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The role of a traditional and western diet on glucose homeostasis in Greenlandic Inuit carriers and non-carriers of type 2 diabetes variant in the TBC1D4 gene: A protocol for a randomized clinical trial

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ABSTRACT

Introduction: The lifestyle of Inuit in Greenland and worldwide is undergoing a transition from a fisher-hunter to a westernized society and meanwhile the prevalence of type-2 diabetes (T2D) has increased dramatically. Studies have shown that a common nonsense p.Arg684Ter variant in TBC1D4, which is frequent in Greenland, confers genetic susceptibility towards high risk of T2D. The aim of the study is to investigate whether a traditional marine diet, with high fat and low carbohydrate, will improve glycemic control in Greenland Inuit compared to a western diet. Moreover, we want to examine if the response is more pronounced in carriers of the p.Arg684Ter variant.

Materials and methods: We will conduct a randomized, clinical cross-over trial with two dietary intervention periods of four weeks duration. The diet intervention comprise provision of >20%E and instruction for the remaining part of the diet. We expect to include 30 homozygous carriers and 30 homozygous non-carriers of the p.Arg684Ter variant, aged 18–80 years, across three Greenlandic towns. The primary outcome is plasma (p)-glucose 2 h post an oral glucose tolerance test and we aim to have 80% power, at α = 0.05, to detect a difference of 1.1 mmol/L. We will also include supporting measures of glucose homeostasis, assess other markers of the metabolic syndrome and perform metabolome and microbiome profiling. The statistical analysis will be performed as complete case analyses using linear mixed models.

Ethics and dissemination: The study received approval by the Ethics Committee of Greenland (KVUG 2018-26) and will be disseminated via international peer-reviewed journal articles and conferences.

Trial registration number: Clinicaltrials.gov identifier no. NCT04011904.

1. Introduction

Indigenous people worldwide are in a social, demographic and cultural transition with changes in lifestyle and living conditions [1]. The Inuit are a historically isolated population, for whom the information about genetic structure, social transition and disease pattern is exceptionally good. It is therefore ideal to study the consequences of social transition on changing disease pattern in the Inuit population [1,2].

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The transition in lifestyle of the Inuit in Greenland includes a shift from a traditional fisher-hunter society, with high physical activity and a diet based on foods from the natural environment, to a westernized lifestyle [3], which is associated with a high risk of lifestyle diseases such as type-2 diabetes (T2D) [4–6].

Studies of the Greenlandic Inuit before the 1980s found a low prevalence of T2D compared to Western populations [4], but recent surveys find that the prevalence of T2D (9%) and pre-diabetes (19%) has increased notably in the adult population [4,7]. In many studies worldwide, an increase in T2D and other lifestyle diseases has been ascribed to social transition and in particular urbanization [8]. Paradoxically, the highest prevalence of T2D is seen in the least urbanized areas in Greenland [7,9,10] where the proportion of Inuit in the population is higher. A common nonsense p.Arg684Ter variant in the TBC1D4 gene is frequent in the Inuit. The TBC1D4 gene encodes a protein that plays an important role in glucose homeostasis by regulating the insulin-dependent trafficking of the glucose transporter 4, which mediates glucose clearance from the bloodstream into skeletal muscle and fat tissues. A recent study by Moltke et al. [5] found that the p.Arg684Ter variant gene was strongly associated with insulin resistance in skeletal muscle, deterioration of postprandial glucose homeostasis, and a high risk of T2D [5]. Carrier of the p.Arg684Ter variant may therefore be specifically susceptible to lifestyle changes that are associated with an increased risk of T2D.

The traditional Inuit foods pattern is characterized by a high ratio of protein and fat relative to the intake of carbohydrate [11]. The association between a western diet and the risk of T2D has been ascribed to a high content of simple carbohydrates and saturated fat [12]. Furthermore, it has been estimated that animal products, mainly from marine animals, account for up to 86% of the energy intake from the traditional Greenlandic diet [13]. Marine animal products are characterized by a high content of long-chain n-3 polyunsaturated fatty acids, which have been associated with favorable improvements in a number of markers for the metabolic syndrome [14]. It is therefore, likely that the dietary changes away from traditional practices in Greenland may contribute to the increase in T2D incidence in the Inuit.

The objectives of the study are to investigate whether a traditional marine diet compared to a westernized diet can improve glycemic control in Greenland Inuit. In addition, we aim to study whether carriers of the p.Arg684Ter variant is affected more by the diet than the non-carriers. The present paper describes the rationale and design of the intervention study. Such an intervention might also be valuable from socioeconomic and cultural standpoint as an Indigenous group such as the Inuit can use their traditional diet to respond to an important health issue. This also underscores that the intervention is a potential health-intervention method, which could have a high acceptability in the Inuit population.

