Cumulative Dissertation

Nonpharmacological interventions for weight reduction in people with type 2 diabetes

submitted by

Dr. med. univ. Anna OBERMAYER

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Prof. PD Dr. Harald SOURIJ, MBA

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Statutory Declaration

I hereby declare that this thesis is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this thesis. The acknowledgement has been made in the text to all other materials used. Throughout this thesis and in all related publications I followed the "Guidelines of the Medical University of Graz on Good Scientific Practice".

Graz, April 2023 Anna Obermayer

Disclosures

This cumulative dissertation is based on the following three papers with Anna Obermayer as an exclusive first author in the following SCI-listed journals:

1. **Obermayer, A**., Tripolt, N. J., Aziz, F., Högenauer, C., Aberer, F., Schreiber, F., Eherer, A., Sourij, C., Stadlbauer, V., Svehlikova, E., Brunner, M., Goswami, N., Kojzar, H., Pferschy, P. N., Pieber, T. R., & Sourij, H. (2021). EndoBarrier™ Implantation Rapidly Improves Insulin Sensitivity in Obese Individuals with Type 2 Diabetes Mellitus. Biomolecules, 11(4), 574. https://doi.org/10.3390/biom11040574

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2. **Obermayer, A**., Tripolt, N. J., Pferschy, P. N., Kojzar, H., Jacan, A., Schauer, M., Aziz, F., Oulhaj, A., Aberer, F., Sourij, C., Obermayer-Pietsch, B., Stadlbauer, V., & Sourij, H. (2022). INTERmittent FASTing in people with insulin-treated type 2 diabetes mellitus - the INTERFAST-2 study protocol. Diabetic medicine: a journal of the British Diabetic Association, e14813. Advance online publication. https://doi.org/10.1111/dme.14813

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'Sola dosis facit venenum.'

(Only the dose makes a thing not a poison)

Paracelsus (1493–1541)

Content

Abbreviations

ADF	alternate day fasting
BMI	body mass index
CGM	continuous glucose monitoring system
CGR	C-peptide/glucose ratio
C-peptide	connecting peptide
DM	diabetes mellitus
DXA	dual-energy X-ray absorptiometry
eGFR	estimated glomerular filtration rate
HbA1c	haemoglobin A1c
IF	intermittent fasting
IR	insulin resistance
LDL	low-density lipoprotein cholesterol
MetS	metabolic syndrome
OAD	oral antidiabetic drug
oGTT	oral glucose tolerance test
PCOS	polycystic ovary syndrome
RMR	resting metabolic rate
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
TDID	total daily insulin dose
TRE	time restricted eating

Zusammenfassung

Mit der steten Zunahme an Personen mit Übergewicht und Diabetes mellitus Typ 2 werden einfache und kosteneffektive Therapien notwendig, um die Betroffenen bei Ernährung, Blutzuckerkontrolle und Gewichtsmanagement zu unterstützen. Die Verringerung des Körpergewichts ist eine Schlüsselstrategie zur Verringerung des Diabetesrisikos. Bei adipösen und prädiabetischen Personen verbessert eine Gewichtsabnahme von 5-7 % typische Diabetes-Risikoparameter, darunter Nüchternglukose, Insulin und Insulinsensitivität. Obwohl diese Ratschläge einfach klingen, ist es in der täglichen Praxis sehr schwierig, eine dauerhafte Gewichtsabnahme zu erreichen. Eine Verzögerung der Resorption der Nahrung, wie durch den duodenal-jejunalen Bypassliner Endobarrier™, oder eine zeitbegrenze Nahrungsaufnahme könnten dabei helfen. Eine gründliche Erforschung klinischer Parameter mittels körperlicher Untersuchung, Blutabnahmen und Dual-energy X-ray absorptiometry bei Verwendung des Endobarrier™ und während des Fastens kann dazu beitragen, die Ernährungsempfehlungen für Personen mit Typ 2 Diabetes zu personalisieren und anzupassen.

Ziel dieser Arbeit war es, unterschiedliche Möglichkeiten der Modulation des Stoffwechsels bei Personen mit Typ 2 Diabetes zu erforschen und ihre praktische Anwendung zu testen.

In der Endobarrier-Pilotstudie mit 10 Personen wurden die Auswirkungen der Implantation des duodenal-jejunalen Bypass Liners (Endobarrier™) auf den Glukosestoffwechsel bei Personen mit Adipositas und Typ 2 Diabetes untersucht. Der Endobarrier™ wurde für 36 Wochen implantiert und die Effekte auf Blutzucker und Gewicht untersucht. 24 Wochen nach Explantation wurden die Parameter noch einmal gemessen um die längerfristigen Effekte nach der Entfernung des Endobarrier™ zu untersuchen.

Das Dissertationsprojekt „Interfast 2“ untersuchte den Einfluss von 12 Wochen intermittierendem Fasten im Vergleich zur Standardtherapie bei Personen mit insulinbehandeltem Typ 2 Diabetes mellitus. Es handelte sich um eine unverblindete, randomisierte, kontrollierte Studie mit 46 Personen. Die Fastengruppe praktizierte intermittierendes Fasten an 3 nicht aufeinanderfolgenden Tagen (75% Kalorienrestriktion), hatte aber keine Kalorienrestriktion an den restlichen 4 Tagen. Das primäre Ziel war die Untersuchung der Auswirkungen des intermittierenden Fastens bei Personen mit insulinbehandeltem T2DM. Das sekundäre Ziel dieser Studie war es, klinische und

metabolische Veränderungen bei Personen mit T2DM nach intermittierendem Fasten zu identifizieren.

Der Endobarrier™ erreichte bereits nach 4 Wochen eine Gewichtsreduktion, die über die gesamten 9 Monate in der Nachbeobachtung erhalten blieb und auch 6 Monate nach der Explantation noch anhielt. In der Interfast 2-Studie zeigte sich eine Verringerung des HbA1c, des Gewichts und der Insulindosis in der Fastengruppe im Vergleich zu Kontrollgruppe. In der Fastengruppe blieben bei einer deutliche Reduktion der Fettmasse sowohl Muskel- als auch Knochenmasse erhalten. Bewegung und Ruheumsatz waren in beiden Gruppen vergleichbar und änderten sich nicht signifikant über den Studienzeitraum. Intermittierendes Fasten ist eine praktikable Ernährungsoption für Personen mit insulinbehandeltem T2DM mit der Aussicht, den HbA1c-Wert zu senken und gleichzeitig die tägliche Gesamtinsulindosis und das Körperfett zu reduzieren.

Sowohl der Endobarrier™ als auch intermittierendes Fasten bieten nicht-pharmakologische, unterstützende Möglichkeiten, wie Personen ihr Gewicht und ihren Blutzucker wieder selbst in die Hand nehmen können.

Abstract

With the steady increase in people with obesity and type 2 diabetes mellitus, simple and cost-effective therapies are needed to support people with their blood glucose control and weight management. Reducing body weight is a key strategy to reduce the risk of diabetes. In obese and pre-diabetic individuals, weight loss of 5-7% improves typical diabetes risk parameters, including fasting glucose and insulin sensitivity. Although this advice sounds simple, in daily practice it is very difficult to achieve lasting weight loss. Delaying the absorption of food, as by the Endobarrier™ duodenal-jejunal bypass liner, or time-limited food intake could help. A thorough exploration of clinical parameters by means of physical examination, blood sampling and dual-energy X-ray absorptiometry when using the Endobarrier™ and during fasting may help to personalize and adapt dietary recommendations for people with type 2 diabetes.

The aim of this work was to explore different ways of modulating metabolism in people with type 2 diabetes and to test their practical application.

The Endobarrier pilot study of 10 people investigated the effects of implantation of the duodenal-jejunal bypass liner (Endobarrier™) on the glucose metabolism in people with obesity and type 2 diabetes. The Endobarrier™ was implanted for 36 weeks and the effects on blood glucose and weight were studied. At 24 weeks after explantation, the parameters were measured again to investigate the longer-term effects after removal of the Endobarrier™.

The PhD thesis project "Interfast 2" investigated the effect of 12 weeks of intermittent fasting compared to standard therapy in people with insulin-treated type 2 diabetes mellitus. It was an unblinded, randomized controlled trial with 46 people. The fasting group practiced intermittent fasting on 3 non-consecutive days (75% caloric restriction) but had no caloric restriction on the remaining 4 days. The primary objective was to investigate the effects of intermittent fasting in people with insulin-treated T2DM. The secondary objective of this study was to identify clinical and metabolic changes in individuals with T2DM after intermittent fasting.

