Demystifying Microbiome Research: New Opportunities for Health & Business

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Agenda

• What is the Microbiome?

• Advances in Microbiome Research

• Relevant Endpoints for Microbiome Studies

• Clinical Substantiation of Probiotics

• Concluding Remarks
Microbiome – Definition

• What we know as the microbiome was coined by Joshua Lederberg as a means to “signify the ecological community of commensal, symbiotic, and pathogenic micro-organisms that literally share our body space and have been all but ignored as determinants of health and disease” (1)

• The term microbiota is the specific microbial population in our body (bacteria, viruses, archea, protozoans, and fungi)

• The microbiome is viewed as the catalog of these microbes genome (i.e. genes)\(^{(2)}\)
Microbiome – History & Function

- Investigation of the diversity of the microbiome began as early as the 1680’s when Antonie van Leeuwenhoek compared his oral and fecal microbiota, noting that there were striking differences between the 2 samples \(^{(3)}\).

- The mutually beneficial relationship between vertebrates and microbes has evolved over millions of years and these micro-organisms have selected for specialized areas of our body such as the gut, mouth, and skin.

- Approximately 90% of the cells in our body are made up of the microbes that colonize our gut.

- The gut microbiome community can influence many aspects of our physiology such as the metabolism/absorption of nutrients, as well as play a preventative role in the pathogenesis of various diseases.

Grenham et al., 2011
Advances in Microbiome Research

Nutrition and dietary supplements can impact the gut microbiome. Thus research into the relationship between our gut microbiota, our physiology, and dietary supplements has become more widespread. Notable advances include;

• *Hilderbrandt et al 2009.*, supplying mice with a high-fat diet led to decreased *Bacteroidetes* and increased *Firmicutes* and *Proteobacteria* in the murine gut, and this was independent of weight gain

• *Sela & Mills 2010.*, the bioactive molecules dissolved in breast-milk influences the development of an infants gut microbiota.

• *Smith M et al 2013.*, severely malnourished children have deregulated gut microbiota that make it difficult to utilize nutrients from food.
Advances in Microbiome Research

Our gut microbiome may also play a role in metabolism and a personalized approach to medicine;

• *Turnbaugh P et al 2009.*, our metabolism has been associated with a core gut microbiome and deviations from this core assembly of microbiota is linked with different physiological states (i.e. obese vs. lean individuals)

• *Knight et al 2015.*, crowd-funded projects, such as the American Gut (participants have their microbiomes sequenced) may help replenish our ancestral microbes and/or discover new ones that will help maintain wellness

• *Knight R et al 2016.*, development of a “personalized” mouse models that can harbor an individual’s microbiome so the effects of interventions (nutrients or drugs) can be tested
Psychobiotics: The microbiome and the Gut-Brain Axis

- **Messaoudi et al 2011.**, L.helveticus and B.longum reduced psychological distress
- **Steenbergen et al 2015.**, multi-species probiotic reduced negative thoughts associated with sad mood
- **Schmidt et al 2015.**, prebiotic reduced stress response and increased processing of positive vs. negative attentional vigilance
- **Allen et al 2016.**, B. longum reduced self reported stress and improved brain activity
- **Kato-Kataoka et al 2017.**, L. Casei strain Shirota reduced physiological and physical symptoms of stress
- **Sampson T et al 2016.**, a mouse with the microbiota from patients suffering from Parkinson’s developed the same disease progression
Oral Microbiome Research

- Parahitiyawa NB, et al. 2010. Over 6 billion bacteria spanning over 700 species inhabit the mouth

- Health Canada, 2008. Oral health linked to overall health (cardiovascular; diabetes; etc)

- James KM, et al. 2016. Specific probiotic combinations prevent C. albicans biofilm formation by downregulating genes critical to pathogenesis

Relevant Endpoints for Microbiome Studies

• Collect metagenomic data from microbiome screening (QIIME, 16S rRNA, shotgun genomic analysis). This can be done in individuals via fecal collection and subsequent extraction of RNA/DNA sequences (7).

