Gut microbiota and obesity (microbiota, obesity, prebiotics)

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The etiology of obesity reflects complex interactions among genetic and environmental factors, with current high energy diets and sedentary lifestyle considered to be foremost among the latter.
The human gastrointestinal microbiota contains $10^{14}$ cells and represents one of the most comprehensive ecosystems on earth. It contains 17 bacteria families, 45 varieties and more than 1000 specie. This microorganism colonise nearly 200-400 m$^2$ of the intestinal epithelium.

The intestinal bacteria specie belong to 8 of 55 known types, the main of which are the *Firmicutes, Bacteroidetes* and *Actinobacteria* and *Proteobacteria*. Approximately 60-80% is mutational microorganisms that occur in various quantities depending on the host. The GI tract is variable, hence the microorganisms in its various parts are distinct. In the lower parts of the GI the transit time is slower, the habitat more favourable thus the most diverse bacterial biocenosis develops. The researches resulted that the large intestine contains 800 species of 9 types bacteria specie and 1 type Archaea. In the several parts of the large intestine the group of microorganisms is differentiated. The ascending colon contains a large substrate concentration, in this part the oligo- and polysaccharides ferment, generally into lactic acid and short chain fatty acids (SCFA). In the transverse colon the microorganisms increase slower and in the descending colon microorganisms conducting the processes of proteolysis.
The colonization of the human gastrointestinal tract begins within a few hours after birth but is not identical in all infants. The initial impact on the microbiota of the digestive system of children is determined by the way of labor, hospital environment, type of food, mother and child diseases and drug use. In the early years of life the gastrointestinal tract is colonized by bacteria belonging to the genus *Lactobacillus*, *Staphylococcus*, *Enterococcus*, *Escherichia*, *Enterobacter*, *Bifidobacterium*, *Bacteroides*, *Eubacterium* and *Clostridium*. An intensive phase of colonization of the human gastrointestinal tract usually lasts until two years of age, after that time the child gut microbiota begins to resemble to that which is in adults.
Strains of *Firmicutes* and *Bacteroidetes* account for more than 90% of the total population of the intestinal microbiota. At the level of genus the dominant type are obligate anaerobes: *Bacteroides, Eubacterium, Clostridium, Ruminococcus, Peptococcus, Peptostreptococcus, Bifidobacterium* and *Fusobacterium*, as well as facultative anaerobes: *Escherichia, Enterobacter, Enterococcus, Klebsiella, Proteus, Lactobacillus*. This composition has a “character of climax”. 
The gut microbiota has many beneficial functions, among them is help in digestion, affects the immunity of the body, stimulates the development of microvilli, takes part in the fermentation of dietary fiber and prebiotics that produces very beneficial to the human body short-chain fatty acids (SCFA) (butyric, propionic and acetic acids) as well lactic acid. Microbiota may play a beneficial role in the metabolism of potentially harmful substances such as cholesterol, nitrosamines, heterocyclic amines and bile acids.
Microbiota may also be a source of antigens and harmful compounds, and even pathogens. The most preferred state for a human is a state of natural balance in. Adverse changes to human health in the composition of microbiota is referred as "dysbiosis". Consequence of dysbiosis may be a leakage of the intestinal barrier and the reduction of the total quantity of SCFA. Dysbiosis may precede the clinical manifestations of intestinal diseases and is tied to the occurrence of colorectal cancer and inflammatory bowel diseases. Dysbiosis can also lead to serious systemic disorders.

Influence of diet on correct development of the gut microbiota

Ridaura (Ridaura et al., 2013) found that the intestinal microbiota of lean and obese people induces a similar phenotype in mice, namely, that the microbiota transplanted from a lean individual (donor) causes the decrease in a fat mass in an obese mice (recipient) where mice were fed with reduced fat content (4 wt%) and a high content of plant polysaccharides. In addition research was done on four pairs of adult female twins lean and obese from which the microbiota was transferred to germ-free mice.
Influence of diet on correct development of the gut microbiota

In animals that received microbiota from obese people obesity has developed, whereas mice that microorganisms colonized the intestine from lean person had normal body weight. Also a research was performed to check whether isolates from stool specimens of slim twin will colonize the intestine of germ-free mice colonized already by microbiota derived from the obese twin. It turned out that the isolates from the slim twin prevented the development of obesity in germ-free mice with the microbiota from the obese twin.