The Endobarrier™ achieved weight loss as early as 4 weeks, which was maintained throughout 9 months of follow-up and persisted 6 months after explantation. In the Interfast

2 study, there was a reduction in HbA1c, weight and insulin dose in the fasting group compared to the control group. In the fasting group, both muscle and bone mass were preserved with a significant reduction in fat mass. Exercise and resting metabolic rate were comparable in both groups and did not change significantly over the study period. Intermittent fasting is a viable dietary option for individuals with insulin-treated T2DM with the prospect of lowering HbA1c levels while reducing total daily insulin dose and body fat.

Both the Endobarrier™ and intermittent fasting offer non-pharmacological treatment options for individuals to control their body weight and blood glucose.

1 Introduction

Diabetes mellitus

Diabetes mellitus, a disorder of the carbohydrate metabolism, comprises a group of heterogenic disorders characterized by chronic hyperglycemia.[1] Diabetes mellitus, was named after one of its key symptoms: “diabetes” ancient Greek for “to siphon” or “to pass through” and “mellitus” translating to “honey sweet” – describing the honey sweet taste of the urine “passing through” the body of the patient trying to lower the blood sugar by flushing out excess glucose.[2] Polyuria, the condition of producing vast amounts of urine, is often one of the first symptoms of diabetes mellitus.[3]

The prevalence of diabetes is rising globally and has nearly quadrupled since 1980. The chances of halting the rise at the level of 2010 until 2025 are highly unlikely.[4] Diabetes cases are rising faster globally than any other disease.[5] With exponentially increasing numbers, the diabetes-related complications and mortality are a challenge for health care systems worldwide.[6] Diabetes is associated with increased mortality from cancer, infections and reduced life expectancy depending on blood glucose management.[7] Projections assume 700 million people will suffer from diabetes mellitus by 2045 with 374 million more experiencing impaired glucose tolerance.[8]

Diabetes mellitus is diagnosed by measuring the fasting blood sugar level or using an oral glucose tolerance test (oGTT).[1] Fasting blood glucose levels (after not eating for at least 8 hours) should be < 100 mg/dL and 2 hours after consuming 75 g of glucose for the oGTT blood glucose levels should return to < 140 mg/dL. If fasting blood glucose levels are in the range of 100-125 mg/dL, it is considered an impaired fasting glucose and if 2 hours after the oGTT blood sugar levels remain between 140-199 mg/dL, an impaired glucose tolerance is diagnosed. Both of these conditions are considered a prediabetic state and a possible indicator for developing diabetes in the future. Confirmed fasting blood glucose taken from a venous sample of ≥126 mg/dL, any blood drawn during the day not related to food intake with ≥200 mg/dL or a venous blood sample 2 hours after the start of the oGTT with glucose levels ≥200 mg/dL will lead to the diagnosis of diabetes mellitus.[1]

Though new subclassifications of diabetes are emerging,[9] which might provide more tailored treatment, diabetes mellitus is commonly divided into 3 categories: type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM) and other types of diabetes mellitus like gestational diabetes, maturity-onset diabetes of the young (MODY) and others. Even though the symptoms are similar, the pathophysiology of these types of diabetes mellitus is remarkably different.[2]

Regardless of the etiology, untreated high blood glucose levels lead to micro- and macroangiopathy. Microangiopathy is seen as nephropathy with albuminuria and progressive kidney impairment, as retinopathy with the potential of blindness and as neuropathy with consecutive polyneuropathy, whereas macroangiopathy increases the risk of myocardial infarction, stroke and peripheral arterial occlusive disease.[1]

Pathophysiology of type 1 diabetes mellitus

In type 1 diabetes, absolute insulin deficiency as a result of a destruction of the beta cells or insulin secretion problems causes blood sugar to rise.[2] The insulin deficiency leads to ketone production and can result in weight loss, metabolic decompensation, diabetic ketoacidosis and eventually death if no external insulin is supplied. While a strict “starvation” diet was advocated to temporarily avoid glucosuria and metabolic decompensation, life expectancy for children with T1DM was limited to a few years before insulin therapy became available.[3]

Pathophysiology of type 2 diabetes mellitus

T2DM is often used as a broad classification of various disorders leading to an elevation of blood sugar.[10] It affects primarily middle-aged and elderly people and is frequently influenced by diet and lifestyle.[2] Genetic predisposition, physical inactivity and body fat increase can contribute to the development of T2DM.[11] About 80% of patients with T2DM are overweight or obese. T2DM is a chronic disease; often manifestation gradually develops over many years with unspecific symptoms such as fatigue, reduced performance and thirst. Hyperglycemia leads to symptoms such as polyuria, polydipsia and impaired vision.[1]

Chronic hyperinsulinemia in obese people is often associated with insulin resistance (IR). Rodin et al. hypothesized in a publication from 1985 that excess food intake raises insulin levels, stimulates fat storage and leads to obesity, but insulin sensitivity remains normal at the beginning. As the hyperinsulinemia continues over time, body tissues become less sensitive to insulin stimulation and higher concentrations of insulin are required to achieve the same effects on glucose and lipid metabolism. Over time, even supramaximal concentrations of insulin are insufficient to control blood glucose levels leading to symptoms of overt glucose intolerance and a vicious cycle of accumulation of body fat and continuously rising insulin levels.[11]

A new, refined classification of diabetes was proposed by Ahlqvist et al. in 2018 by using six variables (antibodies, age, BMI, HbA1, beta cell function and IR) to subgroup diabetes mellitus into 5 categories:

- Severe autoimmune diabetes (SAID)
- Severe insulin deficient diabetes (SIDD)
- Severe insulin resistant diabetes (SIRD)
- Mild obesity-related diabetes (MOD)
- Mild age-related diabetes (MARD)

While SAID corresponds to T1DM with low insulin secretion, SIDD, SIRD, MOD and MARD would represent subtypes of T2DM. These subclassifications would allow a quicker identification of patients at risk for complications and the potential for a closer tailoring of treatment in the future.[5]

Treatment options in type 2 diabetes mellitus

Lifestyle intervention

Nutritional therapy is cost effective and efficient in the prevention and management of diabetes.[12,13] Metabolic abnormalities preceding T2DM, like high fasting blood glucose, hypertension and dyslipidemia often seen in the metabolic syndrome (MetS) can be managed

with lifestyle interventions.[14] In the Diabetes Prevention Program (DPP), weight loss was shown to be the dominant predictor of a reduction in the incidence of diabetes. When adjusted for diet and physical activity, every kilogram of bodyweight lost was associated with a risk reduction of 16%.[15] Currently there is no single eating plan that caters to all personal preferences, comorbidities or socioeconomic situations to prevent T2DM.[16] Low carbohydrate diets have shown promise in improving glycaemic control and reversing T2DM.[17–19] Education and counselling are essential and need to be personalized to achieve individual goals. A HbA1c decrease of up to 2.0% [20 mmol/mol] can be achieved in T2DM within 3-6 months with intense interventions.[12]

The goals of nutritional therapy are to support healthy eating, to improve HbA1c, to address the needs of the patient and to decrease or maintain body weight without taking away the pleasure of eating. Macronutrient composition should be based on personal needs.[12] Self-monitoring of the intake of carbohydrates in people with T2DM is recommended and necessary to calculate appropriate dosages if insulin therapy is used. While glucose is the brains' main energy source, it is not an essential nutrient and can also be covered by protein and fat intake. The consumption of sugar should be minimized and whole foods preferred over processed foods. A reduction of carbohydrates was shown to improve glycemia and can be achieved with various eating patterns. Protein needs to be consumed in adequate amounts to prevent muscle loss. Trans fats should be avoided and saturated fat consumption minimized. In line, epidemiological studies have shown polyunsaturated fats to be associated with a lower risk of T2DM. In people with diabetes the consumption of sufficient amounts of dietary fibers has shown an association with lower all-cause mortality. While the common goal of all eating patterns is to reduce calories, the benefit of interventions with low fat intake appear to be mainly related to the weight loss, while interventions with lower carbohydrate intake improve weight and glycemia.[12,20] A reduction in HbA1c, blood pressure and body weight can lead to a longer life expectancy in people with T2DM.[21]

Achieving the highest quality of life while simultaneously delaying or preventing complications is the main objective in treating T2DM. It can be achieved by careful monitoring blood glucose, minimizing cardiovascular risk and providing a patient-centered treatment implementing and encouraging proactive self-care. An individualized approach with careful consideration of the patients' needs and goals is necessary. Nutritional therapy and physical activity are key aspects. Further steps like smoking cessation and psychological

support should be encouraged. With a growing number of glucose-lowering medication options, hyperglycemia symptoms can be alleviated and quality of life increased. Successful glycemic management can reduce the risk of both microvascular complications as well as macrovascular events.[12]

