All about Gut Health: Understanding the Role of Prebiotics, Probiotics and Postbiotics

Kate Scarlata MPH, RDN
October 26, 2021 | 2:00 PM EST
Learning Objectives

1. Describe the difference between live and active cultures, probiotics, prebiotics and postbiotics
2. Detail key factors one should consider when selecting probiotics for a specific benefit
3. Analyze three foods that contain prebiotics and discuss what clients should look for on food labels.
4. Describe how the gut and brain interact via our gut microbes
**Gut Microbiota**

The microorganisms inhabiting the gastrointestinal tract. The composition of this microbial community is host specific.

**Gut Microbiome**

The entire collection of genes found in all of the microbial cells living in the gastrointestinal tract.

**Metabolome**

The specific metabolites in biological samples (tissues, cells, fluids, or organisms) under normal conditions in comparison with altered states promoted by disease, drug treatment, dietary intervention, or environmental modulation.

---

Gut Microbiome

- 100 trillion microbes
- Includes bacteria, viruses, fungi, archaea
- Influence human physiology, metabolism, nutrition (produce-riboflavin, folate and cobalamin) and immune function

Functions of the Microbiome

- Pathogen protection—e.g., competes for nutrients
- Maintenance of intestinal barrier—protects against increased intestinal permeability
- Nutrient + drug metabolism
- Immune modulation
- Produces and communicates with hormonal products as an endocrine-like organ
- Impacts brain function via gut-brain axis

Some examples: microbes are capable of producing SCFAs which are used as nutrients for colonocytes, create neurotransmitters potentially impacting mood, support cholesterol metabolism, and regulate various hormones involved in appetite.

"Microbiota-modulating dietary interventions include many fermented foods and [fiber]-rich dietary regimens, as well as probiotics, prebiotics and synbiotics, some of which are available as drugs and medical devices, as well as foods."

Primer for “-biotics”

**Probiotic:** “Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.”

**Prebiotics:** “a substrate that is selectively utilized by host microorganisms conferring a health benefit.”

**Postbiotic:** “preparation of inanimate microorganisms and/or their components that confers a health benefit on the host.”

**Synbiotic:** “a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host.”

Probiotics: It’s in the Details

The word “probiotic” comes from Greek, and it means “for life”

• Probiotics include characterized strains with a scientifically demonstrated effect on health

• Probiotics are known by genus, species, and strain: for example, *Lactobacillus acidophilus* ABC

• Different strains of the same species may have different health effects

• Dose is important! A probiotic consumed at a higher dose may not necessarily have a > health benefit than one consumed at a lower dose

• Select dose shown in the research to confer a health benefit

Fermented Foods

Fermented foods are those made through desired microbial growth and enzymatic conversions of food components.

Live microbes present in traditional fermented foods and beverages such as kombucha, yogurt, sauerkraut, and kimchi typically do not meet the required evidence level for probiotics.

Live Active Culture vs Probiotic

• Live active cultures are living microbes used to make fermented foods + beverages

• **Are cultures + probiotics the same?**
  Cultures provide technological benefits while probiotics provide health benefits. Sometimes a culture is also a probiotic + sometimes it is not

• **Where to find them:** Lactobacillus bulgaricus and Streptococcus thermophilus are widely used to ferment milk into yogurt to create unique nutritional and flavor benefits
Fermented Food

“Fermentation processes and products are believed to have been developed **9000 years ago** in order to preserve food for times of deficiency, improve flavor, and reduce poisonous effects.”

Contains live culture: fresh kimchi, water or cured olives, traditional salami, fresh sauerkraut

Does **not** contain live cultures: tempeh, most wine + beer, sourdough bread

https://isappscience.org
## Why Consume Fermented Foods?