It has been shown that bacteria strains derived from slim persons transferred to germ-free obese mice, can prevent the formation of obesity when these mice diet consists of fiber, increased amounts of polysaccharides, and small amount of fat. This indicates that the composition of the intestinal microbiota, and its effect on reducing the development of obesity is closely correlated with the consumed diet.

Analysis of the microbiota of these mice showed increased participation of strains of *Bacteroides* in germ-free mice colonized with samples from the slim twin. This indicates that strains of *Bacteroides* and their quantity may have a significant impact on reducing the development of obesity, but it should be noted that it is important to determine not only the genus type but also the species of a given strain. Increased abundance of *Bacteroides* was correlated with low fat diet that contained higher levels of fruit and vegetables; however correlation disappeared when diet proportions of ingredients were reversed.
As described, enterotypes are based on co-occurring associations of specific genera and species. Shotgun metagenomic analysis of fecal bacterial composition of large cohorts of healthy people has suggested three enterotypes dominated by Bacteroides, Prevotella and Ruminococcus (or other Firmicutes genera).
Importantly, although differences in enterotype were initially reported to be independent of geography, gender, age or body mass index, substantial differences in bacterial assemblages and functional genes were recently reported in a comparative study of US residents and those in either the Venezuelan Amazon or rural Malawi. These differences may reflect (at least in part) differences in long-term diet pattern, which have been associated with human enterotypes. Specifically, the Bacteroides enterotype was associated with higher dietary consumption of protein and saturated fats, whereas the Prevotella enterotype was associated with low intake of protein and fats but high ingestion of carbohydrates and simple sugars.

However, other studies have not confirmed the same pattern, instead suggesting a trade-off between Prevotella and Bacteroides within individuals.
The type and proportions of the microorganisms present in the gut enterotype determines the metabolic products which have important consequences for the host. These metabolites can be either beneficial or harmful.

One of them are short-chain fatty acids (SCFA) formed by the fermentation of indigestible polysaccharides in the large intestine by specific groups of bacteria.

SCFA meet to have numerous positive functions.

These include: butyric acid that stimulates intestinal epithelial tissue, nourishes the intestinal cells and affects their proper maturation and differentiation; propionic acid has a positive effect on the growth of hepatocytes; acetic acid has a positive effect on the development of peripheral tissues.

SCFA regulate glucose and lipid metabolism, stimulate the proliferation and differentiation of intestinal enterocytes, lower pH effect in the intestinal contents, and thus help out in the absorption of minerals by increasing their solubility.
It was shown that in spite of SCFA as a source of energy, it contribute towards reducing the formation of obesity by inhibiting fat accumulation in adipose tissue, increased energy expenditure and increasing production increase of hormones associated with the feeling of satiety.

Influence of butyric acid on regulation of energy homeostasis of the organism may be associated with stimulation of the leptin synthesis in adipocytes, induction of GLP-1 secretion by L cells of intestine and increased fatty acid oxidation. In examining the influence of metabolites of the gut microbiota on the human body it was confirmed that the additional source of energy to the host (human) may be propionic acid used in the synthesis of glucose and lipids.
In 1998, the World Health Organization (WHO) classified obesity to the rank of epidemic on a global scale. In terms of frequency, obesity precedes the occurrence of AIDS and malnutrition. Alarming phenomenon is the growth of the obesity epidemic in children.
Adipose tissue, until recently, was considered only as a reservoir of body energy substrate. Today, it is known that it is an important part of the endocrine system. Pathologically increased amounts of fat in the body result in numerous disorders in the proper functions of the systems, organs, and tissues. Particularly dangerous complications may occur in the cardiovascular system, respiratory, endocrine, and psychosocial problems.

It is estimated that 80% of civilization diseases is caused by problems associated with excessive body weight. Statistics predict the continuous deterioration of the situation, which is a challenge for the public health sector in many countries of the world. The problem of obesity relates to people of all ages, and the causes have a very complex character, from bad habits, through environmental impact (to stress and genetic factors). A major problem is the obesity transfer from childhood to adulthood.

![Diagram of Obesity and Its Complications](image)

- Insulin Resistance
- Complex dyslipidemia
- Glucose intolerance
- Hyperinsulinemia
- Impaired fasting glycemia
- Type 2 diabetes
- Low-grade Inflammation
- Fibrinolysis disorder
- Endothelial dysfunction
- Hypertension
- Atherosclerosis
Many studies have shown that obesity is also associated with significant changes in the composition and function in metabolism of the intestinal microbiota. It is recognized that particularly important behavior is to keep correct proportion of *Bacteroidetes* and *Firmicutes* strains.