An HbA1c of 53 mmol/mol (7%) is considered a reasonable goal for blood sugar management in T2DM. Individual factors, such as frailty and comorbidities of the patients as well as the risk of adverse events, such as hypoglycemia and weight gain need to be considered. An interdisciplinary approach including doctors, nurses, sports scientists and dieticians is necessary to avoid therapeutic inertia. While pharmacological interventions such as sodium-glucose transport protein 2 inhibitors (SGLT2 inhibitors), dipeptidyl-peptidase-4 inhibitors (DPP4 inhibitors) and glucagon-like peptide 1 receptor agonists (GLP-1 RA) have expanded the options of pharmacological treatment and can improve heart failure and kidney disease, the first-line therapies in T2DM are still Medical Nutritional Therapy and physical activity.[22]

Medical Nutritional Therapy aims to preserve the pleasure, experienced while eating and at the same time accomplish blood glucose management and minimize cardiovascular risk. Eating patterns should be individually selected and accommodate personal preferences as well as metabolic needs in order to lead to healthy eating habits and sustainable lifestyle changes. Weight reduction can be achieved with nonsurgical strategies with intensive, consistent counselling and a combination of dietary interventions and physical activity.[22] While a minimum of 150 minutes of moderate activity or 75 minutes of intense activity are recommended for cardiovascular health, these amounts of physical activity are considered inadequate for weight maintenance or weight loss without reducing the amount of calories consumed.[23,24]

Ideally, the treatment should start before the manifestation or diagnosis of T2DM at an early stage of impaired fasting glucose or impaired glucose tolerance. The normalization of body weight to a BMI of < 25 kg/m² and an increase of physical activity are the main goals. The manifestation of T2DM and further therapy escalation can often be delayed, which may explain the low numbers of people with T2DM during times of famine. Even remission of newly manifested T2DM can be achieved in young people with T2DM.[1] Dieting, defined as the endeavor to reduce calories to lower body weight, has been the main focus of addressing

obesity and T2DM.[25] Diets with very low calories can help manage weight, reduce blood sugar and can even put people with T2DM in remission but require discipline and patience.[26] Although a reduction of calories physiologically leads to decreased body weight, people struggle with diets and results are often not maintained. 95% of dieters regain their weight after a few years and two thirds of dieters will regain more weight than they originally lost. Dietary failure can therefore lead to mental health problems and disordered eating such as binge-eating. The adherence to the diet has been considered an important factor in the successful weight loss as well as seeing the dietary modification as a lifestyle change rather than a limiting phase. People on conventional diets report increased temptation and difficulty in moderating quantities of food. Patients often report binging tendencies after dietary violations.[27]

Bariatric surgery can lead to massive weight loss and reduce overall mortality in T2DM. However, the irreversible change in the patients' anatomy as well as surgery per se bear the risk of adverse events.[28,29] Younger patients with shorter diabetes duration, lower HbA1c and better beta cell function are more likely to achieve remission of T2DM with bariatric surgery.[30] A high fasting C-peptide as a surrogate marker for the beta cell reserve is associated with a higher chance of remission and can be used to weight the risks and benefits of bariatric surgery in T2DM.[31] If diet and exercise are not enough to achieve the blood glucose goals, oral antidiabetic (OAD) medication is the next step.[1]

Oral antidiabetic medication (OAD)

Metformin

The first choice of OAD for obese people with T2DM is metformin, a biguanide used to treat diabetes since 1957.[1,32] Metformin can improve blood glucose control by reducing the hepatic glucose output without changes to the insulin signalling.[33] It delays the resorption of glucose from the gut, increases the glucose-uptake in the muscle and curbs appetite. While the exact mode of action of metformin is still a matter of debate, it can cause gastrointestinal side effects in some patients and needs to be cautiously used in people with lowered estimated glomerular filtration rate (eGFR). Of note, metformin has also shown a reduction in cancer mortality.[34] Metformin does not cause drug-induced hypoglycemia. If metformin

is not enough to achieve sufficient glucose control, a second OAD or a GLP-1 receptor agonist (GLP1-RA) can be introduced.[1]

Sulfonylurea

While sulfonylurea drugs are a cost-effective medication to lower blood glucose by enhancing the ability of the beta cells to secrete insulin, around 5 to 10 % of patients will stop responding to the medication every year and 50% of initial responders will not continue to respond to sulfonylurea after 10 years.[35] They lead to an increase in weight, can cause hypoglycemia and - like thiazolidinediones - play a decreasing role in the treatment of T2DM in Germany and Austria.[1]

Thiazolidinediones

Thiazolidinediones belong to a group of oral antidiabetic medicines causing lowered blood glucose and increased insulin sensitivity. While they are known for their glucose lowering ability and glycemic durability, they can cause fluid retention, worsen congestive heart failure and result in weight gain.[22]

Sodium-glucose transport protein 2 inhibitors

Sodium-glucose transport protein 2 inhibitors (SGLT2) provoke to a reduction of glucose reabsorption in the kidney leading to pharmacologically induced glucosuria, which reduces blood glucose levels. They are nephroprotective and show a reduction in cardiovascular mortality, but can also lead to urinary tract and fungal infections.[1] The efficacy of the blood glucose reduction depends on the renal function and can lead to weight loss, lower blood pressure, but does not increase the risk of hypoglycemia. Further cardiac and renal benefits are currently investigated. SGLT2 inhibitors need to be paused during infections an increase the risk of fungal infections and diabetic ketoacidosis.[22]

Dipeptidyl-peptidase-4 inhibitors (DPP4 inhibitor) and glucagon-like peptide 1 receptor agonists (GLP-1 RA)

Glucagon-like peptide 1 (GLP-1) is an incretin, a gut-derived hormone, which stimulates insulin secretion, inhibits glucagon secretion and reduces appetite leading to weight loss and protracted emptying of the stomach.[36,37] It is inactivated by dipeptidyl-peptidase-4. Dipeptidyl-peptidase-4 inhibitors prevent the degradation of GLP-1, thus raising the level of GLP-1.

DPP4 inhibitors are taken orally, while GLP-1 RA are injected subcutaneously. Neither DPP4 inhibitors nor GLP-1 receptor agonists cause hypoglycemia, but can lead to gastrointestinal side effects such as nausea or diarrhea.[1]

If blood sugar is still insufficiently controlled by OADs and GLP1 RA do not show a sufficient HbA1c reduction, insulin can be used to achieve the target blood glucose concentrations.

Insulin

History, production and function of insulin

History

100 years ago in January 1922, insulin became available as a treatment option and changed the lives of millions of people forever.[3] While many people contributed to the discovery of insulin, it was isolated and purified in Toronto in 1921 by Frederick Grant Banting, Charles Herbert Best, John James Rickard McLeod and James Bertram Collip. For their contributions, Banting and McLeod received the Nobel Prize in Physiology/Medicine in 1923 and shared half of their money with Best and Collip. They name of the hormone reflects the origin of its production in the islets (Latin “insula”) of Langerhans in the pancreas.

The amino acid sequence of the two-chain polypeptide hormone was established 28 years later by Fred Sanger, earning him his first Nobel Prize in Chemistry in 1949. In 1959, insulin levels in the blood were measurable for the first time using a radioimmunoassay procedure developed by Yalow and Berson. While Berson died in 1972, Yalow was awarded the Nobel Prize in Physiology/Medicine in 1977.[33] Insulin was the first human protein that was fully

chemically synthesized in 1963. Production of human insulin using a recombinant DNA technology was first developed using Escherichia coli in 1979.[3]

Endogenous insulin production

Endogenous insulin is produced by the beta cells in the Langerhans islets in the pancreas. It lowers the amount of blood sugar by allowing glucose to enter tissue cells, e.g. muscle and fat cells. Insulin secretion is mainly stimulated by glucose and carbohydrates and to a lesser degree by free fatty acids and amino acids. Hormones like GLP-1, GIP as well as sympathetic and cholinergic influences play an important role in the regulation of the insulin levels.[33] It also influences amino acid metabolism, glycogen production, fatty acid synthesis and mobilization in adipose tissue as well as promoting division and growth of cells.[11]

Insulin consists of 51 amino acids and is formed by an A chain and a B chain connected by disulfide bonds.[3] Endogenous insulin secretion can be measured by using connecting peptide (C-peptide) as a surrogate parameter even in insulin-treated patients.[38] C-peptide is the 31-amino acid sequence originally connecting the A and B chains, which has to be enzymatically cleaved from the precursor proinsulin to become active insulin. Therefore, C-peptide is secreted in equimolar amounts to the endogenous insulin.[10] C-peptide has a longer half-life (over 30 min) than insulin (3-5 min) and can be measured in blood or urine samples to assess beta cell function or diagnose insulin producing tumors known as insulinoma.[38,39]