1.1. Hypothesis

We hypothesize that the traditional marine diet will improve post-prandial glucose clearing in Greenland Inuit. We hypothesize that this effect is strongest among carriers of the common nonsense p.Arg684Ter variant.

2. Materials and Methods

The study will be conducted in three towns (Nuuk, Qaanaaq and Qasigiannguit) in Greenland. We expect to include 30 homozygous carriers and 30 homozygous non-carriers of the nonsense p.Arg684Ter variant in the TBC1D4 gene between the age of 18–80 years. We expect to have an equal number of females and males.

The study will be conducted with a randomized, clinical, cross-over design, comprising two dietary intervention periods of four weeks duration, with no wash out in between. The participants will be randomized to start on one of the two diets and cross-over to the other diet after 4 weeks (see Fig. 1). To ensure that we have 50% homozygous carriers of the nonsense p.Arg684Ter variant in the TBC1D4 gene we will recruit from already existing studies: the Population Survey in Greenland 1999 [15], and the Inuit Health in Transition Study from 2005-10 [16], from which participants have been genotyped.

Fig. 1. Study outline – The individuals are recruited from the Population Survey in Greenland 1999 and the Inuit Health in Transition studies and after screening they are randomized to start with either the traditional Inuit diet or a westernized diet. The present study consists of two, 4-week periods and sampling will be conducted before and after each intervention period.
2.1. Participants (Inclusion and exclusion criteria)

Individuals are invited to participate in the study if they have previously participated either in the Population Survey in Greenland 1999 or in the Inuit Health in Transition Study. These studies provided genotype information of all participants, who were recruited from the general population. The participants have to be of Inuit descent and speak either Greenlandic or Danish. This was based on data collected in the previously conducted studies. In order to participate they have to meet the inclusion and exclusion criteria. The participants do not know their carrier status at baseline.

Inclusion criteria:

- Age between 18 and 80 years
- Homozygous carriers of the nonsense p.Arg684Ter variant in the TBC1D4 gene
- Homozygous non-carriers of the nonsense p.Arg684Ter variant in the TBC1D4 gene

Exclusion criteria:

- BMI ≤18.5 kg/m²
- Diagnosis of diabetes (HbA1c ≥ 48 mmol per mol hemoglobin (6.5%)) or pharmacological treatment of diabetes [17].
- Patients with history of severe hypertriglyceridaemia
- Use of systemic per oral glucocorticoids or injected steroids

2.2. Recruitment, screening and informed consent

Eligible candidates are contacted through an information letter encouraging them to contact the investigators by e-mail or telephone. After approximately two weeks, persons who have not replied will be contacted by telephone or home visits by the study staff to learn about their potential interest in participation.

Interested individuals are screened for compulsory inclusion and exclusion criteria. At the screening visit, they receive written and oral information about the study background and study procedures, including dietary registration and fecal collection. Written consent of participation is obtained at the first examination visit before we assess the HbA1c concentration by a DCA Vantage Analyzer (Siemens Healthcare, Denmark) to ensure that included individuals do not exceed the HbA1c limit of 48 mmol/mol. Participants will also be excluded if the responsible clinical physician finds that their participation could be a risk for their health and safety.

2.3. Randomisation, allocation, concealment and blinding

Randomization is performed separately for each participant based on genotype in blocks of variable size to ensure equal randomization throughout the enrolment phase. The randomization sequence is done by a statistician without contact to the participants using the Biorand package in the statistical software R (version 3.5.3). The personnel conducting the study will allocate participants to the sequence of intervention using a list of participant IDs matched with allocated sequences. Blinding participants is not feasible due to the nature of the intervention, however, binding of the allocation sequence will be established during sample analysis and initial statistical data analysis.

2.4. Intervention diets and dietary assessment

The intervention consists of two diets. A traditional Inuit diet rich in marine mammals (such as walrus, seal, and whale), fish, caribou and musk ox, and allowing for some content of grains, fast food and other imported foods. The traditional Inuit diet will be high in fat (≥ 40% of the energy percentage (%) and low in carbohydrate (< 30 %E)). The Westernized diet will consist of high amounts of grains, potatoes, rice and imported meats from livestock animals (beef, pork and chicken). The Westernized diet will be high in carbohydrate (55–65 %E) and lower in fat (30–35 %E). Major parts (> 20 %E) of the diet are provided to the participants mainly as frozen foods in the form of fish, seafood and sea mammals in the traditional Inuit diet and as imported meats, breads and pasta, rice and cereals for the Westernized diet (Table 1). The traditional diet was obtained through local supermarkets (Greenlandic brands) and sponsored by local firms. Furthermore, participants were encouraged to eat locally caught foods. Instructions for the participants regarding incorporation of dietary changes will be performed by trained study staff (Table 2) and the participants are provided with an instruction manual of do’s and don’ts for each intervention period. This included statements such as “eat more ..” and “instead of .. then try to eat ..” suited for each period. For example “eat more seal fish, seal, whales and sea birds” and “instead of eating chicken, try eating shrimps”. There were no specific recommendations made for fruit and vegetable consumption, as we did not want this to be the focus of the study.