Research teams Bäckhed, Gordon and De Filippo also indicate that obesity in humans is likely to be related to the composition of the gut microbiota.
Bäckhed and colleagues determined the share of Firmicutes and Bacteroidetes at obese mice and mice with normal body weight and found that the proportion of Bacteroidetes is significantly lower in obese mice (20%), while in mice with normal weight bacteria was at larger amount up to 40%. In turn Flessner demonstrated that supplying mice with high animal fat and low fiber diet result in a quantity reduction of Bacteroidetes strains, but growth in Firmicutes.

Studies were carried out on a group of twelve obese humans, who had an increased presence of Firmicutes and reduced presence of Bacteroidetes from 1 to 5%. After applying in one group diet with reduced fat content and for others group diet with decreased portion of saccharides, proportions of the major groups of microorganisms changed. In both groups quantity of Firmicutes was gradually declining and Bacteroidetes increased up to 20%.
In order to determine the relationship between the microbiota and the amount of energy, Jumpertz conducted research on the group of 21 volunteers where interchangeably diet of 2400 and 3400 kcal/day was administered. Fecal microbiota composition was monitored. It has been shown that 20% growth of *Firmicutes* strains was accompanied by 20% reduction in the quantity of *Bacteroidetetes*, and changes in the proportions of these strains were directly related to gain in body weight. It seems that important role in participation in gut microbiota has *Bifidobacteria*. It has been proven that in overweight people and sick people with type 2 diabetes abundance of *Bifidobacterium* was significantly lower.

Valentina Tremaroli & Fredrik Bäckhed, Nature 2012
De Filippo compared the composition of the intestinal microbiota in children age 1 to 6 years old, living in extremely different conditions.

The first group of children came from rural areas of Africa (Burkina Faso); the second group consisted of children from Italy (Florence).

The intention of the study was to determine the correlation between the applied diet, and the composition of the intestinal microorganisms.
Diet of children living in Africa was poor in meat, but contained significant amounts of vegetables, starch and dietary fiber (about 672.2 kcal toddler ages 1-2 years old and 996 kcal children ages 2-6 years old), while children from Europe nourish mainly meat, and their diet contains a lot of animal fats, sugars, and was poor in vegetables and fiber (about 1,068.7 kcal children ages 1-2 years old and 1,512.7 kcal children aged 2-6 years old).
F/B = 0.47 ± 0.05
F/B = 2.8 ± 0.06

In the case of children coming from Florence, increased body weight was found and intestinal microbial system was different than in the case of children from Africa. It has been shown that the dominant bacteria of the phylum *Firmicutes* (51 %), and *Actinobacteria* and *Bacteroidetes* were 6.7 % and 27 % respectively. High concentrations of SCFA which has been demonstrated in children from Burkina Faso is an additional source of energy for the host and in these children despite the low calorie intake the normal development is observed.
The gut microbiota vs. obesity - the potential mechanisms

Mechanisms of gut microbiota impact on the development or slowing down of creation of obesity are not fully known.
It is considered that obesity is associated with elevated serum levels of lipopolysaccharide (LPS) which is a component of the cell wall of Gram-negative bacteria. LPS due to proinflammatory properties may be involved in the development of inflammation, present in type 2 diabetes. Intravenous administration of lipopolysaccharide in mice resulted in the development of insulin resistance and weight gain.

*In vivo* correlation was observed between the increase in plasma concentrations of LPS, and the implementation of a high in fat diet. Cani conclude that the fat contained in the food may be an important regulator of the concentration of LPS. It has been proved that the introduction of four weeks of high in fat diet in mice resulted in a two or even three times increase in plasma levels in LPS. This phenomenon was confirmed in people diagnosed with obesity and type 2 diabetes.
In the genesis of obesity a vital role may play intestinal alkaline phosphatase (IAP), which is involved in the degradation of lipids derived from food, but also plays an important role in the detoxification of LPS (dephosphorylation of lipid part of LPS). Furthermore, increased activity of the IAP is associated with a reduced endotoxemia which is caused by metabolic dysfunctions. It was determined that the expression of alkaline phosphatase IAP may be controlled by the gut microbiota.

