Small intestinal ketogenesis – potential significance for type 2 diabetes in obesity

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NCT

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Overall scientific question:
Do obesity cause disturbances in intestinal food-induced ketogenesis and does this influence the development of type 2 diabetes mellitus?

Specific issues:
1. Does food intake-induced ketogenesis exist in the small intestine of obese individuals?
2. Are insulin resistance, GLP-1 release and SGLT1 affected in obese individuals without type 2 diabetes in the same way as those with type 2 diabetes?
3. If no to 2: Where is the difference?

Area overview
Obesity (obesitas) has increased dramatically in the last 3 decades. Obesity also comes with diseases, including type 2 diabetes, high blood pressure and changes in lipid status. It is now common knowledge that obesity with type 2 diabetes can be treated with a certain type of gastrointestinal surgery, the best known being gastric bypass surgery. This operation means that the food ends up directly in the small intestine and has long been used for weight-reducing purposes. The fact that this operation, in addition to its effect on body weight, also has a good effect on diabetes has been noticed in recent years. Of course, there are also treatment failures and side effects, including micronutrient deficiency, linked to this surgery. But on the whole, gastric bypass brings a longlasting weight loss and in most cases also relief of the co-morbidity, e.g. diabetes type 2. A prerequisite, however, is that you take life-long substitution of micronutrients (calcium, iron, vitamin C and D etc.).

Gastric bypass effect on obesity and type 2 diabetes. Gastric bypass provides a powerful and long-lasting reduction in body weight. The operation also improves type 2 diabetes, in most cases immediately after the operation, i.e. before the body weight has been affected. (Wolfe et al 2016) This suggests that the altered gastrointestinal anatomy has a direct effect on blood glucose regulation. The mechanisms are not fully known and include reduced secretion of certain gastrointestinal hormones (for example GLP-1; used in some modern diabetes drugs such as liraglutide and semaglutide) in a positive way. Furthermore, the distribution and composition of the intestinal microorganisms changes. The operation thus activates several different mechanisms that probably work together. However, there is no complete physiological explanation for why this surgery improves the diabetes disease.

Ketone bodies during fasting/starvation. This project-plan deals with ketone bodies, ie mainly the substances beta-hydroxybutyrate and acetoacetate. Acetone is also represented, but is a gas-soluble factor that mainly ends up in the exhaled air and thus leaves the body. Ketone bodies are normally formed in the liver during fasting/starvation when the body's blood sugar level becomes very low. In this situation, ketone bodies take over as an energy source in the blood and the production does not decrease until the blood sugar is back to the original levels. It is well known that systemically increased levels of ketone bodies (measured in peripheral venous return) have been shown to affect diabetes disease, sometimes lethally through ketoacidosis. One way to physiologically increase the body's ketone bodies is through a ketogenic diet (essentially a high-fat diet with a reduced number of

calories). NOTE: We are careful not to confuse these systemic phenomena with the gut-produced ketone bodies (see below).

Intestinally produced ketone bodies. It has been shown that the mucosa in the middle small intestine, the jejunum, changes relatively quickly so that intake of fatty food causes the mucosa to produce ketone bodies. This is thus contrary to the common view, according to above, that ketone bodies are formed during fasting/starvation. The intestinally produced ketone bodies end up in the portal vein, i.e. the blood drainage that goes directly to the liver. There it is diluted with blood from other organs, which is why you see a moderate or no increase in a blood sample taken in a peripheral vein. What then do jejunal ketone bodies in the portal vein? We have recently described that gastric bypass reduces the ability of the jejunum to manufacture "ketone bodies". This releases a brake on the production of GLP-1 (Wallenius et al 2020). This is probably because gastric bypass surgery individuals dramatically reduce their intake of fat in favor of (slow) carbohydrates.