1. Enhanced taste and texture

2. Increased microorganism content that may improve gastrointestinal health, lowering the risk of type 2 diabetes and cardiovascular disease

3. Convert unsaturated fatty acids to conjugated linoleic acid, which has anti-inflammatory effects

4. Enhanced availability of B vitamins, Vitamin K, magnesium, and zinc

5. Creation of isoflavones genistein and daidzein from soy foods

6. Reduction of anti-nutrients such as phytates

7. Enhance polyphenols -- extracted out of the food by microbe fermentation (Polyphenols can function as prebiotics)

8. Increase gut microbial diversity and reduce inflammatory markers

---

RCT: Fermented Food vs High Fiber

N=36 healthy adults (18/arm) were randomly assigned to a 10-week diet that included either fermented (around 6 servings) or high-fiber (40 grams) diet

Combined with -omics measurements of microbiome and host, including extensive immune profiling

Results found diet-specific effects:

• High fiber diet noted to change microbiome function

• Fermented food (yogurt, kefir, fermented cottage cheese, kimchi and other fermented vegetables, vegetable brine drinks, and kombucha tea) led to an increase in overall microbial diversity
  • 4 types of immune cells showed less activation in the fermented-food group
  • The levels of 19 inflammatory proteins measured in blood samples decreased
  • One of these proteins, interleukin 6, has been linked to conditions such as rheumatoid arthritis, Type 2 diabetes and chronic stress

Selecting a Probiotic

Note: food and supplements may supply probiotics

Key factors to selection:

• Backed by science
• Provides effective dose
• Provides the benefit you are seeking
• Properly labeled with CFU* amount, GSS (genus, species, strain) dose and proper storage

https://isappscience.org
*Colony-Forming Units
Know the “G-S-S”

- Probiotics are known by genus, species, and strain
- Different strains of the same species may have different health effects
- The dosage consumed should match the level shown in an efficacy study to confer a benefit

https://isappscience.org/for-scientists/resources/probiotics/
Strain Specific Effects

2 DBRCT studies combined, n=538 adult women with minor GI complaint

Primary endpoint: the effect of the consumption of a Fermented Milk Product (FMP) over 4 weeks on Subject’s Global Assessment (SGA) of gastrointestinal well-being

Assessment of FMP with Bifidobacterium animalis subsp. lactis CNCM I-2494 and lactic acid bacteria 125 g BID compared to control (non-fermented dairy product without bacterial strains) has been shown to reduce the frequency of minor digestive symptoms (gas, rumbling, abdominal discomfort and bloating) when consumed twice a day for 2 and 4 weeks

*This effect is not influenced by dietary fiber intake or physical activity at the start of study*

Probiotic Selection Resources

Bear in mind: This guide is geared toward disease management. Not all probiotics are utilized to benefit disease specific symptomology but rather goal of prevention of disease or maintenance of health.
Criteria are used to classify a compound as a prebiotic:

- The growth and/or activity of the intestinal microbes can be selectively stimulated by prebiotics and improve health
- Resistant to acidic pH of stomach
- Cannot be hydrolyzed by mammalian enzymes
- Should not be absorbed in the GI tract
- Most prebiotics are carbohydrates but NOT all!!

Prebiotics

- Majority are a subset of CHO groups—oligosaccharide carbohydrates:
  - GOS + Fructans
- Others include non-carbohydrate prebiotics, such as cocoa-derived flavanols - e.g., Flavanols can stimulate lactic acid bacteria
### Prebiotics You Might Find on a Food Label or in Foods

<table>
<thead>
<tr>
<th>Prebiotic Examples</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOS, Oligofructose and Inulin</td>
<td>Onion, garlic, wheat, additives: chicory root extract, inulin, FOS</td>
</tr>
<tr>
<td>GOS</td>
<td>Legumes, nuts + seeds</td>
</tr>
<tr>
<td>Isomaltooligosaccharides - IMOs are a mixture of ( \alpha-(1 \rightarrow 6) ) and ( \alpha-(1 \rightarrow 4) )-linked glucose oligomers, synthesized by an enzymatic reaction from starch (corn, tapioca).</td>
<td>Sweetener and bulking agent found in cereals, granola bars; IMOs naturally exist in honey, and fermented foods, such as soy sauce, miso, and sake.</td>
</tr>
<tr>
<td>Guar gum-polysaccharide extracted from endosperm of the plant <em>Cyamopsis tetragonolobus</em>.</td>
<td>Guar Gum is commonly used in dairy, bakery, cereal, and meat products.</td>
</tr>
<tr>
<td>RSs and maltodextrin (Resistant maltodextrin is highly water-soluble dextrin that is produced by treating cornstarch with numerous acid, enzymatic, and heating processes, and used in a variety of applications.)</td>
<td>RS-uncooked oats, cooked and cooled rice and potatoes, Hi-maize resistant starch is isolated from white, cornstarch with high amylose content, resists digestion in small intestine used in some products.</td>
</tr>
<tr>
<td>B-glucan</td>
<td>Oat and barley are the 2 highest sources of beta-glucans today in the diet.</td>
</tr>
<tr>
<td>Xyloooligosaccharides</td>
<td>XOSs are commonly found in dairy products, cereals, bars, sports drinks, and isotonic beverages</td>
</tr>
</tbody>
</table>