In obese people with type 2 diabetes changes in the intestinal barrier was detected, namely an increase of about cellular permeability. It has been shown that the increase in intestinal permeability observed in obese mice can be associated with a change in the expression, localization and distribution of proteins belonging to the tight-junctions of the small intestine.
Another potential factor linking gut microbiota to obesity is blocking the expression of fasting–induced adipose factor (FIAF) by the microbiota. FIAF inhibits the activity of lipoprotein lipase (LPL) enzyme responsible for the storage of energy in fat. The decreased expression of FIAF determines the increased LPL activity and enhances the process of storing energy in the form of fat. There is evidence that gut microbiota modulates the activity of the endocannabinoid system and thus has an effect on the function of the intestinal barrier. These studies revealed an important role of the intestinal barrier in the etiology of obesity and Type 2 diabetes.
Prebiotics

Since gut microorganisms to some extent are responsible for the formation of obesity therefore modulation of microbiota is seen as a potential tool in the prevention and treatment of that disease. It was presented that the growth of beneficial microbiota, and therefore sealing the intestinal barrier and changes in the metabolism of endotoxin in the blood can be modulated by the addition of prebiotics to the diet.
The concept of prebiotics – yesterday

„nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health”


„a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health”

The concept of prebiotics – today

„non-viable food component that confers a health benefit on the host associated with modulation of the microbiota”

FAO Technical Meeting on Prebiotics (2007)

„selectively fermented ingredients that result in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health”

6th Meeting of ISAPP (2008)

„the selective stimulation of growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host”

Prebiotics

Prebiotics are not hydrolyzed and absorbed in the upper parts of the gastrointestinal tract whereby unchanged reach the large intestine where they are nutrients for the health beneficial bacteria.

To substances having prebiotic properties currently we include

- fructooligosaccharides,
- glucooligosaccharides,
- isomaltooligosaccharides,
- maltooligosaccharides,
- lactulose,
- raffinose,
- soy oligosaccharides,
- stachyose,
- xyloooligosaccharides,
- inulin,
- resistant starch.
Prebiotics

Recently research is conducted to confirm prebiotic properties of new substances such as resistant dextrins derived from potato starch. It has been shown that these formulations have a bifidogenic effect and stimulate the growth of beneficial for the body guts microbiota, thus limiting the growth of Clostridium strains.

Studies conducted on rats and healthy persons confirmed that prebiotics reduce hunger and increase the feeling of satiety. Positive effects of modulation of gut microbiota, in particular to the production of SCFA, and increased level of PYY (this peptide is synthesized and secreted by the L-cells of the ileum and colon, and has a stimulant effect on satiety center) and GLP-1, resulting in a reduced glycemic, reduction of insulin resistance, reduced fat cells, and takes part in the perception of satiety. Adding to diet a mixture administration of inulin and xylooligosaccharides resulted in lowering of the LPS level in a blood plasma.
It turned out that after a diet consisting of RS4 amount of these bacteria increased. Also reduced share of *Firmicutes* bacteria was observed and thereby increasing *Bacteroidestes* and *Actinobacteria*. Addition of fructooligosaccharides and inulin mixture (10g/d) to the diet stimulated of the growth of bifidobacteria, in particular *Bifidobacterium adolescentis*. 
Resistant starch

Resistant starch (RS) includes the portion of starch that can resist digestion by human pancreatic amylase in the small intestine and thus, reach the colon.

The general behaviour of RS is physiologically similar to that of soluble, fermentable fibre, like guar gum. The most common results include increased faecal bulk and lower colonic pH and improvements in glycaemic control, bowel health, and cardiovascular disease risk factors, so it has shown to behave more like compounds traditionally referred to as dietary fibre.

Resistant starch is found in many common foods, including grains, cereals, vegetables (especially potatoes), legumes, seeds, and some nuts.
Resistant starch may not be digested for four reasons:

- this compact molecular structure limits the accessibility of digestive enzymes, various amylases, and explains the resistant nature of raw starch granules. The starch may not be physically bio accessible to the digestive enzymes such as in grains, seeds or tubers,

- the starch granules themselves are structured in a way which prevents the digestive enzymes from breaking them down (e.g. raw potatoes, unripe bananas and highamylose maize starch),
Resistant starch

Resistant starch may not be digested for four reasons:

- starch granules are disrupted by heating in an excess of water in a process commonly known as gelatinization, which renders the molecules fully accessible to digestive enzymes. Some sort of hydrated cooking operation is typical in the preparation of starchy foods for consumption, rendering the starch rapidly digestible. However, if these starch gels are then cooled, they form starch crystals that are resistant to enzymes digestion. This form of “retrograded” starch is found in small quantities (approximately 5%) in foods such as “corn-flakes” or cooked and cooled potatoes, as used in a potato salad,

- selected starches that have been chemically modified by etherisation, esterisation or cross-bonding, cannot be broken down by digestive enzymes.
Resistant starch is the sum of starch itself and products of her decomposition, that are neither being digested nor absorbed in the small bowel of healthy human.