Hypothesis

Our hypothesis (partially proven) is that the local ketone body formation (ketogenesis) inhibits the release of the hormone GLP-1. This hormone is released from the intestine into the blood in the portal vein, via the liver and on to all the body's cells. GLP-1 controls, among other things, insulin sensitivity (a very important factor in type 2 diabetes) and is an important factor in slowing down food intake. Furthermore, parallel to this, there is a brake on the expression of the enzyme that absorbs glucose across the mucosa (SGLT1). The local ketogenesis in the intestinal mucosa thus slows down 2 factors of particular importance for glucose homeostasis: partly GLP-1 mediated insulin sensitivity in general in the body, partly the SGLT1 mediated glucose transport across the mucosa. Recently, we demonstrated in healthy volunteers that this fat-induced ketone body formation de-facto exists. We also found clear signs of disturbances in glucose uptake in the intestine, but at the systemic level the values were adequate (Wallenius et al 2021, Elebring et al 2022). The question in the present project is: What does intestinal ketogenesis look like in individuals with obesity? Are there changes that contribute to the development of, among other things, type 2 diabetes mellitus (see above)?

Project description

Summary

Human subjects, either without or with non-insulin-treated diabetes type 2 + possibly with any other metabolic disorder (see below). Otherwise, the individuals must be medication-free (exceptions may be allowed) and physically mobile. Patients with insulin-treated diabetes are avoided so that they are not exposed to any risks (especially hypoglycemia). Participants in this study receive two complete diets for 2 weeks each from the laboratory. The diet is put together by a dietitian specializing in obesity/diabetes and is individual. In one case the diet is of the type "high-carbohydrate diet" and in the other case "high-fat diet". The idea is that the research subjects should subsist on these diets for the respective 14 days. The energy content of the diet periods must be the same and correspond to the participants' daily needs. Each participant take one diet for 2 weeks. After a break of at least 3 weeks, the second diet is taken for 2 weeks. In each diet, a meal test is done after 12 days and after the 14th day an enteroscopy with a sample of the intestinal mucosa. In addition to this, blood samples and questionnaires are taken to describe the general state of health and gastroenterological status. This is an exploratory study, which is why the number of research subjects is limited to 20 individuals.

Research persons

Research is carried out on 20 obese individuals who are metabolically healthy (approx. 10/20) or have type 2 diabetes (co-morbidities such as high blood pressure, blood lipid disorders and the like are accepted if they have been stabilized >1 month with adequate pharmacology). All study participation is voluntary. We participate in the Regional Obesity Center at Sahlgrenska University Hospital. We thus come into contact with all obese patients from the Västra Götaland region who are referred for treatment. Some of these are contacted by phone by a research nurse to provide further information. The research nurse then asks the patient about interest in participation. If the answer is yes, the patient is called to a physical meeting with a blood sample and a dietitian. Study physicians assess presumptive participants' conditions to participate and, after receiving a written response to the researcher's request, place suitable candidates on the study list.

Inclusion criteria:
- Individuals with obesity
- Age between 18 and 65
- BMI 35 – 45 kg/m2 without or with type 2 diabetes

Exclusion criteria:
- Insulin-treated diabetes
- Sequelae of previous diabetes-associated disease (foot, eyes and kidneys, or cardiovascular incident, etc.)
- Diabetes, hypertension, lipid disorder that has not been stabilized for at least 1 month by adequate pharmacological treatment
- Continuous NSAID use
- Preferably otherwise medication-free (exceptions can be made)
- Not operated on in the abdomen (appendectomy excluded)
- Previously known organic gastrointestinal disease, except for gastroesophageal reflux disease (GERD)
- Smokers
- Pregnancy and breastfeeding
- History of drug abuse or other circumstances deemed to jeopardize the patient's ability to participate in the research project