Health Benefits of Prebiotics

- Increase bioavailability of calcium
  - Calcium absorption is stimulated by the chemical changes and increases in acid fermentation of the prebiotic dietary fibers by various bacteria
- Reduction of potential deleterious metabolites (ammonia, indoles, sulfides)
  - Prebiotic intake with protein consumption reduces potential deleterious metabolites
- Reduction of pathogenic microbes
  - (e.g. E. coli, Salmonella ssp, Campylobacter) populations, via lowering colonic pH below pathogenic bacteria threshold, competition for limited nutrients, enhanced immune system, limited colonization sites
- Modulate immune function + improved intestinal permeability

Prebiotics: Chain Length Matters

Certain microbes have the capacity of fermenting different carb chain length (DP*)

Inulin (with DP of ≤60) can be fermented only by a few species, whereas a large number of microorganisms are able to degrade FOS (with DP of ≤10)

Location of fermentation differs: FOS** are rapidly fermented in the proximal colon whereas inulin appears to have a more sustained fermentation profile that potentially enables protective effects in the distal colon


*DP=degree of polymerization. **FOS=fructo-oligosaccharides
The Newer Kid on the Block: **Postbiotics**

**Postbiotic**: “preparation of inanimate microorganisms and/or their components that confers a health benefit on the host”

- Effective postbiotics must contain inactivated microbial cells or cell components, with or without metabolites, that contribute to observed health benefits

- To qualify as a postbiotic, the microbial composition prior to inactivation must be characterized, and so preparations derived from undefined microorganisms are not included in the definition
  - Many traditional fermented foods are created with undefined, mixed cultures, and such a product could not be used for the preparation of a postbiotic

The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics

Sappo Salminen,1,2,3 Maria Carmen Collado,2,4 Akibito Endo,1 Colin Hill,1,3 Sarah Lebeer,4,5 Eamonn M. M. Quigley,6,7,8 Mary Ellen Sanders,9 Roaan Shamir,7,10 Jonathan R. Swane,11,12 Hania Sznajder,13 and Gabriël Vinderola14

Abstract | In 2019, the International Scientific Association for Probiotics and Prebiotics (ISAPP) convened a panel of experts specialized in nutrition, microbial physiology, gastroenterology, pandiatrics, food science and microbiology to review the definition and scope of postbiotics. The term ‘postbiotics’ is increasingly used in the scientific literature and on commercial products, yet is inconsistently used and lacks a clear definition. The purpose of this panel was to consider the scientific, commercial and regulatory parameters encompassing this emerging term, propose a useful definition and thereby establish a foundation for future developments. The panel defined a postbiotic as a “preparation of inanimate microorganisms and/or their components that confers a health benefit on the host”. Effective postbiotics must contain inactivated microbial cells or cell components, with or without metabolites, that contribute to observed health benefits. The panel also discussed existing evidence of health-promoting effects of postbiotics, potential mechanisms of action, levels of evidence required to meet the stated definition, safety and implications for stakeholders. The panel determined that a definition of postbiotics is useful so that scientists, clinical trialists, industry, regulators and consumers have a common ground for future activity in this area. A generally accepted definition will hopefully lead to regulatory clarity and promote innovation and the development of new postbiotic products.

The past few decades have demonstrated unprecedentedly the importance of the human microbiota to both short-term and long-term human health. Early programs of the microbiota and immune system during pregnancy, delivery, breastfeeding and weaning is important and determines adult immune function, microbiome and overall health. We have also seen rapid growth in the number of products that claim to affect the functions and composition of the microbiota at different body sites to benefit human health.