Resistant starch is the difference between amount of the starch exposed to the action of amylolytic enzyme complex and the amount of starch decomposed to glucose during hydrolysis performed by those enzymes.

\[
RS = TS - (RDS + SDS)
\]

Where:
RS – resistant starch
TS – total starch
RDS – rapidly digestible starch
SDS – slowly digestible starch
Resistant starch

- **RS1**: Physically inaccessible starch present in cells of plants with undamaged cell walls
- **RS2**: Granules of raw starch of some plant species, e.g. potato or banana
- **RS3**: Retrograded or crystalline nongranular starch formed after cooking
- **RS4**: Physically or chemically modified starches (RS4)
  - Amylose lipid complexed starch
  - Resistant maltodextrins

References:
- Fuentes-Zaragoza et al., Starch-Stärke, 63, 406 (2011)
Resistant dextrins are defined as short chain glucose polymers, without sweet taste and performing strong resistance to hydrolytic action of human digestive enzymes. In accessible throughout the whole products (resistant dextrin Nutriose, Fibersol) bigger percentage presence of (1-2)-, (1-3)-, (1-6)- α and β-glycoside bonds than in native starch which is the source of getting them.
During warming of starch in high temperature, with or without addition of catalyst (usually acidic) dextrinization of starch is observed. Dextrinization is a complex process taking chemical side of it into account. It covers depolymerization, transglucolyzation and repolymerization.

From all presented reactions the most characteristic and dominating one is transglycolisation. Formation of bonds other than typical for starch (1-4) and (1-6) causes that the received product becomes unavailable for human digestive enzymes and shows properties of resistant starch.
Resistance of starch molecules with $\alpha$-1,2- and $\alpha$-1,3- linkages or highly clustered bonds to enzymes present in the digestive tract.

Ohkuma et al., US. Patent 5,620,873 (1997)

Resistance of starch molecules with $\alpha$-1,2- and $\alpha$-1,3- linkages or highly clustered bonds to enzymes present in the digestive tract.

dietary fibre

Potential prebiotic
Objective

- combination of two factors responsible for starch resistance (temperature and chemical modification) to produce preparations with a higher content of the fraction inaccessible to human digestive enzymes

- examination the possible connections between the method of dextrin production, their structure and their resistance to enzymatic digestion

- application of enzyme-resistant dextrins as products with potential prebiotic properties, selectively stimulated growth and activity of selected probiotic bacteria, and not contributed to growth of undesirable intestinal microflora
Methodology

Preparation of the enzyme-resistant dextrins from potato starch

Physico-chemical characteristics of dextrins

Assessment of prebiotic properties of dextrins
Preparation of dextrins

Enzyme-resistant tartaric and citric acid-modified dextrin were prepared following the method of Kapusniak et al. (2008).

**CA-DEXTRIN**
HCl (0.1%) + citric acid (0.1%), 130°C, 240 min.

**TA-DEXTRIN**
HCl (0.1%) + tartaric acid (40%), 130°C, 120 min.
<table>
<thead>
<tr>
<th>Physico-chemical characteristics of dextrins</th>
<th>Method</th>
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<td>pH of aqueous solutions of dextrins</td>
<td>potentiometric method</td>
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<td>water solubility at 37°C</td>
<td>Schoch method</td>
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<tr>
<td>reducing sugar content</td>
<td>3,5-dinitrosalicylic acid (DNS) method</td>
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<td>chemical modification</td>
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<tr>
<td>degree of substitution (DS)</td>
<td>Klaushofer et al. method</td>
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<td>molecular weight distribution</td>
<td>High-performance size-exclusion chromatography (HPSEC)</td>
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<td>chain length distribution</td>
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<td>indigestible fraction content</td>
<td>AOAC 2001.03 and Englyst methods</td>
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Assessment of prebiotic properties of dextrins

The growth of bacterial monocultures in broth with enzyme-resistant dextrins

The growth of mixtures of probiotic and intestinal bacteria co-cultured in broth with enzyme-resistant dextrins

Prebiotic index

The products of fermentation of enzyme-resistant dextrins

Probiotic bacteria

*Lactobacillus casei* Shirotia
*Lactobacillus casei* DN 114 001
*Lactobacillus rhamnosus* Lakcid®
*Bifidobacterium animalis* DN 173 010
*Bifidobacterium bifidum* Bb12