Insulin Function

Insulin levels respond to feeding and fasting and represent the switch between an anabolic to a catabolic state of the body. Rapidly rising insulin levels lower postprandial blood glucose peaks, suppress hepatic gluconeogenesis and promote the storage of body fat. In times of scarcity, low insulin levels facilitate the release of energy stored as fat and glucose production in the liver preventing low blood glucose (hypoglycemia), which would pose a risk to the brain requiring a constant glucose supply.[33] Glucose can be generated with glycogenic amino acids and triglyceride lysis resulting in glycerol.[40] To guard against periods of starvation, accumulation of fat by hyperinsulinemia is an essential evolutionary adaptation.[41]

Between 1921 and 1922, Banting and Macleod managed to extract insulin and successfully used it for the first time in critically ill children. Despite sufficient calories, children with an absolute insulin deficiency are not able to use the rising blood glucose levels as energy or store it as fat and the resulting ketoacidosis leads to coma and death. In January 1923, an emaciated, insulin deficient child was restored to an ordinary physique with daily insulin injections for the first time.[33] Blood sugar dropped by 75% after the first injection and glycosuria and ketonuria improved dramatically.[42]

The researchers sold the patent for insulin to the University of Toronto for one Canadian dollar with Banting declaring insulin does not belong to him, it belongs to the world. Eli Lilly and Company started manufacturing Insulin followed by the Nordisk Insulin Laboratorium. Originally made using beef and pork extracts, a major milestone was achieved with human insulin becoming the first recombinant protein therapeutic with further developments for insulin analogues. Today, insulin can be administered as an injection with syringes, with a pen or with an insulin pump. In 2015, the global market for insulin was estimated at 27 billion dollars and an expected growth of 8% every year. [33]

Longer acting insulin and insulin analogues

In 1936, Hagedorn and Jensen managed to combine protamine with insulin and to create an insulin which delays release of insulin monomers. In 1946, neutral protamine Hagedorn (NPH) insulin was introduced with an action of 4-12 hours. Constant reformulations and improvements extended the action time to 20-30 hours and beyond, leading up to research into insulin which can be applied only once a week.[3] Long acting insulin is used as "basal insulin", covering the bodies' basal insulin requirements as opposed to "bolus insulin" which is used for mealtimes. Basal insulin can be used once or twice a day. Concentrated formulations have reduced the volume which needs to be injected with higher doses. If blood glucose levels cannot be adequately controlled with basal insulin, an intensified therapy using rapid acting insulin becomes necessary.[22]

Rapid acting insulin and insulin analogues

The first human synthetic insulin analogue known as insulin lispro switched the position of proline and lysine (position 28 and 29 in the B-chain) and provided faster absorption. Faster

acting insulins provide more flexibility regarding the injection time before meals.[3] The amount of carbohydrates consumed at the meal needs to be taken into consideration to calculate the amount of "bolus insulin" needed. Similar to basal insulin, if too much basal insulin is injected, blood sugar will drop below recommended thresholds and may lead to potentially dangerous hypoglycemia.

Premixed insulins

Premixed ratios of insulin subtypes are available to keep the number of injection low for elderly patients, but provide less flexibility and carry a higher risk of hypoglycemia compared to a basal insulin monotherapy.[3]

Combination products of insulin and GLP1 agonists, trying to minimize the weight gain due to insulin treatment in T2DM are also available.[3]

The efficacy of insulin treatment depends on correct titration and appropriate use. Proper training, administration and timing are essential.[22] Side effects and contraindications for insulin include hypoglycemia, lipodystrophy of body fat at injection sites and IR.[1] Insulin therapy can lead to weight gain due to iatrogenic hyperinsulinemia and needs to be carefully selected and adjusted to minimize adverse effects.[43,44] IR in people with T2DM and insulin therapy is associated with increased risk of mortality, major adverse cardiovascular events and diabetic kidney disease.[45]

Insulin resistance

In the consensus conference of the American Diabetes Association on IR in 1997, IR has been described as a defect in insulin sensitivity of tissues in people with diabetes mellitus. IR is the impairment of the cells' ability to react to endogenous or exogenous insulin. The effects can transcend to other biological actions of insulin such as lipid metabolism, effects on the vascular system and specific gene expression.[24]

IR syndrome is a constellation of observations and lab parameters including a compromised glucose tolerance, central obesity, lipid metabolism disorders and elevated blood pressure. IR is the main feature of IR syndrome but can also typically occur without the syndrome for

example in polycystic ovary syndrome (PCOS) or during pregnancy and systemic glucocorticoid therapy. IR, independent of other parameters like waist circumference or weight can be used as a predictor for T2DM. IR improves with a hypocaloric diet, even before weight loss can be measured. Weight loss further helps to restore insulin sensitivity.[24] Lower insulin sensitivity is associated with various health problems such as obesity and can be used as a predictor of cognitive decline in people with prediabetes independent of elevated glucose levels.[46]

IR can arise due to various factors. Some appear to be inherited, while others are acquired due to an individual's lifestyle. Defects in the receptors, signaling pathways and reduced receptor density are among many reasons that have been identified.[33] While the hyperinsulinemic euglycemic glucose clamp is the most accurate measurement of IR, surrogate markers are necessary to simplify the detection of IR.[47] Ralph DeFronzo, a pioneer in establishing the concept of IR, developed the glucose and insulin clamp techniques in 1979. He invented the hyperglycemic clamp method to assess beta-cell sensitivity as well as the euglycemic insulin clamp to measure tissue sensitivity to insulin. In the hyperglycemic clamp the plasma glucose concentration is elevated to 125 mg/dl and kept constant with variable glucose infusions allowing for the measurement of the subsequent plasma insulin levels. In the euglycemic clamp the plasma insulin levels are maintained at 100 muU/ml using insulin infusions and the glucose infusion rate necessary to stabilize the blood glucose levels provides a measure of the tissue sensitivity to the insulin injected. [48,49]

Judith Rodin, a psychologist and student of Ralph DeFronzo postulated a sequence of hyperphagia, hyperinsulinemia and IR in 1985.[11] IR necessitates higher amounts of insulin which further worsens IR leading to a vicious cycle. One of the key strategies should therefore be the focus on minimizing the hyperalimentation.[1]

Hyperinsulinemia and IR have a dynamic relationship, but despite a clear association, the question of causality remains unclear. Hyperglycemia is the consequence when the beta cells cannot compensate blood glucose levels with adequate amounts of insulin anymore. IR as well as hyperinsulinemia are seen in the development of T2DM earlier than hyperglycemia. However, which one would be considered the chicken and which the egg regarding hyperinsulinemia and IR? If hyperinsulinemia is the consequence of IR, an increase of plasma insulin concentration is necessary to achieve lower blood glucose levels, even if it

leads to adverse metabolic effects. If hyperinsulinemia is the origin and not the consequence of IR, the therapies will need to focus on reducing plasma insulin concentration.[50]

New non-pharmacological approaches in the treatment of T2DM

Endobarrier™

The Endobarrier™ is an impermeable duodenal-jejunal bypass liner developed by GI Dynamics (Lexington, MA, USA).[51] It is 60 cm long and made of a fluoropolymer material which prevents food from coming into contact with jejunal mucosa. The Endobarrier™ is kept in place by an nitinol anchor in the duodenal bulb. It can be inserted and removed via endoscopy and delays digestion and resorption.[52] A reduction of BMI and improved glucose parameters were observed after implantation.[53–55]

Intermittent Fasting

Intermittent fasting (IF) is not considered an eating pattern but rather a modification of the eating periods. The focus lies on the time of food consumption rather than macronutrients. Many people stop diets due to the increased effort to monitor their caloric intake.[56,57] Popular forms of intermittent fasting cover restricting food for certain hours of the day or alternate day fasting, dividing the week into eating and fasting days.[12]

Intermittent fasting might be a new dietary approach for people with T2DM. However, blood glucose levels have to be monitored closely especially with medication that could induce hypoglycemia such as sulfonylurea and insulin. Blood sugar levels below 70 mg/dL should be avoided and blood glucose levels of 180 mg/dL and beyond need to be checked for adherence to fasting and medication changes.[58,59] Medication management in people with T2DM should be overseen by a dedicated specialist with a focus on endocrinology and diabetology.[59,60] Until 2010, four studies were conducted on IF in human participants.[61–64] Case reports of IF as alternative to insulin were published in 2018[65], with intermittent fasting later described as a user-friendly option of treatment for T2DM.[66] Reviews and meta-analyses showed IF to be associated with more weight loss compared to a standard diet, but

further studies were required to demonstrate the safety and efficacy of IF in T2DM and to explore the possible physiological processes leading to the observed results.[67–69]