• Employ the use of novel bioinformatic tools (e.g., InnateDB and NetWorkAnalyst) to evaluate the interaction of genes, proteins, and cytokines that may be modulated by alterations to the microbiome (8).

• Molecular markers of gastric emptying (via radiopaque markers), GI inflammation (fecal calprotectin or lactoferrine), and microbiome stability (i.e., microbiome sequence before/after a round of antibiotics) (15).

• Validated gut-health questionnaires (bowl habits, GI symptoms, and quality of life).
Clinical Substantiation of Probiotics

The KGK Augmented Randomized Controlled Trial:

- **Study Design:** trial design that follows a two stage process
  - **Stage 1:** Capture valuable information as to optimal inclusion criteria, dosing, and responders
    - Meta-analysis of several N-of-1 trials
  - **Stage 2:** Confirmatory trial, utilizes information gathered from stage 1 to provide optimal inclusion criteria in a Randomized double-blind study

Other points to consider:

- **Endpoints:** Disease endpoints that relate to drugs commonly used
  - KGK proposes development of a global health index for probiotics
- **Population:** Challenge of recruiting healthy populations with sufficiently low intake
Summary

• The microbiome is the catalog of all the microbes and their respective genes that colonize our gut (and other areas of the body)

• Diet and supplements can significantly impact the composition of the microbiome and may be used to optimize its function

• A healthy microbiome is critical to maintain wellness and may contribute to the prevention of chronic conditions

• Clinical substantiation of supplements that support health and wellness will continue to be of great importance
About KGK

Founded in 1997 and built on a foundation of scientific excellence, KGK is the leading contract research organization in the Nutraceutical Industry. Working to substantiate claims of dietary supplements, functional foods, and other natural health products, KGK incorporates the highest quality standards and state-of-the-art scientific technique and technologies to satisfy our client needs.

With clinics in Canada and the USA, KGK Clinical Trial Centers can efficiently develop and manage your research throughout North America. KGK is renowned internationally for our ethical and specialized study of natural health products.
References

References


References

• [22] Nuzzo, R. Statistical Errors: P-values, the gold standard of statistical validity, are not as reliable as many scientists assume. Nature. 2014:506.
References

How botanicals can benefit the gut microbiota and its host?

Antoine Bily, Pascale Fança-Berthon

Demystifying microbiome research: new opportunities for health & business

Engredea, Thursday March 9th 2017
The human microbiota

The term microbiota refers to the microbial population present within the human body, including bacteria, viruses, archea, protozoans, and fungi.

Represents 10 times more cells than human cells: 1-2 kg of material

$10^{14}$ Microorganisms in the gut

Gut microbiota genome = microbiome = 100 to 150 times more genes than our own genome

It is a hot topic
What are its fundamental characteristics?

It is unique

1000 species estimated to be part of the human microbiota
But only 200 species per individual; each combination of species is unique

But functional activities of the microbiota very similar from a healthy subject to another

It is possible to classify people according to their enterotype i.e., the relative abundance of 3 bacterial groups: Bacteroides, Prevotella and Ruminococcus

What are its main roles?

The whole community of gut microbiota serves many functions:

- **Metabolic**
  - Production of vitamins
  - IEC differentiation
  - Digestion of dietary carcinogens
  - Fermentation of non-digestible Substrates
  - Production of SCFAs

- **Antimicrobial secretion**
- **Protective**
  - Colonization resistance
  - Innate and adaptive immunity
  - Inflammatory cytokine oversite

- **Sites and nutrients competition**
  - Immune system and barrier function

- **Energy Source**

- **Structural**
  - sigA production
  - Intestinal villi and crypts
  - TJs
  - Mucous Layer

Grenham et al. Front Physiol, 2011
What is dysbiosis?
Dysbiosis definition

During healthy, homeostatic conditions the microbiota is composed of a diversity of organism. However, environmental factors can lead to alterations of the ecologic organization of the gut microbiota via:
- Pathobiont expansion
- Reduced diversity
- Loss of beneficial microbes

