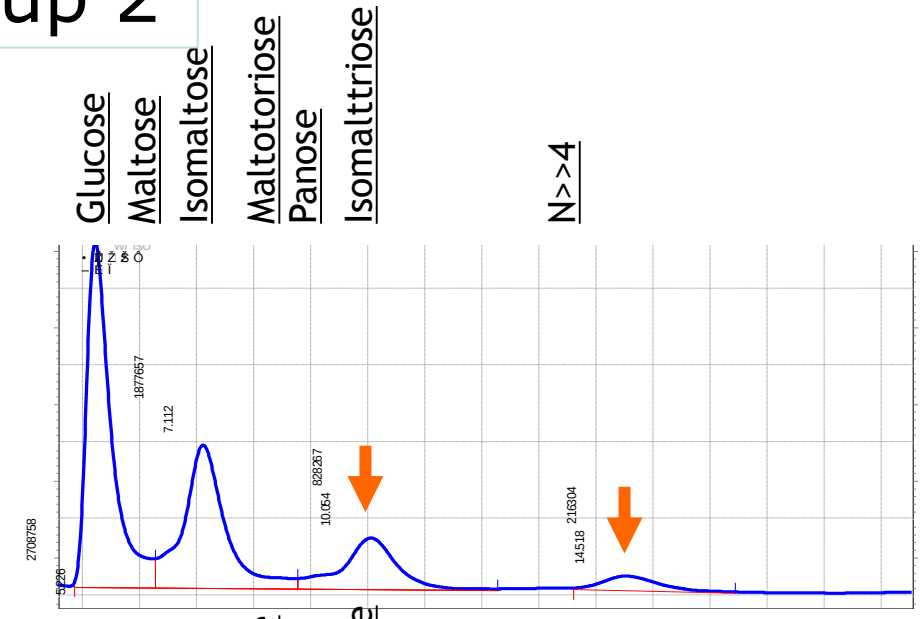
Panose
(α-1,4, α-1,6)

α-1,4-linkages

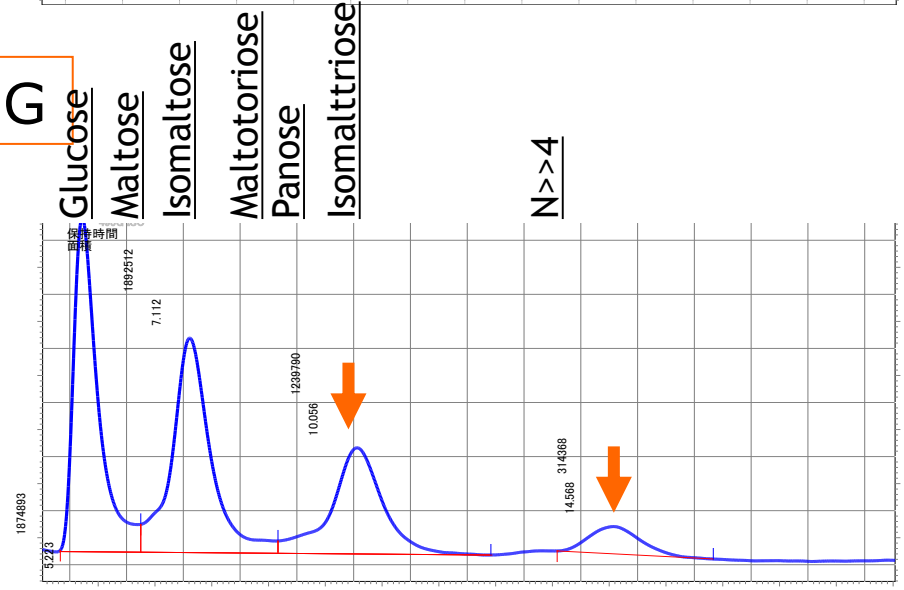
Group 2 mutant

Group 2

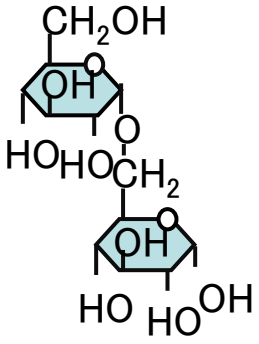
WT



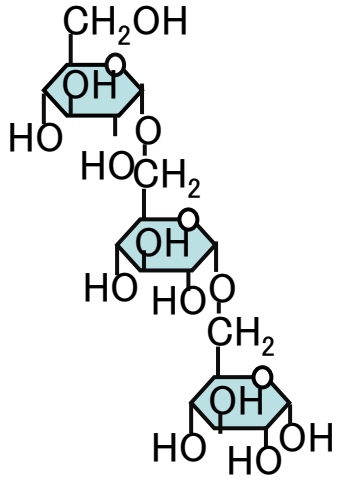
S495G



Substrate：25% isomaltose



Isomaltose
(α-1,6)



Isomaltotriose
(α-1,6, α-1,6)

α-1,6-linkages

Improving IMO production capability with protein engineering