A clinically significant weight loss of over 5%[70] can be achieved with IF, providing an easier option for people struggling with classic caloric restriction.[71–76] Intermittent fasting can be performed as alternate day fasting (ADF), dieting for 2 days a week or as time restricted eating (TRE).[71,77–79] Fasting days in ADF can be either zero calories[80,81] or limited to 25% or roughly 500 kcal.[61,82–84] The meals can be spread out during the day or contained to specific hours.[82,85] While people choose the time of eating freely, some choose dinner due to the social aspect of eating together.[86] The 5:2 diet is a modification of IF allowing for eating on 5 days during the week and fasting on 2.[87–89] When food intake is not restricted by the day, but rather by usually 4 to 8 hours during the day, it is called TRE.[90–94]

Results of ADF[81,83,95–97] and 5:2[98,99] showed 4-8 % of weight loss in obese people over 8-12 weeks. For longer periods of time between 24 and 52 weeks, ADF[84] as well as 5:2[87–89,100] showed the most weight loss at 12 weeks which could indicate an efficacy apex. TRE in comparison showed less weight loss (< 5%) in 12 weeks.[90,93,101] Neither sex nor menopause affected the weight reduction.[102] People in a normal BMI range lost on average 0.2 kg every week with intermittent fasting.[96] Intermittent fasting can also be used in people with IR.[103] Meta-analyses comparing IF and continuous caloric restriction were performed showing similar effects on weight and metabolic improvements.[104–106]

In weight loss induced by caloric restriction, the majority of the weight is lost via fat mass, while one fourth is a loss of lean body mass.[107–110] No difference in loss of lean mass was observed when combined with ADF.[111] In TRE, resistance training helped with muscle mass maintenance.[90,112] Trials have shown no changes in fiber[84,85,87,90,113] or beverage[90,114] consumption. The effects on blood pressure are not clear yet with some studies showing a reduction in blood pressure[115] and others showing no difference.[116] Sympathetic nerve activity as well as noradrenalin levels and natriuresis have been discussed as possible mechanisms.[117,118] It is surprising to find HDL levels unchanged in IF[58,81,100] which are typically lower during weight loss.[119] HDL can, however, be raised with endurance training.[120,121] Fasting beyond 18 hours lead to higher levels of triglycerides and free fatty acids due to lipolysis.[122–124]

While much on the information on IF comes from Ramadan fasting, it can only partially be compared with other forms of IF due to Ramadan shifting the time of eating to the night

while IF performed as ADF, 5:2 or TRE. The latter usually have eating periods during the day. Meal timing might play a critical role in the outcomes of IF.[125] In their pioneering research on intermittent fasting, Varady et al. showed that energy intake was reduced in obese people by an average of 300 to 600 kcal per day even without calorie counting.[90,101] One study showed a reduction in calories consumed on eating days during 5:2.[126] While time restricted eating has shown no benefit over simple calorie restriction regarding weight loss in people with obesity, IF might be a new approach for people with diabetes.[127]

Until 2022, only one trial investigated intermittent fasting in T2DM.[58] While fasting glucose remains stable in people without diabetes[128], the level of fasting insulin was shown to be reduced with IF.[58,84,87,90,95,99,115] The effect was stronger in people with elevated fasting insulin levels.[58,84,90,95] An improvement of IR was shown in some studies[87,90,95,115] but an improvement in HbA1c was not seen in people without elevated HbA1c levels as seen in T1DM or T2DM.[84,90,93,111,116]

Oxidative stress and inflammation are key aspects of T2DM.[129–134] IF was shown to reduce oxidative stress.[61,87,90,115] This could be a factor in improvement in IR while fasting.[135,136] Antioxidants can be used to improve insulin sensitivity in overweight people, which could be one of the reasons why IR can be improved when oxidative stress is reduced.[137,138] While side effects like constipation, fatigue, diarrhea and headaches are seen in intermittent fasting[139,140], they can be mitigated with sufficient hydration.[141,142] While many people are worried, IF could lead to the development of eating disorders, one study on ADF and one in TRE showed that IF did not lead to binging or purging.[139,140] People with eating disorders were not enrolled in trials on IF yet. Thyroid hormone levels were not changed in people with[143] or without[112] mild hypothyroidism.

While reduced resting metabolic rate (RMR) is a common concern in weight loss achieved with caloric restriction[144] IF had no or minor effects on the RMR in weight maintenance[62,92,112] and small decreases of around 150 kcal in weight loss of 5-7% of body weight.[84,100]

Obese adolescents are a special target group for IF[145–147] While some researchers see adolescence as a vulnerable time for eating disorders[148–150], others argue medically guided and supportive weight loss would not lead to eating disorders in obese adolescents.[151] Therefore, further research in this special group is necessary.

IF can be integrated into daily life.[71,78,81,93,111,152–154] Sufficient protein should be consumed during IF[155–157] to counteract muscle mass loss.[158,159] While noncaloric drinks can be consumed during fasting times, excessive amounts of diet soda can lead to sugar cravings.[160]

Combined with IF, a change in eating behavior should be supported in people trying to lose weight, helping them to track their nutrients, increasing physical activity and monitoring weight changes.[161–163] Mobile apps could have the potential to support behavioral changes.[164]

Intermittent fasting is not recommended for children, pregnant or lactating women, young people in a healthy weight range, people with eating disorders, underweight individuals or cachectic elderly people. It is recommended for severely obese adolescents, normal or overweight adults, people suffering from hypertension and/or lipid disorders, insulin resistant people and people with prediabetes, T1DM and T2DM using medical supervision.[165] A systematic review in March 2022 concluded, IF could be used as an auxiliary treatment in MetS to improve glucose and lipid parameters as well as significantly reducing IR.[166]

While more frequent smaller meals are often recommended in early-stage T2DM[167] more recent data has shown a positive effect of fewer meals in people with insulin-treated T2DM. Jakubowicz et al. argued that fewer meals during the day could upregulate clock gene expression.[133] A comment was published on the trial, noting an alternative explanation for the outcome of the study, claiming the protocol to be similar to intermittent fasting with 18 hours and 30 minutes of fasting after a carbohydrate reduced dinner.[168] The corresponding author Oren Froy agreed with the observation but argued the increased breakfast and reduced amount of carbohydrates for dinner provided during the trial might be the main driving factor resulting in the positive effect on the HbA1c-levels.[169–172]

In a review article on the clinical application of intermittent fasting in Nature Reviews Endocrinology in February 2022, Varady et al. highlighted the importance of studies on intermittent fasting in people with diabetes mellitus.[165]

2 Aims and Hypothesis

The three publications selected for this cumulative dissertation were chosen to accurately illustrate the evolution of ideas during the course of the PhD and the steps leading to a successful randomized controlled trial and the corresponding publication of the results.

With the numbers of people with diabetes mellitus increasing every year the need of easily accessible and inexpensive treatment options is growing.[4] New pharmacological therapies have vastly improved the options of blood glucose management for people with T2DM, but new approaches to lifestyle management are also needed.[22]

The publication "EndoBarrier™ implantation rapidly improves insulin sensitivity in obese individuals with type 2 diabetes mellitus" lay the groundwork for the "Interfast 2" study, providing important information on non-pharmacological treatment in patients with obesity and T2DM.[51] At the same time, the publication of the first "Interfast study" in healthy, non-obese and non-diabetic participants using alternate day fasting showed a reduction of weight and an improved fat-to-lean ratio suggesting intermittent fasting could become a clinically relevant intervention for people trying to lose body weight and improve blood glucose management. This publication paved the way to the development of the "Interfast 2 study" protocol.[80]

In an earlier publication, we were able to provide evidence of prolonged being safe in people with T1DM treated with insulin therapy and participants with T2DM without insulin therapy, however we investigated only one prolonged fasting session.[173,174]

The published study protocol "Interfast 2" was shaped by data and insights generated in the "Endobarrier" study and submitted to the Austrian Science Fund (FWF). After revisions and a preliminary data collection, it was approved for funding and published online in Diabetic Medicine in February 2022. The printed version appeared in volume 39 issue 6 in June 2022.

Finally, the publication of the results of the "Interfast 2" trial presents the culmination of the information gathered during the PhD studies and the work on the trial and provided many new interesting research questions.

Endobarrier:

Justification of the research question:

The primary aim of the study was to explore short and long-term effects of the EndoBarrier™ implantation on the glucose metabolism of obese participants with T2DM.[175]

Hypothesis:

The EndoBarrier™ modulates the glucose metabolism in obese people with T2DM and could induce weight loss and improve metabolic parameters.