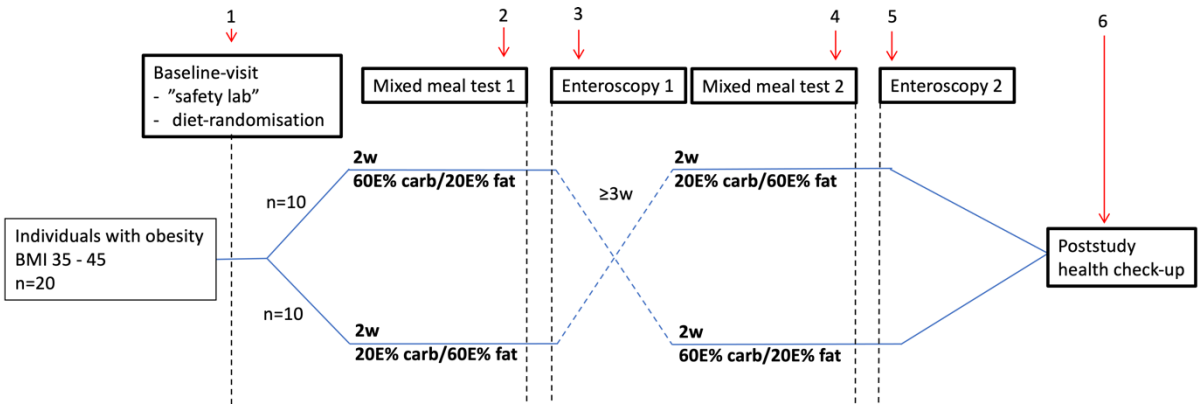

Figure 1. Study overview with visits 1 to 6.

Visit 1: The research person meets a research nurse and a dietician
Review of eating habits and physical activity. In addition, height + weight is measured and the research subject may provide blood and urine samples corresponding to a general health check (Hb, glucose/insulin, salt balance, blood fats, liver and inflammation markers and glucose/protein in urine). Blood amount max 20 ml, time required 1 hour. Health status and possibly medicines are assessed by study doctors who put presumptive subjects on the study list.
The research subject is provided with food for two 14-day periods with the same energy content as the research subject normally consumes, but where the main energy source is either fat (60% of total energy intake, 20% is carbohydrate-based) or carbohydrate (60% of total energy, 20% is fat-based), in random order. The goal is for the individual to maintain the weight during the study period. The individual diet is constructed by a dietician with a specialty in obesity/diabetes. The research subjects receive ready-made food for one week at a time. During the respective diet period, the research subject must not use alcoholic beverages, but otherwise live a normal life. After diet period 1, the research subject must have a 3-week "washout" period.

Visit 2 + 4: On day 12 of each diet, a meal test (Mixed Meal Test) is performed
Ev. morning medications are taken 45-60 minutes before the laboratory visit. The research person arrives at the laboratory at 8.30 a.m., otherwise fasting. A venous catheter is placed in one arm crease for blood sampling and the research person must answer questions about any gastrointestinal complaints in connection with the current diet (GSRS questionnaire). At approximately 09:00, the research subject may eat a brunch for approximately 15 minutes (sandwich, juice, fried egg and sausage; 550-600kcal). Venous blood samples are taken before and 15, 30, 45, 60, 90 and 120 minutes after the meal. At each sampling occasion, the research person has to estimate hunger or satiety on a visual analogue scale. The research person may then leave the laboratory and eat their regular lunch at 12 to 1pm.
Analyses: At each blood sampling, the concentration of glucose, ketone bodies, insulin and other intestinal hormones in the metabolic control (e.g. glucagon, GIP, GLP-1, PYY etc.) are analyzed and possibly presence of intestinal bacterial components (endotoxinaemia). The examination takes approximately 2.5 to 3 hours in total. In total, a maximum of 70 ml of blood is taken per diet period (which corresponds to a total of 1/6 of a normal blood donation).

