a place of mind
THE UNIVERSITY OF BRITISH COLUMBIA

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***Title: Impact of dark chocolate consumption on glucose levels of people with diabetes***

**Research Question:** Does dark impact blood glucose levels in people with diabetes?

**Objectives**

1) To determine whether a dark chocolate sweetened with stevia, erythritol, and inulin compared to a standard dark chocolate bar impacts blood glucose levels in individuals with diabetes (T1D and T2D);
2) to understand and manage glucose levels in people with diabetes after consumption of different blends of chocolate.

**Hypothesis:**

a) A dark chocolate sweetened with stevia, erythritol, and inulin, compared to a standard dark chocolate bar will not impact blood glucose levels in individuals with diabetes (T1D and T2D)

**Background Information**

Diabetes is a growing concern in the world with an estimated 9.3% of adults, ages 20-79, with it in 2019, type 2 diabetes accounting for 90% of this total (1). In Canada, approximately 7.5% of adults have type 2 diabetes, 4.7% diagnosed and 2.8% undetected (2). Furthermore, an estimated 12.4% of adults in Canada have prediabetes, with 5-10% of those becoming diabetic each year (2). In order to prevent this, interventions aimed at weight loss, dietary change, and increased physical activity have been found to be effective (3). Additionally, a common recommendation for individuals with type 2 diabetes is to limit sugars and sweets as it may cause a high blood glucose response (4).

In diabetes, the postprandial phase is characterized by a rapid and large increase in blood glucose levels, and the possibility that these postprandial “hyperglycemic spikes” may be relevant to the pathophysiology of late diabetes complications is recently receiving much attention (5).

Postprandial hyperglycemia is a major component of glucose toxicity at the cellular and tissue level. Acute elevations of glucose after nutrient ingestion are associated with a variety of glucose-mediated tissue defects, including oxidative stress, lipid peroxidation, and advanced glycation end products (AGEs) formation leading to endothelial dysfunction and inflammation (6). This state not only initiates the development of early microvascular and macrovascular complications, but it also can contribute to a rapid progression to diabetes by causing glucose toxicity in muscle and pancreatic beta cells. The effects of hyperglycemia are often irreversible and lead to cell dysfunction.

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Therefore, strategies aiming at the specific reduction of postprandial hyperglycemia must be utilized to minimize clinical damages (7).
Chocolate is often avoided due to the sugar content, however, high-polyphenol chocolate has a beneficial effect on hyperglycaemia. (8) In a study looking at consumption of isocaloric doses of dark, milk, and white chocolate, blood glucose levels were higher 30 minutes after ingestion of the white and milk chocolate compared to the dark chocolate (9). This offers potential for individuals with diabetes to consume chocolate products depending on sugar and cocoa content. Daily consumption of flavonoid-rich chocolate have shown improved fasting plasma glucose levels and insulin resistance, three times greater than in milk chocolate (10). It has also been shown that a 15-day administration of polyphenol-rich dark chocolate decreased blood pressure and increased insulin sensitivity in healthy subjects (11). Similar results after 4-week consumption of polyphenol-rich dark chocolate; improved fasting glucose and decreased insulin resistance (12). By consuming high-quality cocoa products such as dark chocolate, insulin function could be improved, blood sugar better controlled, and possibly, the incidence of prediabetes is lowered (13). Therefore, it appears that the health benefits from chocolate are predominantly from the dark chocolate form.
The sugar-free chocolate from Ross Chocolates is formulated with a blend of inulin, erythritol, and stevia. These alternatives to sugar are not expected to cause a significant change in blood glucose levels following consumption. A study evaluating the consumption of sucrose, fructose, and isomalt-sweetened chocolates in type 2 diabetes patients found that while the sucrose and fructose-sweetened chocolates induced a similar glycemic effect, the isomalt-sweetened chocolate produced a lower glycemic effect (14). Therefore, since it has never been tested scientifically before, it would be novel and beneficial to assess if Ross Chocolates' blend of sweeteners would lead to a lower or null glucose spike in people with diabetes. Confirmation of this key piece of evidence could open the door for longer term studies easing concern over sugar-free dark chocolate postprandial hyperglycemia.