Interfast-2:

Justification of the research question:

The aim of the project was to investigate the impact of 12-weeks of intermittent fasting (IF) compared to usual care on metabolic parameters and physiologic mechanisms in a group of participants with insulin-treated type 2 diabetes mellitus T2DM.[82]

Hypothesis:

Intermittent fasting could improve glycemic control and reduce HbA1c, weight and insulin dose in people with insulin-treated type 2 diabetes.

Novelty value and limitations of the research topic

While less invasive alternatives to bariatric surgery and intermittent fasting have risen in popularity, further research is necessary to assess safety and efficacy in people with T2DM. Insights generated in studies with participants with T2DM might help in shaping future studies, dietary recommendations and adjuvant therapy options.

3 Results and Data

This section recapitulates the results as published in the following articles:

- Obermayer, A., Tripolt, N. J., Aziz, F., Högenauer, C., Aberer, F., Schreiber, F., Eherer, A., Sourij, C., Stadlbauer, V., Svehlikova, E., Brunner, M., Goswami, N., Kojzar, H., Pferschy, P. N., Pieber, T. R., & Sourij, H. (2021). EndoBarrier™ Implantation Rapidly Improves Insulin Sensitivity in Obese Individuals with Type 2 Diabetes Mellitus. Biomolecules, 11(4), 574. https://doi.org/10.3390/biom11040574

- Obermayer, A., Tripolt, N. J., Pferschy, P. N., Kojzar, H., Jacan, A., Schauer, M., Aziz, F., Oulhaj, A., Aberer, F., Sourij, C., Obermayer-Pietsch, B., Stadlbauer, V., & Sourij, H. (2022). INTERmittent FASTing in people with insulin-treated type 2 diabetes mellitus - the INTERFAST-2 study protocol. Diabetic Medicine: a journal of the British Diabetic Association, e14813. Advance online publication. https://doi.org/10.1111/dme.14813

- Obermayer, A., Tripolt, N. J., Pferschy, P. N., Kojzar, H., Aziz, F., Müller, A., Schauer, M., Oulhaj, A., Aberer, F., Sourij, C., Habisch, H., Madl, T., Pieber, T., Obermayer-Pietsch, B., Stadlbauer, V., & Sourij, H. (2022). Efficacy and Safety of Intermittent Fasting in People With Insulin-Treated Type 2 Diabetes (INTERFAST-2)-A Randomized Controlled Trial. Diabetes care, dc221622. Advance online publication. https://doi.org/10.2337/dc22-1622

Results of *EndoBarrier™ Implantation Rapidly Improves Insulin Sensitivity in Obese Individuals with Type 2 Diabetes Mellitus*

The study protocol was published in Diabetes Therapy.[175] Participants had a mean age of 48 ± 9 years, a diabetes duration of 7 ± 6 years and an average body weight of 121.2 ± 18.5 kg. Body weight was significantly reduced already 4 weeks after insertion to 116.3 ± 18.2 kg, remained lower for the entire 36 weeks until removal (115.1 ± 21.4 kg) and the positive effects on weight and glucose metabolism remained even 24 weeks following the explantation of the device with an average weight of 117.2 ± 20.8 kg at the end of the study.

The mean glucose infusion rate during Botnia clamps was used to assess insulin sensitivity showing a significant improvement 4 weeks after insertion, during the 36 weeks the Endobarrier™ was used and showed significantly higher insulin sensitivity even 24 weeks after the explantation. The Food Frequency questionnaire that was used showed a significant reduction in calories consumed after the insertion of the device. Four serious adverse events were recorded due to a case of dehydration, a participant developing a duodenal ulcer, a case of nausea and vomiting leading to intravenous fluid replacement and a case of hemorrhoid bleeding. We noted gastrointestinal side effects in 40 % of the participants similar to other trials using the Endobarrier™.[176]

All participants finished the study without premature removal of the device.[51] While the Endobarrier™ was shown to be more effective than simple calorie restriction the change in body weight was smaller compared to surgical interventions which can lead to a body weight reduction of over 30%.[177,178] It could be used as a preparation for bariatric surgery in high risk patient with obesity and T2DM.[179]

Results of *INTERmittent FASTing in people with insulin-treated type 2 diabetes mellitus – the INTERFAST2 study protocol*

With intermittent fasting becoming a popular alternative to classic calorie restriction the Interfast 2 Study protocol for intermittent fasting in insulin treated type 2 diabetes mellitus was developed. The main aim of the study was to determine the effect of 12 weeks of intermittent fasting compared to usual diet and care. It was designed as an open, single-centre study randomizing participants to either a fasting group or a control group. Participants with T2DM, an HbA1c of ≥ 53 mmol/mol (≥7.0%) and at least 0.3 IU/kg body weight were included. A continuous glucose monitoring system (CGM), dietary counselling as well as measurements of the resting metabolic rate, body composition using dual-energy X-ray absorptiometry (DXA), oGTT were provided and blood and stool samples were collected.[180,181] The primary endpoint was defined as the difference in the change in HbA1c from baseline to 12 weeks between the two groups. A co-primary endpoint consisting of the difference in the number of participants achieving a combined end point encompassing a body weight reduction of at least 2%, an insulin dose reduction of at least 10% and an absolute HbA1c reduction of at least 3 mmol/mol (0.3%) between the two groups was selected to depict metabolic changes.[182–184] A hierarchical order was chosen to perform the calculations according to the European Medical Agency guidelines.[185] Intermittent fasting has been shown to reduce body weight and lower triglyceride levels and low density lipoprotein (LDL) cholesterol. It can lower blood pressure and improve IR.[61,111,186,187] Similar to caloric restriction it reduces fat mass and fasting insulin levels.[60] Fasting and feeding affect the circadian rhythm and high blood glucose levels are associated with poor sleep.[188–190] Questionnaires were added to asses sleep and sleepiness, physical activity, quality of life and self-regulatory behaviour.[191–195] T2DM lowers the diversity of the gut microbiome and affects the metabolism and fat storage.[196–201] Further research on intermittent fasting and the effects on sleep and microbiome in people with insulin treated T2DM is required.[165,202,203] A insulin-reduction-protocol was developed to minimize the risk of hypoglycemia during fasting.[204,205] Intermittent fasting has the potential to reduce waist circumference associated with IR.[206] All participants wore a CGM for the duration of the study to minimize the risk of hypoglycemia on fasting and eating days.[207] The protocol was published in Diabetic Medicine.[82]

Results of *Intermittent fasting in people with insulin treated T2DM (INTERFAST-2) - a randomized controlled trial*

From October 2019 to October 2021, 46 participants were recruited and randomized. 22 women and 24 men with a mean age of 63 ± 7, a mean diabetes duration of 21 ± 9 years and a mean HbA1c of 67 ± 11 mmol/mol (8,3%). The average BMI was 34.3 ± 4.5 kg/m^2 with an average total daily insulin dose of 56 ± 27 international units. 22 people were allocated to the fasting group and 24 people to the control group. One screening failure due to HbA1c occurred and the patient was rescreened. Two participants were not able to complete the trial due to the start of an oral glucocorticoid therapy and moving out of the country. The Baselinecharacteristics were matched in terms of anthropometric, glucose parameters and total daily insulin dose (TDID). The fasting group was significantly older and had a longer duration of diabetes than the control group. The primary endpoint a significant difference in the change of HbA1c between baseline and 12 weeks between the two groups was recorded (p=0.012). HbA1c was on average lowered by -7.25 ± 12 mmol/mol and remained similar in the control group with an increase of 0.08 ± 6 mmol/mol. All three aspects of the co-primary endpoint were achieved by 8 participants in the fasting group and none in the control group. 60 % of participants in the fasting group and 25 % in the control group achieved a reduction in HbA1c by 3 mmol/mol or more. 80% in the fasting group and 4.16 % in the control group achieved a reduction of weight of 2 % or more. 75 % in the fasting group and 0 % in the control group achieved a reduction of insulin dose by 10% or more. During the study period no cases of severe hypoglycemia necessitating external help were recorded. The body weight, body fat mass and fat mass to lean mass ratio was significantly reduced in the fasting group compared to the control group. Muscle mass and bone mass did not significantly change over 12 weeks between the groups. Android and gynoid fat mass was also significantly reduced. The resting metabolic rate and the physical activity showed no difference between the groups and remained constant comparing the measurements at baseline and after 12 weeks. Insulin dose was significantly reduced in the fasting group but increased in the control group. Two significant adverse events occurred in the fasting group and three in the control group. Intermittent fasting was show to be feasible and safe in people with insulin treated T2DM using CGM to prevent hypoglycemia and led to lower HbA1c, lower weight and lower TDID.[208]

4 Discussion

Endobarrier

The pilot trial using the duodenal-jejunal bypass liner Endobarrier™ was able to demonstrate a significant decrease in body fat mass using body composition measurements by DXA-scans in obese patients with T2DM as well as a rapid improvement of insulin sensitivity assessed by Botnia clamps within 4 weeks of implantation.[51] The HbA1c showed no statistically significant reduction, possibly due to the changes in medication necessary to prevent hypoglycemia. Improvements in glucose parameters with the bypass liner have been shown in a number of trials [209–211].