This is dysbiosis, a switch from a mutualistic beneficial relationship to a pathological unbeneficial relationship:

- Mutualistic effects
- Pathogenic effects
- Regulatory
- Proinflammatory
- Beneficial Homeostasis
- Dysbiosis e.g. IBD and CRC
- Host health
  - Tolerance
  - Robust regulation
  - Barrier protection
  - Antimicrobial activity
  - Tissue repair
  - Cell renewal
- Disease
  - Intolerance
  - Lack of regulation
  - Barrier defects
  - Microbial invasion
  - Tissue pathology
  - Cell damage

Petersen and Round. Cellular Microbiology, 2014
Obesity-related dysbiosis and IR & steatosis

- Loss of diversity
  - Pathobiont expansion
  - Loss of beneficial microbes

- Leaky gut – hypermeability

- Low grade inflammation
  - Adipose tissue
    - Muscle
    - Liver

- Altered glucose & lipid metabolism
  - Steatosis

How can we reverse dysbiosis?

**Probiotics** are “live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host” (Definition FAO/WHO 2012)

**Prebiotics** are “selectively fermented ingredients that promote the selective stimulation of growth and/or activities of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host” (Definition from Gibson & Roberfroid et al, 2010)

**Symbiotics**

**Postbiotics** are “non-viable bacterial products or metabolic byproducts from probiotic microorganisms that have biologic activity in the host” (Definition Patel & Dening, 2013)

**REBIOSIS:** Establishing a microbial community back to a healthy state

**Botanicals**?
Positive influence of a *Fraxinus* extract on steatosis *via* gut microbiota modulation
What is *Fraxinus angustifolia*?

- *Fraxinus angustifolia* is a tree usually referred to as narrow-leaved ash

- Historically, *Fraxinus* was largely limited to the Mediterranean Basin, where local populations used it in food and infusions, and for its hypoglycemic effect¹

- Glucevia® is an extract derived exclusively from the samara of *Fraxinus angustifolia*

  **Product Description:** Purified extract of *Fraxinus* samara
  **Standardization:** > 10% Nuzhenide and GL3 (= secoiridoids) via HPLC
  **Substantiation:** Clinically shown to reduce blood glucose. Shown to reduce steatosis in *In vivo* animal models

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Metabolic syndrome and Liver Health

- **Non-alcoholic fatty liver disease (NAFLD)** is one of the causes of fatty liver, occurring when fat is deposited (steatosis) in the liver due to causes other than excessive alcohol use.

- **Non-alcoholic steatohepatitis (NASH)** is the most extreme form of NAFLD, and is regarded as a major cause of cirrhosis of the liver of unknown cause.

- Cause is still unknown but **obesity** and **insulin resistance** likely play a strong role in pathology.

- NASH is generally recognized as the liver’s expression of Metabolic Syndrome.


World Gastroenterology Organization, 2012
Arslan. World J Gastroenterol, 2014
**Scientific substantiation – in vivo**

**Title:** Glucevia® limits weight gains and hyperglycemia in high-fat diet-induced obese mice

**Population:** C57BL/6J male mice (5 weeks of age)

**Duration:** 16 weeks

**Design:**
- High Fat Diet [60% fat] \((n=20)\)
- HFD + 0.5% Glucevia® \((n=10)\)
- Low Fat Diet [60% fat] \((n=20)\)

**Outcomes:**
- Fasting blood glucose
- Fatty livers

**Result:** Significantly lowers the development of hyperglycemia after administration of a high fat diet by 33% \((p<0.001)\)

**Result:** Significantly reduces fatty liver by 50%

Ibarra et al. (2011) Phytomedicine,
**Title:** Action of an extract from the seeds of Glucevia® on metabolic disorders in hypertensive and obese animal models

**Population:** Zucker rats (8 weeks old)

**Design:** Administered, for 5 weeks, a diet containing:
- Negative control: No additive (n=6)
- Positive control: Metformin (n=6)
- Glucevia (100 mg/kg bw/day) (n=6)