Bacteria strains were isolated from feces of three health children (1-year and 8-year) and three adults (30-year) (*Kordyl-Kolata M.*, 2010)

*Escherichia coli*
*Enterococcus*
*Clostridium*
*Bacteroides*

The nine strains

- growth medium as specified by Wynne et al. (2004) modified by removing sources of carbon and replacing them with resistant dextrin at a concentration of 1%
- inoculum – 3%
- control – bacterial cultures in medium containing 1% glucose
- incubation – 37°C, 168 hour, aerobic or anaerobic conditions (co-culture, Concept 400 Anaerobic Workstation)
- dynamics of bacterial growth – Koch’s plate method
Assessment of prebiotic properties of dextrins

- The growth of bacterial monocultures in broth with enzyme-resistant dextrins
- The growth of mixtures of probiotic and intestinal bacteria co-cultured in broth with enzyme-resistant dextrins
- Prebiotic index
- The products of fermentation of enzyme-resistant dextrins

**Determination of fermentation products by High Performance Liquid Chromatography (HPLC)**

Organic acids, aldehydes, and ethanol concentrations were determined using Thermo/Finnigan Surveyor HPLC system equipped with refractive index (RI) and ultraviolet (UV) detectors. Analyses were performed using a BioRAD AMINEX HPX-87H (300 x 7.8 mm) column. Operation conditions were as follows: mobile phase: 0.005 M H$_2$SO$_4$; flow rate: 0.6 ml/min; column temperature: 60°C.

- 24-hour cultures of probiotic and intestinal bacteria
- HPLC system equipped with BioRAD AMINEX HPX-87H (300 x 7.8 mm) column and refractive index (RI) detector
- operation conditions: column temperature – 60°C; mobile phase: 0.005 M H$_2$SO$_4$; flow rate – 0.6 ml/min
- control – bacterial cultures in medium containing glucose
### Assessment of prebiotic properties of dextrins

<table>
<thead>
<tr>
<th>The growth of bacterial monocultures in broth with enzyme-resistant dextrins</th>
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<tr>
<td><strong>PI</strong> = ((Bif/\text{Total}) - (Bac/\text{Total}) + (Lac/\text{Total}) - (Clos/\text{Total}))</td>
</tr>
<tr>
<td><strong>PI</strong> – prebiotic index</td>
</tr>
<tr>
<td><strong>Bif</strong> – bifidobacterial numbers at sample time/numbers at inoculation,</td>
</tr>
<tr>
<td><strong>Bac</strong> – bacteroides numbers at sample time/numbers at inoculation,</td>
</tr>
<tr>
<td><strong>Lac</strong> – lactobacilli numbers at sample time/numbers at inoculation,</td>
</tr>
<tr>
<td><strong>Clos</strong> – clostridia numbers at sample time/numbers at inoculation,</td>
</tr>
<tr>
<td><strong>Total</strong> – total bacteria numbers at sample time/numbers at inoculation</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The growth of mixtures of probiotic and intestinal bacteria co-cultured in broth with enzyme-resistant dextrins</th>
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<tbody>
<tr>
<td>Prebiotic fermentation of resistant dextrins were analyzed using quantitative equation (prebiotic index – PI). The PI equation is based on the changes in key bacterial groups during fermentation. The bacterial groups incorporated into this PI equation were bifidobacteria, lactobacilli, clostridia and bacteroides. The equation assumes that an increase in the populations of bifidobacteria and/or lactobacilli is a positive effect while an increase in bacteroides and clostridia are negative.</td>
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<thead>
<tr>
<th>Prebiotic index</th>
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<td>The products of fermentation of enzyme-resistant dextrins</td>
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</table>
An important factor in the production of potentially prebiotic preparations is the relationship between the content of the undigested fraction and water solubility. It has been shown that pyrodextrins containing a greater proportion of the undigested fraction are less readily soluble in water, which may be due to repolymerization or the formation of non-starch compounds. In turn, study Kapusniaka et al. reported that heating potato starch without acid at 130°C for 3h led to a low-solubility product (2.7%), while heating starch in the presence of hydrochloric acid considerably improved solubility (up to about 67%). This was caused by the hydrolysis of glycosidic bonds and the formation of shorter glucan chains, or even oligosaccharides and simple sugars. An additional treatment with citric acid led to a dextrin with 63% solubility. The water solubility of the dextrin obtained in the presence of tartaric acid was approximately 68%.
High-performance size-exclusion chromatography (HPSEC)