Assessment of habitual food intake at baseline will be conducted with a previously validated food frequency questionnaire [3]. A modified version of the food frequency questionnaire will be used at the end of each intervention period to assess diet during the intervention periods. In addition, participants will be asked to keep a simple log of ingestion of delivered products to estimate compliance.

2.5. Measurements

All three examination days consists of similar examinations and data collection (Table 3). Participants arrive for clinical examination after an overnight fast of at least 8 h. Lifestyle questionnaires, including a food frequency questionnaire and questions about medication use are performed for baseline characterization of the participants. After this a fasting blood sample is obtained and an oral glucose tolerance test (OGTT) is performed with blood sampling at 30 and 120 min. Anthropometric measurements (height, weight, waist circumference, blood-

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Intervention products consumed ad libitum for substituting.</th>
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<tbody>
<tr>
<td>Traditional diet</td>
<td>Westernized diet</td>
</tr>
<tr>
<td>Product</td>
<td>Product</td>
</tr>
<tr>
<td>Seal meat</td>
<td>Pork</td>
</tr>
<tr>
<td>Whale blubber</td>
<td>Beef</td>
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<tr>
<td>Salmon</td>
<td>Chicken</td>
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<tr>
<td>Trout</td>
<td>Salmi sausage</td>
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<tr>
<td>Halibut</td>
<td>Mortadella sausage</td>
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<tr>
<td>Shrimps</td>
<td>Chocolate cereal product</td>
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<tr>
<td>Red fish</td>
<td>Oat meal</td>
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<tr>
<td>Cod</td>
<td>Rye bread</td>
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<tr>
<td>Cat fish</td>
<td>White bread</td>
</tr>
<tr>
<td>Other fish</td>
<td>Pasta</td>
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<td></td>
<td>Rice</td>
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<table>
<thead>
<tr>
<th>Table 2</th>
<th>Study products - instructions.</th>
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<tbody>
<tr>
<td>Traditional diet</td>
<td>Westernized diet</td>
</tr>
<tr>
<td>Eat more marine mammals, game, birds and fish</td>
<td>Eat less marine mammals, game, birds and fish</td>
</tr>
<tr>
<td>Eat more roots and berries</td>
<td>Eat more beef, pork, lamb and chicken</td>
</tr>
<tr>
<td>Eat less bread, potatoes, pasta and rice</td>
<td>Eat more cereals, rye bread and white bread, pasta and rice</td>
</tr>
<tr>
<td>Drink less sugar sweetened beverages</td>
<td>Limit alcohol intake</td>
</tr>
<tr>
<td>Reduce use of sugar in coffee and tea</td>
<td></td>
</tr>
<tr>
<td>Eat less junk food</td>
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pedance) were conducted between the 30 and 120 min blood sampling. In addition, participants will be equipped with a 24-h continuous glucose monitoring device (non-invasive) for 14 days at the start of each intervention period. Furthermore, fecal samples will be collected at all examination visits. All data will be collected by trained staff using standard operating procedures. Samples will be stored at −20 °C at the respective study site in Greenland for 12 weeks and during transportation to Denmark, and then at −80 °C until analysis. The study coordinator will be responsible for collecting data from different sources (laboratory, external partners).

2.6. Outcomes and hypotheses

2.6.1. Primary outcome

We expect that postprandial glucose homeostasis will be more efficient in the traditional diet period. The primary outcome of the study is blood glucose 2-h post OGTT after each of the dietary intervention periods. To further examine glucose homeostasis and insulin resistance, we will measure fasting glucose, insulin (both fasting and postprandial), c-peptide, and HbA1c, and perform 14-day continuous glucose monitoring using the Freestyle Libre Pro system (Abbott Diabetes Care, Germany). All laboratory analyses will be conducted using standard clinical procedures.