However, Miras *et al.* argued the rapid changes during the first few weeks of the treatment could be due to changes in the eating habits of the patients rather than the device itself.[212] During the first two weeks after the implantation participants were instructed to first consume liquids and soft foods to prevent any solid food getting from obstructing the Endobarrier™. We observed a significant reduction in the calories consumed, assessed with a food frequency questionnaire over the course of the study until explantation. Of note, a lactulose/mannitol test performed at baseline, after 4 weeks and 9 months showed no change in gut permeability due to the implantation of the Endobarrier™.

While the Endobarrier™ was originally designed for a 12 months use up to 24 months, this pilot trial reduced the treatment period to 9 months to minimize the risk of hepatic abscess, which is supported by Betzel *et al.* arguing the largest benefit could be achieved after 9-12 months.[213] We found a partial increase in body weight and HbA1c 6 months after explantation of the bypass liner similar to the data of Deutsch *et al.*[211].

However, the improvement in insulin sensitivity compared to baseline remained even 6 months after explantation. The reimplantation of the Endobarrier™ for a second time is technically possible.[214] A 4 year follow-up recorded no further weight loss after the initial removal of the device but recommended larger controlled studies to be conducted.[215]

The Endobarrier™ induces a delayed absorption of nutrients, which leads to weight loss, but could also result in potential deficiencies of ferritin and vitamin B12.[216] While the bypass liner was shown to be more effective than calorie restriction alone[177], bariatric surgery has

shown a much more pronounced overall body weight reduction of more than 30%[178]. Therefore, the minimally invasive endoscopic placement of the Endobarrier™ provides an option for patients with T2DM who might be opposed to bariatric surgery or it could be used as a pre-surgery treatment for high risk patients.[210]

A removal of the device through endoscopy is less invasive compared to surgery, but the risk of serious adverse events remains due to the metal anchor and potential occlusion of the device.[217] While no cases of hepatic abscesses were recorded in our study and no device had to be removed before the planned explantation, around 40% of participants experienced gastrointestinal side effects with similar numbers recorded in other studies.[176]

In summary, the Endobarrier™ is a reversible and minimally invasive intervention to help patients reduce body weight and increase insulin sensitivity and can be seen as an intermediate step between nutritional, pharmacological and surgical therapies in people with obesity and T2DM.

Intermittent fasting in type 2 diabetes mellitus

In the Interfast 2 trial, three non-consecutive days of IF showed a reduction in HbA1c, body weight and total daily insulin dose compared to a control group in people with insulin treated T2DM. Out results are in line with data by Carter *et al.*[89], who showed a HbA1c reduction due to IF and the study was used to calculate the sample size needed for the Interfast-2 study.

Data on intermittent fasting and insulin therapy was still scarce in 2018 when the protocol was developed. While Li *et al.* showed a one-week fasting program consisting of only 300 kcal of food a day in people with T2DM, patients with additional insulin therapy were excluded from the trial. The focus of the Interfast-2 trial was decided specifically on people with insulin treated T2DM and a total daily insulin dose of ≥0.3 IU of insulin per kilogram of body weight.

Meta-analyses confirmed, IF can have a similar HbA1c reduction in T2DM comparison with classic continuous calorie restriction, but weight loss might favor the IF approach in this specific patient cohort.[67,68] Participants of the Interfast-2 study often noted they found intermittent fasting to be easier to follow compared to other diet modifications. IF could even

provide benefits beyond weight loss including ketone production, beta oxidation and autophagy.[218]

IF has shown to reduce the calorie intake even when ad libidum eating was available.[90,101] Participants of the Interfast-2 study often described less hunger after a fasting day making them less likely to eat back calories omitted on the fasting days. The risk of hypoglycemia was managed by using a CGM device as seen in Ramadan studies. Classic Islamic Ramadan fasting is limited to the hours of sun during the day allowing unlimited food and drink intake during the night. Liquids are normally not allowed during the fasting times in Ramadan which is a stark contrast to the Interfast-2 study where drinking of non-caloric and sugar free liquids such as tea and coffee without sugar or milk and water was encouraged during fasting times. While Ramadan could be considered an TRF eating pattern the switch from eating during the day to the night shows a significant difference to standard intermittent fasting.[207] Regardless of the time of food consumption the education of patients with T2DM and insulin therapy concerning hypoglycemia symptoms and responses is of paramount importance for the safety of patients using insulin therapy.

The Endobarrier™ study revealed a clear necessity to meticulously document the insulin dose due to the direct effects of insulin on the HbA1c. The interrelationship of HbA1c, insulin dose and body weight makes the interpretation of study results challenging. Hence, we introduced a co-primary endpoint in Interfast-2 depicting all three aspects at the same time. Furthermore, documentation of meals and the use of the CGM device required a high amount of adherence from the participants. CGM was not blinded for neither the fasting group nor control group. Therefore, participants were able to observe the effects of their diet on their blood glucose levels which could have affected the study results.

While alternate day fasting can be performed with zero calories on fasting days, we allowed participants of the fasting group to consume up to 25% of their caloric intake as breakfast and/or lunch to reduce the risk of hypoglycemia and to increase adherence. This provided not only an intervention similar to ADF and 5:2 fasting but by limiting the food to two meals or less during the day participants achieved the mandated 18 hours of fasting. With the data we have collected we now would feel comfortable omitting the 25% of calories on the fasting days and explore ADF in people with T2DM but participants did not receive financial compensation for the fasting, therefore adherence was already strained. Participants were

motivated to join the study to lose weight, improve HbA1c levels and try intermittent fasting for the first time with clinical supervision.

The study was conducted between 2019 and 2022 during the Covid-19 pandemic. T2DM was identified as risk factor for severe covid-19 infections and impeded recruitment for the study.[219] Exceptional care was taken to minimize the risk during patient visits. All in-person interactions were conducted according to the hospitals rigorous safety measures. Patients were advised to contact research staff in case of a possible covid-19 infection. None of the 46 patients in the study contracted a covid-19 infection during the 3 years of the conduction of the study.

In summary, the Interfast-2 study demonstrated, three non-consecutive days of IF to be a safe and feasible intervention for 12 weeks in people with insulin treated T2DM. It reduced HbA1c, body weight and insulin dose while no changes in physical activity levels and resting metabolic rate were recorded.

Biomarkers and future perspectives and research questions

The Endobarrier™ and Interfast-2 study provided many insights into the non-pharmacological effects of delayed absorption and intermittent fasting on people with T2DM. At the same time, they revealed further research questions on the use of laboratory measurements as potential biomarkers such as insulin and leptin and the role of the microbiome on glucose metabolism.

Insulin and insulin resistance

Hyperinsulinemia during fasting as measured by baseline c-peptide and during the oGTTs was seen in both the fasting and control groups. A publication by Rodin et al. in 1985 suggested that hyperinsulinemia indifferently of blood glucose levels is associated with an increase in hunger, enjoyment of sweeter taste and higher food intake. The elevated insulin levels seen in obesity could create a feedback loop with hyperinsulinemia leading to hyperphagia, which - unless compensated by burning of calories - would in turn lead to

weight gain thus creating a vicious cycle of insulin and weight escalation. With insulin rising in the anticipation of food, acute hyperinsulinemia can be triggered in people seeing, smelling or simply thinking about food and could contribute to consumption and preference for sweeter food resulting in possible weight gain.[11]

Anticipatory cephalic hormonal responses (including insulin and other hormones) were described by Skvortsova et al. in 2021, inducing hunger by signaling via the vagus nerve and allowing for the consumption of larger meals.[220] Prof Martin Heni recipient of the 2022 Minkowski Price showed the brain to be an insulin-sensitive organ and provided evidence of brain insulin resistance modulating weight, metabolism and food intake.[221] Higher brain insulin sensitivity is also associated with long term weight loss and changes in body fat distribution.[222,223] High brain insulin sensitivity could influence the effect of lifestyle intervention.[224,225]

Rodin et al. hypothesized that postprandial serum insulin levels would be highest after 2 to 3 hours, making people hungry again.[11] This hypothesis was reconfirmed in 2021 by Wyatt et al. providing evidence that in healthy individuals the postprandial glycaemic dips 2-3 hours after eating can predict appetite and energy intake. The data revealed increased hunger reported by the participants, less time until the next meal and a greater intake in energy over 24h following a larger dip in glucose levels.[226] Furthermore, priming with glucose rich food enhanced the intake of food even in a satiated state and was able to induce hunger regardless of food consumptoon.[227] Therefore in people experiencing IR the satiety might be better preserved in low-carbohydrate diets compared to low-fat diets.[228]

The insulin response to glucose was shown to be stronger compared to fructose leading Rodin et al. in 1985 to assume people eating glucose would get hungrier and eat more compared to fructose. 24 hours after consuming a preload of fructose or glucose, participants who had eaten glucose, consumed almost 500 kcal more, when presented with ad libidum food compared to the fructose group. Although fructose and glucose have identical caloric content, these findings suggest, the type of food we eat affects our hunger and the number of calories we consume in the near future possibly due to the effects of insulin.