Results. Characteristics of potato starch dextrins

### Table of Characteristics

<table>
<thead>
<tr>
<th>Dextrin</th>
<th>Peak no.</th>
<th>aver. Mw (g/mol)</th>
<th>aver. DP</th>
<th>% of fraction</th>
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</thead>
<tbody>
<tr>
<td>CA-dextrin</td>
<td>1</td>
<td>$2.73 \times 10^6$</td>
<td>16871</td>
<td>1.9</td>
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<tr>
<td></td>
<td>2</td>
<td>$3.56 \times 10^3$</td>
<td>22</td>
<td>86.0</td>
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<tr>
<td></td>
<td>3</td>
<td>161</td>
<td>1</td>
<td>12.1</td>
</tr>
<tr>
<td>TA-dextrin</td>
<td>1</td>
<td>1828</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>154</td>
<td>1</td>
<td>1</td>
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</table>
High-performance anion-exchange chromatography (HPAEC)

Results. Characteristics of potato starch dextrins

<table>
<thead>
<tr>
<th>Dextrin</th>
<th>Carbohydrate profile (Peak DP)</th>
<th>% distribution</th>
<th>Average CL</th>
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</thead>
<tbody>
<tr>
<td>CA-dextrin</td>
<td>DP1</td>
<td>4.58</td>
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<tr>
<td>CA-dextrin</td>
<td>DP2</td>
<td>4.90</td>
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<td>CA-dextrin</td>
<td>DP3</td>
<td>7.28</td>
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<tr>
<td>CA-dextrin</td>
<td>DP4-6</td>
<td>15.65</td>
<td></td>
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<tr>
<td>CA-dextrin</td>
<td>DP7-34 (13)</td>
<td>66.95</td>
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<tr>
<td>TA-dextrin</td>
<td>DP1</td>
<td>24.83</td>
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<tr>
<td>TA-dextrin</td>
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<td>1.6</td>
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<tr>
<td>TA-dextrin</td>
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</tr>
<tr>
<td>TA-dextrin</td>
<td>DP4-6</td>
<td>16.68</td>
<td></td>
</tr>
<tr>
<td>TA-dextrin</td>
<td>DP7-34 (7)</td>
<td>48.18</td>
<td></td>
</tr>
</tbody>
</table>
Results. Characteristics of potato starch dextrins

Based on enzymatic tests, it was postulated that the dextrin obtained in the presence of excess tartaric acid may be classified as RS4 starch. This was also confirmed by previous studies, which indicated that heating potato starch in the presence of tartaric acid led to a high degree of chemical modification. In turn, in the case of dextrin obtained in the presence of citric acid, hydrolysis induced by hydrochloric acid largely dominated chemical modification with citric acid. This was confirmed both by previous FTIR tests and by determining the degree of substitution (DS) of dextrin molecules by citric acid (0.0073%), which means that only about 7 hydroxy groups out of 1000 could be esterified. Thus, dextrins obtained from potato starch met the basic prebiotic criterion – they were not degraded by the digestive enzymes of the upper sections of the gastrointestinal tract.
Results. The growth curves for probiotic bacteria grown in the medium containing D1-dextrin or D2-dextrin

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Both probiotic and intestinal strains used resistant dextrins as a source of carbon and energy. The highest cell counts were found for the strains *Lactobacillus rhamnosus* Lakcid (5.63 × 10⁸ CFU/mL) and *Bifidobacterium bifidum* Bb12 (4.08 ×10⁸ CFU/mL).
Results. The growth curves for intestinal bacteria grown in the medium containing D1-dextrin or D2-dextrin.
Results. The increase in the number of cells of probiotic and intestinal bacteria \((N_K - N_0)\)
Results. Percentage of probiotic and intestinal bacteria grown together in the medium containing D1-dextrin or D2-dextrin

No significant differences were observed between the growth of bacteria isolated from the faeces of 1-year-olds and that of bacteria acquired from 8-year-olds. In contrast, the growth rate of the strains isolated from the feces of 30-year-olds was much smaller in media with dextrin D1, which may indicate greater selectivity of this dextrin in respect of *Clostridium*, *Bacteroides*, and *Enterococcus* strains isolated from people at different age.
Results. Prebiotic index

Of the two studied dextrins, D1, which was produced in the presence of citric acid, exhibited a higher prebiotic index than D2, which was produced in the presence of tartaric acid. This proves that dextrin D1 more strongly stimulates the growth of *Bifidobacterium* and *Lactobacillus* strains.