## Research Method

Twenty (N=20) individuals at University of British Columbia with physician diagnosed T1D (N=10) or T2D (N=10) (HbA1c 6.5-8.5%), between the ages of 18-75 years, with a BMI between 25-40 kg/m$^2$ will be recruited through online social media and newspaper advertising.
**Inclusion criteria** will be: i) physician-diagnosed T1D or T2D of ≥1 year; ii) current HbA1c of 6.5-8.5%; iii) BMI: 25-40 kg/m2; iv) blood pressure of <160/99 mm Hg assessed according to guidelines; v) non-smoking; vi) not on hormone

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replacement therapy, corticosteroids, or anti-inflammatory medications; and vii) 18–75 years old. **Exclusion criteria will include** i) Are taking more than 2 glucose lowering medications; ii) Are ongoing medical treatment for diseases such as cancer, auto-immune or inflammatory disease, liver or kidney disorders; iii) Have allergy, intolerance or aversion to cocoa, stevia, erythritol, inulin, or any other dietary restrictions (e.g., vegan) that will prevent them from following the standardized study diets; iv) Are unable to follow remote guidance by internet or smartphone; v) Are unable to follow the controlled diet instructions; vi) Are unable to read or communicate in English.

Based on our previous studies ~55% of males and ~70% of females with T1D or T2D who volunteer will meet these eligibility criteria so we anticipate no recruitment issues. We will allow statins and anti-hypertensive medications if on a stable dose for 3 months because the majority of T2D patients are on at least one of these medications; excluding them would leave a small pool for recruitment and limit the generalizability of our findings. Interested participants will provide their e-mail to establish direct and individual communication with research team.

A double-blinded crossover pilot trial is proposed. Eligible participants will complete 2 experimental trials separated by approximately 2-7 days, consuming no-sugar dark chocolate from Ross Chocolates and a conventional dark chocolate bar.

Due to COVID-19 breakout, this study is proposed to be done remotely since participants belong to risk group and will probably maintain in quarantine for unpredictable time. Information and guidance for the trial will be done by email, telephone calls and Zoom meeting. RedCap-UBC will be used to manage information and deliver questionnaires. Participants will be recruited after completing an online Eligibility questionnaire.
Participants will receive in their homes a box containing a OneTouch Verio IQ® kit Blood glucose monitoring system (OneTouch Verio IQ® meter, lancing device, sterile lancets, carrying case), glucose strips, alcohol swabs, 1 bar of Ross dark chocolate (34g each) and 1 bar of a conventional dark chocolate (34g each) and instructions on how to proceed during each trial. Chocolate bars will be identical and wrapped equally and labeled as A and B, by Ross Chocolates (prepared in the same mold and packaged identically) who are not involved in any other aspects of the study, allowing for blinding of research team and participants. Participants will complete two experimental trials: 1) consumption of 1 bar (34g) of Ross dark chocolate 2) consumption of 1 bar (34g) of conventional dark chocolate. The order of the bars will be randomized so there will be an equal chance of getting bar A or bar B first (similar to drawing a name out of a hat or flipping a coin). Participants will be advised to perform both trials on the exact same time of day, in the morning after an overnight fast, refraining from diabetic medications. They will be asked to register glucose levels before and after eating the chocolates. A OneTouch Verio

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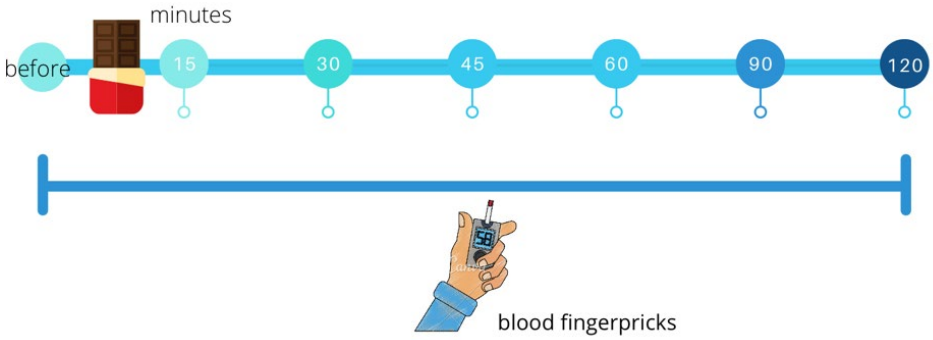
IQ® meter will be used to measure blood glucose. Time points will be as follows: before consumption, 15 min after, 30min after, 45min after, 60min after, 90min after, 120min after. Values will be registered on a Glucose Form and stored on the meter and sent to research team by email. Participants will then be asked to Complete Taste Test Questionnaire sent by RedCap link through e-mail.