2.6.2. Secondary outcomes

2.6.2.1. Cardio-metabolic health. Analyses of other cardio-metabolic markers will be included in addition to examining insulin resistance and glucose homeostasis. An assessment of dyslipidemia will be conducted and we hypothesize that a traditional Inuit diet increases total and HDL-cholesterol compared to a westernized diet. We will also assess low-grade inflammatory markers (CRP, IL-6 etc.), which are expected to be reduced by the increase in long-chain n-3 polyunsaturated fatty acid intake following the Inuit diet. Finally, we will measure blood pressure, which is also expected to decrease due to the high content of long-chain n-3 fatty acid in the Inuit diet.

2.6.2.2. Gut microbiota and metabolomics. To explore possible underlying mechanisms determination of the gut microbiota will be performed using untargeted shotgun sequencing. We hypothesize that the traditional Inuit diet compared to a westernized diet will induces changes of gut microbiota composition and functional potential, partly due to differences in fiber intake, but also fatty acid composition. This might mediate some of the effect of the diet and we will use both blood and fecal metabolome analyses to verify potential metabolites mediating the effect of the gut microbiota. The metagenomic data will be used to evaluate the potential presence of well-established patterns that are related to metabolism in the human gut e.g. the relative abundance of Bacteroidetes and Firmicutes as well as the presence of pathways to create short chain fatty acids acetate, propionate and butyrate. These patterns have also been related to consumption of traditional Greenlandic foods [18].

2.6.2.3. Weight and body composition. Height, weight, waist, and hip circumference will be measured. Moreover, body composition will be determined using a bioelectrical impedance scale. We expect the participants to be weight stable in the two dietary intervention periods, and that neither the interventions affect body composition. However, we measure this to adjust for potential individual changes during the intervention period, which might affect insulin resistance and glucose homeostasis.

2.6.2.4. Compliance biomarkers. To objectively assess compliance, we will include an assessment of circulating fatty acids profiles [19]. We expect a higher long-chain n-3 polyunsaturated fatty acid content following the Inuit diet, and an increase in n-6 polyunsaturated fatty acid after the traditional western diet. Adherence to the diet may also be assessed by plasma alkyresorcinols to verify the low or high intake of grains. These compliance markers may be used to examine dose-response associations between exposure and outcomes.

2.7. Sample size estimation

The power calculation for the study is based on the primary outcome (change in 2-h blood glucose from start to end of intervention period). Estimations are based on 80% statistical power to detect a difference in 2 h glucose of approximately 1.1 mmol/L or equivalent 0.6 standard deviation in metabolic quantitative traits within the subgroup of homozygous carriers and 0.4 standard deviation differences in the whole group [20]. The level of significance is set at < 0.05. With a 15% drop-out rate after randomization it is calculated that we need 30 homozygous carriers and 30 homozygous non-carriers.

2.8. Data handling and statistical analyses

All analyses will be performed as complete case analyses and considering the cross-over design, imputations for drop-outs is not considered feasible for the primary or many secondary outcomes. The main analysis of intervention effect outcomes (including the primary outcome) will be conducted using linear mixed models with participant and study site as random effect factor. The primary analysis will examine the overall effect of the dietary intervention. Secondly, we will also do a test for effect modification between diet intervention and genotype, by a stratified analysis based on genotype as well as interaction tests. Subsequent sensitivity analyses will be adjusted for non-modifiable risk factors such as sex and age. Moreover, effect modification between diet in-
tervention and sex and age will be explored. Additional sensitivity analyses with adjustments for non-modifiable risk factors such as smoking and alcohol intake will also be conducted. If there are large changes in anthropometric variables such as weight or fat mass, we will conduct mediation analysis as a secondary analysis. Finally, we will examine compliance using both questionnaires and changes in compliance biomarkers (whole-blood fatty acid composition) and whether the potential effect of the dietary intervention is compliance dependent.

For the secondary outcomes, gut microbiota and metabolomics, exploratory data analysis will be conducted using both univariate and multivariate models. These analyses are conducted to explore potential mechanisms mediating the effect of the dietary intervention.

3. Discussion

To the authors’ knowledge, the presented intervention is the largest study to date examining the effects of a traditional Inuit diet on glucose homeostasis and metabolic markers, as well as changes in the gut microbiota composition in the Inuit population in Greenland. The Inuit are a historically isolated population with a well characterized genetic profile. Therefore it is considered optimal for an investigation of potential genetic modification of health improvements from a traditional diet compared to a westernized diet, specifically by the p.Arg684Ter variant in the TBC1D4 gene, which has a high frequency in the Inuit population. This study is the first dietary intervention targeting a gene polymorphism with strong effects on T2D risk (OR=10) which is a major health concern in the Inuit [5]. Thus the study may also contribute to solving a major health concern in the Inuit population. In addition, the study population will get a thorough health check as part of the examinations. The novel approach in the study will provide valuable knowledge for future genotype-based interventions. The main objective of the study is to examine the effect of the traditional Inuit diet on glucose homeostasis, but we will also measure changes in a number of other metabolic variables as well as the metabolome and microbiome.