<table>
<thead>
<tr>
<th>Incubation time (h)</th>
<th>CA-dextrin</th>
<th>TA-dextrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>168</td>
<td>0.70</td>
<td>0.88</td>
</tr>
<tr>
<td>96</td>
<td>0.46</td>
<td>0.27</td>
</tr>
<tr>
<td>72</td>
<td>0.18</td>
<td>0.20</td>
</tr>
<tr>
<td>48</td>
<td>0.18</td>
<td>0.03</td>
</tr>
<tr>
<td>24</td>
<td>0.11</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Results. Percentage of fermentation products after 24-h incubation of probiotic bacteria in the medium containing CA-dextrin or TA-dextrin.
Results. Percentage of fermentation products after 24-h incubation of intestinal bacteria in the medium containing CA-dextrin or TA-dextrin.
Conclusions

- The results show that the manner of preparation of dextrins from potato starch, in particular the concentration of organic acid, has a decisive influence on the chemical structure of dextrins and thus also on their behaviour in relation to digestive enzymes.

- At low concentration of the organic acid, small molecules might repolymerise after the initial hydrolysis, leading to larger, highly branched molecules. High concentration of the organic acid are likely to produce highly chemically modified molecules and thus to increase the content of RS4.

- From the present study, it can be concluded that enzyme-resistant dextrins support the growth of both probiotic and non-probiotic bacteria.

- The yield of probiotic bacterial cells is higher than the yield of cells of bacteria isolated from gastrointestinal tract.

- Probiotic bacteria, in the presence of enzyme-resistant dextrins, are able to dominate the environment in the common culture with bacteria isolated from human feces.

- The type and concentration of fermentation products depend on the examined strain.
Dextrins obtained from potato and corn starch to activate the development of the intestinal bacteria *Bacteroidetes* and to limit the increase of *Firmicutes*, are responsible for obesity and the metabolic syndrome (research *in vitro* and *in vivo* on animals).

The purpose of the research is to define the quantity and quota of the dominating intestinal microorganisms of the types *Firmicutes* and *Bacteroidetes* in obese Poland children and to find out if in fact the *Firmicutes* bacteria preponderate. A further intention of the research is to show if under the *in vitro* conditions (laboratory cultures) as well as *in vivo* (on rats) dextrins obtained from potato and corn starch, may activate the development of *Bacteroidetes* and limit the increase of *Firmicutes*. If that is the case, there is a possibility that a similar mechanism could occur by obese children.
In the human intestinal the dominating groups of bacteria are the *Bacteroidetes* and *Firmicutes*, however by obese persons the quota of *Bacteroidetes* is lower and the one of *Firmicutes* higher than by slim persons. The proportions of the intestinal microorganisms may be regulated by an appropriate diet. Therefore applying ingredients that activate the increase of *Bacteroidetes* in a diet could decrease obesity by humans. This active food ingredients may be dextrins obtained from potato and corn starch.

Currently has been proved that the occurrence of particular groups of intestinal bacteria, especially the populations of *Bacteroidetes* and *Firmicutes*, interrelates with obesity. This selectivity of the stimulation of intestinal bacteria development could be provided by the dextrines obtained from potato and corn starch.
Results. Classified them into five varieties of bacteria.

<table>
<thead>
<tr>
<th>Obese children</th>
<th>Slim children</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lactobacillus</strong></td>
<td><strong>Lactobacillus</strong></td>
</tr>
<tr>
<td>20 strains</td>
<td>20 strains</td>
</tr>
<tr>
<td><strong>Bifidobacterium</strong></td>
<td><strong>Bifidobacterium</strong></td>
</tr>
<tr>
<td>20 strains</td>
<td>20 strains</td>
</tr>
<tr>
<td><strong>Prevotella</strong></td>
<td><strong>Prevotella</strong></td>
</tr>
<tr>
<td>20 strains</td>
<td>20 strains</td>
</tr>
<tr>
<td><strong>Bacteroides</strong></td>
<td><strong>Bacteroides</strong></td>
</tr>
<tr>
<td>20 strains</td>
<td>20 strains</td>
</tr>
<tr>
<td><strong>Clostridium</strong></td>
<td><strong>Clostridium</strong></td>
</tr>
<tr>
<td>20 strains</td>
<td>20 strains</td>
</tr>
</tbody>
</table>
Results. The number of microorganisms in lean and obese children

In obese children were dominated by strains *Clostridium* while slim children were dominated by *Bifidobacterium* and *Lactobacillus*. 
So we have to take care of intestinal microorganisms, turning evil for good!
Thank you for your attention