**Outcomes:**
- Glucose was followed during the 5 weeks study

**Results:**
- Significantly reduces fasting blood glucose by 16% (p<0.001)
- No significant difference between Glucevia and Metformin. (Metformin is the reference drug for blood glucose management)

Monto et al. (2014). Food&Function
Title: Preventive Effect of a Glueveia® on Hepatic Steatosis in Obese Type 2 Diabetic Mice

Population: BKS (db/db) female mice (5 weeks of age)
Design: 7 months administration of Control diet (n=10)
Diet with Glueveia® (0.7g/kg of diet) (n=10)

Outcomes:
Metabolic blood parameters (glucose, insulin, cholesterol, triglycerides)
Histological analyses of liver
NAFLD blood parameters

Results
• Significant reduction in Insulin (p < 0.05)
• Significant improvement in Insulin resistance (p < 0.05)
• Significant improvements in fatty liver

Stained sections of livers from diabetic mice administered control or Glueveia®
• Micrographs of H&E (a vs. b)
• Oil red O (c vs. d)

**Acute Human Clinical Study**

**Title:** Acute effects of Glucevia® on postprandial glycemia and insulin secretion on healthy volunteers

**Population:** 16 healthy people (11 male, 5 female; 20-55 y, BMI: 26±2.2 kg/m²).

**Design:** Double blind, randomized, crossover (1 week washout period), acute, placebo controlled clinical trial.

**Dose:** 1,000 mg of Glucevia® or a Placebo (Wheat bran), 50 g glucose for OGTT

**Outcomes:** Blood glucose (0, 15, 30, 45, 60, 90, 120 min)

**Results:** Immediate and significant reduction in blood glucose AUC of 9% (p > 0.05)

**Chronic Human Clinical Study**

**Title:** Glucemia® benefits glucose homeostasis and adiposity related markers in elderly overweight/obese subjects: A longitudinal, randomized, crossover, double-blind, placebo-controlled nutritional intervention study.

**Population:** 17 healthy volunteers (50-80 y, BMI: >25 kg/m²).

**Design:** Double blind, randomized, cross-over (21 days for each arm), placebo controlled intervention study with one week wash-out between arms.

**Interventions:**
- Recommendation for a healthy diet
- 1,000 mg of Glucemia® or placebo

**Outcomes:**
- OGTT before and after each arm
- Markers of glycemia

**Results:**
- Significant reduction in blood glucose (AUC) of 28% in Glucemia group vs. Non-significant reduction of 8% in placebo group.

Zulet et al. (2014) Phytomedicine 21: 1162–1169

Effect of 3-week Glucemia® administration on blood glucose levels
**Results:** Significant (p<0.05) reduction in 2h blood glucose level (-14%) observed

**Relevance:** According to the World Health Organization the 2h blood glucose value is regarded as an indicator of impaired glucose tolerance and diabetes (>140 mg/dL)

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**Results:** Significant difference in the change in fructosamine between groups

**Relevance:** According to EFSA, Fructosamine is relevant marker for glucose control and the most widely used alternative to HbA1c
Where does efficacy come from?

**Title:** Targeted and untargeted metabolomics to explore the bioavailability of the secoiridoids from Glucevia® in human healthy volunteers: a preliminary study

**Population:** 10 healthy volunteers (23.5 y, BMI < 30 kg/m²).

**Design:** open-labelled acute single dose bioavailability study with a targeted and untargeted metabolomics methodology

**Outcomes:**
- Plasma metabolites analysis before and 1, 2, 4, 8 and 24h after 1000mg of Glucevia® intake
- Urine metabolites analysis before and 1, 2, 4, 8 and 24h after 1000mg of Glucevia® intake

**Results:**
No parent compounds (nuzhenide and GL3) found in urine or plasma
Some predicted metabolites detected at low intensity
Detection of potential phenolic conjugates and sulfates derivatives of secoiridoids from phase I and/or microbial enzymes

**Conclusion:**
The effects of Glucevia seem not to be linked to a direct effects of its parent compounds but to an direct or indirect effect of their metabolites (human or gut microbiota-derived).