Improving human health through modulation of microbial interactions during all phases of life is an evolving concept that is increasingly important for consumers, food manufacturers, health-care professionals and regulators. Microbiota-modulating dietary interventions include many fermented foods and fiber-rich dietary regimens, as well as prebiotics, probiotics and synbiotics, some of which are available as drugs and medical devices, as well as foods. The rich, diverse microbial ecosystems and immune cells inhabiting all mucosal and cutaneous surfaces provide targets for intervention, with the goals of reducing the risk of diseases and improving health status. Consensus definitions of probiotics, prebiotics and synbiotics have been published previously. Prebiotics are “non-microorganisms that, when administered in adequate amounts, confer a health benefit on the host”, whereas a probiotic is a “substance that is selectively utilized by host microorganisms conferring a health benefit on the host”. A synbiotic, initially conceived as a combination of both probiotics and prebiotics, has now been defined as “a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host”. The concept of postbiotics is related to this family of terms and is emerging as an important microorganism-mediated tool to promote health.

Probiotics are by definition alive and required to have an efficacious amount of viable bacteria at the

5 potential mechanisms postbiotics offer benefit:
1. modulation of the resident microbiota
2. enhancement of epithelial barrier functions
3. modulation of local and systemic immune responses
4. modulation of systemic metabolic responses
5. systemic signaling via the nervous system

Postbiotic Potential

• Molecules present in postbiotics, such as lactic acid and bacteriocins may provide direct antimicrobial activity

• Postbiotics could also modulate the microbiota indirectly by carrying lactic acid that can be consumed by some microbiota resulting in SCFAs and butyrate

• Postbiotics may also compete with resident microorganisms for adhesion sites

• SCFAs present in a postbiotics have the potential to modify epithelial barrier function and protect against negative lipopolysaccharide-induced changes

<table>
<thead>
<tr>
<th>Data with Postbiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients with IBS with diarrhea (n = 297)</strong></td>
</tr>
<tr>
<td><strong>Patients with recurrent respiratory tract infections (n = 160)</strong></td>
</tr>
<tr>
<td><strong>Patients with cancer and leukopenia following chemotherapy (n = 78)</strong></td>
</tr>
<tr>
<td><strong>Preterm infants 30–35 weeks of gestational age, age 0–3 days (n = 58)</strong></td>
</tr>
</tbody>
</table>

Figure adapted from Salminen S, et al. *Nat Rev Gastroenterol Hepatol*. 2021 Sep;18(9):649-667.
Define: “a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host”

2 subtypes:

**Synergistic synbiotic**

**Complementary synbiotic**
Synbiotics

Complementary Synergistic

Prebiotic Probiotic

Health benefits

Live microorganisms chosen for health benefit

Substrate selectively used by co-administered microbes

Figure adapted from Swanson KS. et al. Nat Rev Gastroenterol Hepatol 17, 687–701 (2020).
The Shaping of Our Gut Microbiota

FETUS  BIRTH/FEEDING METHOD  WEANING  3 YRS-ADULT

uterine

Breast vs formula
Antibiotic exposure
c-section vs. vaginal birth

Solid foods

High fiber diet
Western diet
low fiber, high animal protein

Factors that may impact gut microbiota composition + diversity during life stages

<table>
<thead>
<tr>
<th>Gestational health/DM</th>
<th>Diet</th>
<th>Antibiotics</th>
<th>Probiotics + Prebiotics</th>
<th>Bacteria in amniotic fluid</th>
<th>Lifestyle</th>
<th>Hygiene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode of delivery</td>
<td>Birth weight</td>
<td>Environment</td>
<td>Antibiotics</td>
<td>Maternal flora</td>
<td>Hospital flora</td>
<td></td>
</tr>
<tr>
<td>Mode of feeding</td>
<td>Fatty acids in breast milk</td>
<td>Type of formula</td>
<td>Siblings</td>
<td>Pets</td>
<td>Dust/hygiene</td>
<td>Probiotics</td>
</tr>
<tr>
<td>Diet</td>
<td>Geography</td>
<td>Hygiene</td>
<td>Antibiotics</td>
<td>Probiotics</td>
<td>Sleep</td>
<td>Pregnancy</td>
</tr>
<tr>
<td>Lifestyle</td>
<td>Age related illnesses</td>
<td>Hospital stays</td>
<td>Hygiene</td>
<td>Menopause</td>
<td>Drugs</td>
<td></td>
</tr>
</tbody>
</table>