Study Timeline:




**Baseline Contact**

On the initial contact, by a FIRST email, participants will be informed of the study and asked to complete a Health Screening Questionnaire and sign electronically an Informed Consent. After confirmation of eligibility and given consent, participants will receive a SECOND email with information on the study kit and asked to inform Research team when kit arrives. The kit will contain:

- Study Welcoming, Instructions and Responsibilities
- Glucose levels form
- OneTouch Verio IQ® kit Blood glucose monitoring system (OneTouch Verio IQ® meter, lancing device, sterile lancets, carrying case)
- Glucose strips
- Alcohol swabs
- 1 bar of dark chocolate A (34g each)
- 1 bar of dark chocolate B (34g each)

Once participants confirm study kit was received, they will get a THIRD email suggesting to start the protocol.
Participants will choose 2 different days, one for Day 1 and one for Day 2 (2-7 days apart from each other) and perform the following protocol:

**Day 1**

- ✓ Measure glucose levels before chocolate consumption and write down value on Glucose levels form
- ✓ Consume 1 bar of dark chocolate (A or B) according to instructions from Research team after randomization

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- ✓ Measure glucose levels after consumption (15 min, 30 min, 45 min, 60 min, 90 min, 120 min) and write down values on Glucose levels form
- ✓ Complete Taste Test Questionnaire sent to participant's e-mail

**Day 2**

- ✓ Measure glucose levels before chocolate consumption and write down value on Glucose levels form
- ✓ Consume 1 bar of dark chocolate (A or B) according to instructions from Research team after randomization
- ✓ Measure glucose levels after consumption (15 min, 30 min, 45 min, 60 min, 90 min, 120 min) and write down values on Glucose levels form
- ✓ Complete Taste Test Questionnaire sent to participant's e-mail
- ✓ Send photo of Glucose levels form by email

A Zoom meeting will be scheduled to guide participants with procedures in study if necessary.

**Statistical Analysis**

Changes in blood glucose across time between conditions will be analyzed with a linear mixed effects model including time, condition, and their interaction as fixed effects and participant ID and baseline glucose values as random effects. Area under the curve and incremental AUC will be calculated and analyzed by paired t-test.

**Sample size**

Sample size calculation for a within-between interaction with alpha of 0.05, power of 80%, and an expected moderate effect size (f=0.25) reveals a sample size of 12 is needed to detect a significant difference. To preserve power and enable potential sub-analyses of type 1 vs. type 2 diabetes we will aim to recruit 20 participants for this preliminary study.

**Significance**

The results of this study will help determine whether by consuming a dark chocolate bar sweetened with stevia, erythritol, and inulin will cause a blood glucose spike in people with diabetes (T1D and T2D). This information will provide high quality scientific evidence which will allow individuals with diabetes more food choices, helping them manage diabetes better.

## OUTCOMES MEASUREMENTS AND METHODS

The primary outcome measure is plasma glucose levels following consumption of the dark chocolate bars. The taste of the chocolates will also be evaluated.

Glucose Monitoring

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A glucose monitoring device (OneTouch Verio IQ® kit Blood glucose monitoring system) will be used to measure blood glucose levels before and after consumption of chocolates. Following consumption, measurements will be taken 15 min after, 30 min after, 45 min after, 60 min after, 90 min after, and 120 min after. Values will be registered on a form, stored on the meter and sent to research team by email. All to be done at home with no displacement.

**Taste Test Questionnaire**
Questionnaire is included as attachments and will be sent as link from the RedCap platform.

**Protection of Human Subjects**

All participants will be given a unique study code, with all data and information gathered connected with this code. Only the PI will have access to the master list linking the codes with participant names. Participant information and data will be either stored in a locked filing cabinet, or on an internal UBC network drive accessed only through a dedicated LAN internet connection on a password protected computer in the PIs laboratory with Salto passcard access only to the members of the research lab.
REDCap will be used as project management, storage of information, sending questionnaires and processing results. The REDCap platform is a secure web application for building and managing research data collection instruments. The platform is specifically designed to support online or offline data capture for research studies. The REDCap platform runs on server infrastructure physically located in BC, Canada, at the UBC University Data Centre (UDC).

## POTENTIAL PROBLEMS AND ALTERNATIVE STRATEGIES

Diet compliance: Since the study involves a minor modification to diet, we do not anticipate that any adverse events due to the interventions will be seen.

Blood sampling:
When compared to traditional wet blood and plasma collection using venipuncture, capillary blood sampling is safer and easier, so much so that nearly anyone can perform the procedure, mainly diabetes patients who are used to doing finger pricks for glucose levels. However, risks associated with the process of finger-stick blood draws are minimized by usage of sterile and disposable material as well as hygiene procedures. Participants will receive their own kit with sterile material and instructions on procedure.

Protection of personal data: All participants will be given a unique study code, with all data and information gathered connected with this code. Only the PI will have access to the master list linking the codes with participant names. Participant information and data will be either stored in a locked filing cabernet, or on an

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Informed consent and ethics: Prior to beginning the study Informed consent will be obtained from participants and they will be informed they can withdraw at any time. They will be informed that this study is partially funded by Ross Chocolates through a Mitacs Accelerate Grant. Ross Chocolates is a for-profit company that may stand to benefit commercially from research on how its sugar-free chocolate impacts blood sugar levels.

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