Working Title: Glucevia®, a *Fraxinus angustifolia* Vahl extract, is able to modify the gut microbiota composition and those effects are correlated with steatosis severity in obese and diabetic mice.

**Population:** Mice  
**Design:** Administered, for 12 weeks, a diet containing 60% energy from fat with or without Glucevia® (200mg/kg bw)  
**Outcomes:** Liver fat content, metagenomics study on the fecal samples

Glucevia protected against liver steatosis induced by 12 week high fat diet consumption in mice

The World Gastroenterology Organization histological scoring system for steatosis (Grade 0: <5%, Grade 1: 5-33%, Grade 2: 34-66%, Grade 3: >67%):

**Results:**  
Control: 35.2% - Grade 2  
Glucevia® 22.8% - Grade 1 (-35% vs. control) p = 0.004
Glucevia induced some modifications of the gut microbiota composition in mice fed a HFD during 12 weeks.
The gut microbiota modifications are significantly correlated (positively or negatively depending on the bacterial groups) to the liver steatosis severity.
Continued research program

**Longitudinal clinical study in preparation**

**Population:** Subjects suffering from steatosis and NASH (fibrosis score < 2), n = 50

**Duration:** 6 months

**Main clinical endpoints:** Liver fat content, gut and blood microbiota metagenomic (shot gun)

**Objectives:**

- Establish a proof of concept of efficacy for improvement of NAFLD in humans
- Demonstrate that Glucovia is able to modify the gut microbiome in humans
- Demonstrate the correlation between steatosis severity and the modification of gut microbiome in humans

Strategy for demonstrating the causative role of the gut microbiota in chronic diseases.

How can we reverse dysbiosis?

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(Definition FAO/WHO 2012)

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(Definition from Gibson & Roberfroid et al, 2010)

Symbiotics

REBIOSIS: Establishing a microbial community back to a healthy state

Postbiotics are “non-viable bacterial products or metabolic byproducts from probiotic microorganisms that have biologic activity in the host”

(Definition Patel & Dening, 2013)

Botanicals!

Revisited & enlarged definition of prebiotics
Not an isolated case…
A Rosemary Extract Rich in Carnosic Acid Selectively Modulates Caecum Microbiota and Inhibits β-Glucosidase Activity, Altering Fiber and Short Chain Fatty Acids Fecal Excretion in Lean and Obese Female Rats

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Conclusion

Our results show that plant bioactive compounds such as those contained in the rosemary extract (mainly cirtopencosides) or their derived metabolites are able to modify gut microbiota composition and to promote beneficial changes with an impact into host metabolism and inflammatory response. Therefore these compounds should be considered as prebiotic or as compounds with “prebiotic-like” effects. Further researches are still required to fully characterize their mechanism of action and to demonstrate an effect in humans.
A first prospective, randomized, double-blind, placebo-controlled study on the use of a standardized hop extract to alleviate menopausal discomforts

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Received 4 February 2005; received in revised form 10 October 2005; accepted 11 October 2005

A randomized, double-blind, placebo-controlled, cross-over pilot study on the use of a standardized hop extract to alleviate menopausal discomforts

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Fig. 3. Inter-individual differences in the conversion of isoxanthohumol into 8-prenylnaringenin by intestinal bacteria derived from 51 individuals. Statistical two-step cluster analysis revealed three groups with significantly different means (P < 0.01). Individuals were separated in slow (I), moderate (II) and high (III) 8-prenylnaringenin producers. (After Possemiers et al., 2006.)
Acknowledgments

Inserm UMR 1048/I2MC
Diabetology department,
CHU de Toulouse
Elodie Riant
Aurélie Waget
Rémy Burcelin
Pierre Gourdy

R&D team
Leila Falcao
Melissa Feuillatre

Rocío García-Villalba
Pilar Zafrilla
María-Teresa García-Conesa
Francisco A. Tomás-Barberán
Thank you for your attention!

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