The Brain-Gut Connection

- Enteric nervous system + central nervous system highly linked
- Example: Stressful event—induces an emergent trip to the bathroom—or GI symptoms lead to anxiety

Vagus Nerve
- Neurotransmitters
- Stress
- Mood
- Behavior

- Motility
- Secretions
- Gut microbiota
Gut-Brain Axis (GBA)

- Gut microbiota regulates neurotransmitters/brain chemicals such as: serotonin (alters precursors), GABA, dopamine
- When gut bacteria diversity diminishes, there are systemic consequences, such as GI and psychological distress
Stress-Induced Gut Microbiome Changes

Stress exposure has been shown to change the composition of the gut microbiome and worsen intestinal inflammation.

Stress exposure has a profound effect on microbiome diversity, multiple genera, and mucosal inflammation.

Gut microbiota contribute to dysregulation of mucosal inflammatory responses during stress.

Colonization of gut microbes is necessary for normal immune system development, as indicated by the loss of immune function in germ-free mice. Microbiota stimulates immune cells through their metabolites (e.g., SCFA + proliferation of T cells). Probiotic bacteria interact with the intestinal epithelial cells or immune cells associated with the lamina propria, through Toll-like receptors, and induce the production of different cytokines or chemokines. Probiotics are proposed to maintain gut microbial balance by suppressing the growth of potential pathogenic bacteria in the gut.

Gut Health, Immune Function, + Microbes

Specific probiotics influence the acquired and innate immune response by inducing phagocytosis and IgA secretion.

Specific probiotics alleviate intestinal inflammation and down-regulate hypersensitivity reactions.

Specific probiotics shown to inhibit growth of pathogenic microbes: Lactobacillus rhamnosus strain GG can inhibit attachment of Escherichia coli in GI tract.

Specific probiotics have been shown to strengthen intestinal barrier. Lactobacillus species increase mucin + increasing goblet cells which reinforce mucin layer.

Some microbes produce SCFA, e.g., butyrate, acetate which can suppress pro-inflammatory cytokines and bacteriocins which may reduce cancer risk + inflammation.

• **Stability:** resist change in the setting of an ecologic stress (resistance) or to return to an equilibrium state following a stress-related perturbation (resilience)

• **Balance** of microbiota
  - Some microbial distributions may increase risk of infection or disease. E.g. antibiotics can put an individual at risk for *Clostridium difficile*
  - Microbiota can shift with changes in age, diet, geographical location, intake of food supplements and drugs

• **Microbial diversity**
  - The lack of sufficient diversity or evenness in a bacterial community structure appears to diminish its ability to withstand perturbation - e.g., obesity + IBD have reduced diversity

• Lifestyle factors can play a role in *positively* impacting these important gut microbiome attributes...

Exercise: Many Benefits

- GI Tract
  - Less bloating
  - Improved bowel habits
  - Increased tight junction protein barrier

- Gut Microbiome
  - Increased diversity
  - Increased Firmicutes
  - Increased Akkermansia
  - Increased SCFA

- Brain
  - Decreased depression
  - Decreased anxiety

Exercise

Figure adapted from Dalton A et al. Gut Microbes. 2019;10(5):555-568.
Exercise, Gut Microbiome, + Health

- Exercise increases gut microbial produced SCFA, butyrate
  - Study: active women + sedentary controls. Women who performed at least 3 h of exercise per week had increased levels of *Faecalibacterium prausnitzii*, *Roseburia hominis*, and *Akkermansia muciniphila*. *F. prausnitzii* and *R. hominis* are known butyrate producers
  - Exercises reduces transit time—one way it may modulates the microbiome
  - Reduces colon cancer risk -may be in part due to gut microbiome effects (e.g., increase in butyrate, transit effects)
  - Many studies in athletes did not control for diet—major limitation in the research