The main strengths of the study is its randomized controlled cross-over design with an extensive characterization of the study population, including measurements of dietary intake, objective compliance biomarkers and clinical phenotyping. Furthermore, the study uses both dietary advice as well as the providing of food products for the participants to ensure a high compliance to the dietary intervention. The study is a real life intervention, which reflects a realistic pattern of consumption and thus, deliberately not focused on specific food products, but provides a variety of different food products according to the seasonality in the intervention periods. Because of this and ad libitum consumption of products, the study will render the results that are applicable to the Greenlandic Inuit population. However, the study does not take into account other parts of the local food culture such as land use, food preparation and sharing. The participants are not provided with food to cover their full dietary requirements, so there may be some deviations from the planned target diet and varying degrees of compliance among the participants. The cross-over design of the study compliments the decision to include a heterogeneous population across multiple sites in Greenland to ensure a high generalizability to the overall Inuit population in Greenland. However, the use of multiple sites, across different seasons, might also introduce differences in diet, especially in the Inuit diet period, as local food sources and availability change substantially during seasons. We will try to address these differences both in the participants’ dietary registrations and through objectively measured biomarkers of intake. Furthermore, the study includes a wide age range to ensure the best possibilities for recruiting participants with two copies of the minor allele p.Arg684Ter variant. However, this will potentially result in large differences in baseline dietary practices as well as microbiome composition, which could affect the results of the study. We will try to address and explore these differences in the statistical analysis by using stratified analysis e.g. on age.

It may be an optimistic goal that half of the participants should be carriers of two copies of the minor allele p.Arg684Ter variant in the TBC1D4 gene given the limited prevalence. If we cannot recruit enough minor allele participants, we will include more major allele homozygotes, in order to have enough power to test the overall effect of diet.

In conclusion, the current study will provide new insights into how transitions from a traditional diet to a westernized diet affect glucose homeostasis and overall metabolic health. Furthermore, the study is expected to provide relevant information in relation to whether diet has a role in preventing T2D in the large group of people with the TBC1D4 variant. We expect that the study results will give enhanced insight that will be useful and valuable for future development of genotype-based interventions, in particular in patients with T2D.

3.1. Ethics and dissemination

All study procedures will be in accordance with the Helsinki Declaration and the study is registered in the public database clinicaltrials.gov (NCT04011904). The study was approved by the Ethics Committee of Greenland (KVUG 2018-26). Any modifications to the protocol which may impact on the conduct of the study, potential benefit of the patient or may affect patient safety, including changes of study objectives, study design, patient population, sample sizes, study procedures, or significant administrative aspects will require a formal amendment to the protocol. Such amendment requires approval by the ethics committees and a renewed consent from the participants before continuation of the study.

The results from the study will be disseminated via presentations on national and international scientific conferences and submitted to international peer-reviewed journals regardless of the nature of the results. Furthermore, a short report in Danish and Greenlandic with the main results from the study will be send to participants and made available for relevant public institutions and local media interested in nutrition, prevention and health.

3.2. Patient and population involvement

This research was done without patient involvement. Patients were not invited to comment on the study design and were not consulted to develop patient relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy. However, the authors have continually worked and received feedback from the local communities across Greenland over the last decades and thus include ideas and suggestions from the Indigenous Inuit population in order to support the Greenlandic society, culture and public health. Specifically in relation to the study at hand, knowledge on perceptions on genome based research in Greenland has been collected from Sharing Circles (under publication, to be updated). Furthermore, this research project also strives for enhancing ethical genomic research within Indigenous communities by fostering collaboration with local researchers and support capacity building [21].

3.3. Confidentiality

Information regarding the participants’ physical, biological and personal data will be treated with confidentiality in accordance with the Danish and Greenlandic law. All handling and storing of personal data will be in accordance with applicable law, GDPR and Danish Data Protection Act. no. 502 from May 23rd 2018. The project is registered at the University of Copenhagen, where the records and data are kept in accordance with current legislation. All handling and processing of the data and biological samples are only identified by the project- ID. The “key” that associates the project- ID with personal information is stored in locked rooms and freezers separately from the data and biological
Declaration of competing interest

MEJ has received funding from AstraZeneca, Sanofi Aventis, Boehringer Ingelheim and AMGEN. MEJ owns shares in Novo Nordisk A/S. The other authors have nothing to disclose.

References