Visit 3 + 5: On day 14 of each diet period, an enteroscopy is performed with mucosal sampling. This step is performed to obtain a sample (biopsy) of the mucosa of the small intestine (duodenum and jejunum) and is performed at GEA SU-Sahlgrenska or SU-Östra. The research subject arrives at the hospital after 6-8 hours of fasting (morning medications are taken after the examination). Endoscopy is experienced by some individuals as unpleasant, which is why local anesthesia and sometimes sedation (midazolam + pethidine or rapifen) can be offered according to standard routine in the endoscopy department. Approximately 8 - 12 biopsies are taken at each location. The responsible endoscopist assesses in each individual case whether tissue sampling is possible from a medical point of view. All tissue collection is thus done according to clinical routine. Research staff attend the examination and take care of the biopsy material which is either fixed, deep-frozen (histology and immunofluorescence on given factors such as SGLT1, GRPr, etc) and/or stored for a short time in oxygenated preservation medium (the latter for functional studies on SGLT1 in-vitro i.e. Ussing chamber). Gastroenteroscopy is normally carried out in approximately 15-25 minutes. With preparation time, the length of stay in the endoscopy department is approximately 1 hour.

Visit 6. The research subject's health is checked by phone call.

Preliminary results

We have shown that jejunal ketone body formation can reduce the release of GLP-1 and thus interfere with insulin sensitivity in a manner similar to type 2 diabetes. (Wallenius et al 2019). We have also conducted studies on 16 healthy volunteers with basically the same protocol as in the current study. It was carried out without any complaints, except that 1 woman had to cancel due to an unplanned pregnancy. We then showed that (nb in healthy normal weight people) there is a ketone body formation from the intestine after a fatty meal. We also found clear signs of disturbances in glucose uptake in the intestine, but at the systemic level the values were normal (Wallenius et al 2021, Elebring et al 2022).

Significance

If ketone body formation is important for obesity with type 2 diabetes, then the results can be used to understand the disease and possibly develop a non-injection-dependent pharmacological therapy.

References

Casselbrant A, Wallenius V, Elebring E, Marschall HU, Johansson BR, Helander HF, Fändriks L. Morphological Adaptation in the Jejunal Mucosa after Iso-Caloric High-Fat versus High-Carbohydrate Diets in Healthy Volunteers: Data from a Randomized Crossover Study. Nutrients. 2022 Oct 4;14(19):4123. doi: 10.3390/nu14194123.

Elebring E, Wallenius V, Casselbrant A, Docherty NG, Roux CWL, Marschall HU, Fändriks L. A Fatty Diet Induces a Jejunal Ketogenesis Which Inhibits Local SGLT1-Based Glucose Transport via an Acetylation Mechanism-Results from a Randomized Cross-Over Study between Iso-Caloric High-Fat versus High-Carbohydrate Diets in Healthy Volunteers. Nutrients. 2022 May 7;14(9):1961. doi: 10.3390/nu14091961.

Wallenius V, Elebring E, Casselbrant A, Laurenius A, le Roux CW, Docherty NG, Biörserud C, Björnfot N, Engström M, Marschall HU, Fändriks L. Glycemic Control and Metabolic Adaptation in Response to High-Fat versus High-Carbohydrate Diets -Data from a Randomized Cross-Over Study in Healthy Subjects. Nutrients. 2021 Sep 23;13(10):3322. doi: 10.3390/nu13103322.

Wallenius V, Elias E, Elebring E, Haisma B, Casselbrant A, Larraufie P, Spak E, Reimann F, le Roux CW, Docherty NG, Gribble FM, Fändriks L. Suppression of enteroendocrine cell glucagon-like peptide (GLP)-1 release by fat-induced small intestinal ketogenesis: a mechanism targeted by Roux-en-Y gastric bypass surgery but not by preoperative very-low-calorie diet. Gut. 2020 Aug;69(8):1423-1431. doi: 10.1136/gutjnl-2019-319372.

Wolfe BM, Kvach E, Eckel RH. Treatment of Obesity: Weight Loss and Bariatric Surgery. Circ Res. 2016 May 27;118(11):1844-55. doi: 10.1161/CIRCRESAHA.116.307591.