Small sleep and gut microbiome study n=26

Results: microbiome diversity was positively correlated with sleep efficiency, and total sleep time, and was negatively correlated with the sleep fragmentation.¹

In humans, previous research has shown that partial sleep deprivation can alter the gut microbiome composition in as little as 48 hours.²

High sleep quality was associated with a gut microbiome containing a high proportion of bacteria from the Verrucomicrobia and Lentisphaerae phyla, and that this was associated with improved performance on cognitive tasks.³

Healthy Diet and Gut Microbiome

• Population-based study sample consisted of 4930 participants (ages 25–74; 53% women)- large, Finnish, population-based study sample

• Created a healthy food choices (HFC) score- A healthy food choices (HFC) score was formed by choosing and summing FPQ responses to food items that are recommended in the Nordic Nutrition Recommendations dietary guidelines

• Plant- and fiber-rich dietary choices (higher HFC score) are associated with a more diverse and compositionally distinct microbiota, and with a greater potential to produce SCFAs

• Known SCFA-producing species, Faecalibacterium prausnitzii, Akkermansia muciniphila, and Roseburia intestinalis, were all significantly elevated in individuals with a higher HFC score in this study

Associations of healthy food choices with gut microbiota profiles

Kari K Koponen, 1,2 Anna Golubovskaya, 1,2 Matti O Ruusunen, 1,2 Ali S Halmesmäki, 1,2 Katja Mustonen, 1,2 Pekka Saloivan, 1,2 Annarita Pihlava, 1,2 Riku Salo, 1,2 Kari Mustonen, 1,2 Pirjo Hannula, 1,2 Annarita Pihlava, 1,2

1Population-based study sample consisted of 4930 participants (ages 25–74; 53% women)- large, Finnish, population-based study sample

2Created a healthy food choices (HFC) score- A healthy food choices (HFC) score was formed by choosing and summing FPQ responses to food items that are recommended in the Nordic Nutrition Recommendations dietary guidelines

3Plant- and fiber-rich dietary choices (higher HFC score) are associated with a more diverse and compositionally distinct microbiota, and with a greater potential to produce SCFAs

4Known SCFA-producing species, Faecalibacterium prausnitzii, Akkermansia muciniphila, and Roseburia intestinalis, were all significantly elevated in individuals with a higher HFC score in this study

Eat More Plants!

Western diet + low-grade intestinal inflammation are implicated in a growing number of immune-mediated inflammatory diseases

Higher intake of animal foods, processed foods, alcohol and sugar, associated with higher levels of intestinal inflammatory markers + corresponds to an inflammatory microbial environment

Plant-based foods are linked to short-chain fatty acid (SCFA)-producers, microbial metabolism of polysaccharides and a lower abundance of pathobionts

Summary

• As the gut microbiome science is still in its infancy, the role of probiotics, prebiotics, synbiotics, and postbiotics will continue to evolve.

• An individual’s gut microbiome is as unique as their fingerprint—and individualized treatments will likely be key to success.

• Beyond prebiotics, probiotics, synbiotics and postbiotics, lifestyle factors can impact our gut microbiome, such as:
  • Stress management
  • Sleep hygiene
  • Plant-rich diet
  • Exercise
- Probiotics - select strain specific data, proper dosing for symptomology reduction and/or desired health outcome
- The future: targeted treatments based on gut microbiome and personalized medicine
- Dietitian stay informed! Since diet, pro-pre-synbiotics + fermented foods are a key modifiable factors influencing the composition of the gut microbiota, adjust clinical guidelines as dictated by the science!
Questions & Answers

Kate Scarlata MPH, RDN
www.katescarlata.com
A Generational Approach to Healthy Eating for People with Prediabetes and Diabetes

Presented by Toby Smithson, MS, RDN, LD, CDCES, FAND

November 3, 2021, 2–3 pm ET
Credit Claiming

You must complete a brief evaluation of the program in order to obtain your certificate. The evaluation will be available for 1 year; you do not have to complete it today.

CREDIT CLAIMING INSTRUCTIONS:

2. Click “My Courses” and select this webinar’s title.
3. Click “Take Course” on the webinar description page.
4. Select “Start/Resume” to complete the course and submit the evaluation.
5. Download and print your certificate.