The conclusions stated, many factors including environment, behavior and biological factors influence overeating. With some people producing more insulin due to genetic predispositions or after overeating for a long time, the question remained, how people could

stop eating considering food induced hyperinsulinemia. Rodin et al. assumed a satiety hormone could counteract the eating stimulating effects of insulin but at the time of publication of their research no such hormone was known.[11] In 1994, nearly 10 years after Rodin's publication a satiety inducing hormone "leptin" was discovered.[229]

Leptin and leptin resistance

In 1994, leptin - a hormone secreted predominantly from adipocytes in white adipose tissue - was discovered.[230] In 1998, the administration of leptin as a pharmacological treatment was used to help the first obese child with leptin-deficiency reach normal weight. Whereas children with T1DM "starve" even with sufficient calorie intake due to the lack of insulin and subsequently develop cachexia, children born with primary leptin deficiency experience weight gain and uncontrolled "starvation"-like hunger despite their obesity. Leptin application is used in patients with leptin deficiency and lipodystrophy.[33]

Leptin rises slowly with increasing fat mass. Leptin levels could serve as a non-stigmatizing biomarker for body composition. Obese patients with high leptin levels become insensitive to the appetite suppressing effects of leptin.[229] During fasting, leptin levels drop and signal the need to replenish sustenance to the brain through leptin-sensitive neurons. With the restoration of energy intake, leptin levels rise again. Leptin is serving as a "lipostatic" signal. However, endogenous hyperleptinemia is associated with the loss of ability to suppress appetite.[33,231]

Of note, IF could be used as a non-pharmacological treatment for obesity modulating both hypothalamic leptin and insulin signalling.[232,233] While leptin levels decrease with weight loss, fasting can change serum leptin levels in the first 24 hours prior to any significant changes in body fat mass. A reduction in serum levels of leptin have been reported to be preventable by supplementing glucose, thus suggesting an influence of serum glucose and serum insulin on serum leptin.[231]

When serum insulin levels are kept stable, leptin levels also remain unchanged.[234] While leptin levels reflect body fat, a disproportionate reduction in leptin levels during fasting has been observed. Even after 72 hours of fasting, leptin levels will return to baseline levels

withing 12 hours, as soon as food is consumed.[235] Leptin follows a circadian rhythm and rises during the night minimizing the desire for food consumption while sleeping. These fluctuations resemble the daily patterns of other hormones such as prolactin or thyroid-stimulating hormone.[236]

Insulin stimulates leptin secretion through the adipo-insular axis.[230,237] Higher leptin levels are associated with larger intra-abdominal fat accumulation whereas higher levels of adiponectin were associated with a lower amount of intra-abdominal fat.[238] A reduction in plasma leptin can improve leptin resistance.[239] Leptin levels are also associated with hypertension, independent of BMI.[240] Serum leptin concentrations can be reduced with fasting whereas adiponectin levels appear to be independent on dietary restrictions when ADF was performed.[241]

The adipo-insular axis might represent an evolutionary adaptation to survive times of food scarcity. Grizzly bears (Ursus arctos horribilis) have an especially fascinating metabolism concerning insulin, fat mass and leptin. Despite enormous fluctuations of more than 50% of body mass and rapid fat accumulation, bears do not experience the harmful effects of obesity seen in humans. While bears are insulin resistant in winter, insulin sensitivity rises again after hibernation. The increase in body weight is precedented by an almost insatiable appetite, which can be dampened by leptin-treatment in the bears showing low leptin levels in spring. IR during hibernation might be necessary to diminish the anti-lipolytic effects of insulin, which prevents the clearance of blood glucose mediated by insulin and ensures euglycemia during hibernation.[41] Another important factor affecting hibernation and fasting are changes in the gut microbiome, with an important interaction to the nitrogen metabolism.[242,243]

Microbiome

The microbiota in the gut of bears differs in summer and winter. The diet of a brown bear changes with the seasons and during the 4-6 months of hibernation. During summer, the microbiota promotes fat accumulation, while the winter microbiota is more homogenous. Changes in the bear microbiota have been shown to be associated with the caloric restriction during hibernation and nutritional intake with seasonal variety.[242]

Though the microbiome environmental factors can influence insulin sensitivity in humans, reduced diversity in the gut microbiome is seen in obesity and T2DM. Modulation of the microbiome could therefore be a novel way to treat IR.[244] The microbiome has the ability to expand nutritional resources, produce vitamins and modulate immunology and inflammation.[245] Diet can influence temporary changes and shifts in the microbiome over 24 hours and therefore be used to affect intestinal microbiota.[246,247]

The microbiome is part of the para- and endocrine signaling of the gut-brain-axis.[248] The nervus vagus has been proposed as a major indicator of the gut-brain-axis and associations between the gut-microbiome and the influence on mood disorders could potentially lead options for nutritional therapy and "psychobiotics" in the treatment of depression.[249–251] For people with insulin treated T2DM a significant association between depression and insulin therapy has been shown in a meta-analysis in 2018 highlighting the need for interdisciplinary treatment.[252]

Kawano *et al.* proposed a model of immunological protection against MetS based on the modulation of the microbiome which can be negatively influenced by dietary sugar highlighting the connection between metabolic disease, microbial balance and commensal immune cells.[253] Further research in humans is needed to investigate the effects of fasting on the microbiome.[254,255]

In the current study, microbiome samples were taken during the Interfast-2 study at baseline, after 4 weeks and after 12 weeks and will be analyzed to explore the effect of intermittent fasting on the microbiome in people with insulin treated T2DM. Questionnaires on the psychosomatic competence of patients will be evaluated and data collected from the CGM devices will be analyzed in further detail to gain insight into the glucose fluctuations during fasting and eating days. These results may open up new insights into the complex mechanisms of fasting in people with T2DM.

Thus, the growing knowledge on the effects of the modulation of food consumption whether through delayed absorption or intermittent fasting on insulin, leptin as well as the effects on the microbiome might expand our understanding of metabolism and help us succeed in providing personalized precision medicine to help patients achieve their treatment goals.

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List of publications produced in the course of the PhD, including details on the contributions

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Appendix

This section contains the following papers:

- Obermayer, A., Tripolt, N. J., Aziz, F., Högenauer, C., Aberer, F., Schreiber, F., Eherer, A., Sourij, C., Stadlbauer, V., Svehlikova, E., Brunner, M., Goswami, N., Kojzar, H., Pferschy, P. N., Pieber, T. R., & Sourij, H. (2021). EndoBarrier™ Implantation Rapidly Improves Insulin Sensitivity in Obese Individuals with Type 2 Diabetes Mellitus. Biomolecules, 11(4), 574. https://doi.org/10.3390/biom11040574

- Obermayer, A., Tripolt, N. J., Pferschy, P. N., Kojzar, H., Jacan, A., Schauer, M., Aziz, F., Oulhaj, A., Aberer, F., Sourij, C., Obermayer-Pietsch, B., Stadlbauer, V., & Sourij, H. (2022). INTERmittent FASTing in people with insulin-treated type 2 diabetes mellitus - the INTERFAST-2 study protocol. Diabetic medicine: a journal of the British Diabetic Association, e14813. Advance online publication. https://doi.org/10.1111/dme.14813

- Obermayer, A., Tripolt, N. J., Pferschy, P. N., Kojzar, H., Aziz, F., Müller, A., Schauer, M., Oulhaj, A., Aberer, F., Sourij, C., Habisch, H., Madl, T., Pieber, T., Obermayer-Pietsch, B., Stadlbauer, V., & Sourij, H. (2022). Efficacy and Safety of Intermittent Fasting in People With Insulin-Treated Type 2 Diabetes (INTERFAST-2)-A Randomized Controlled Trial. Diabetes care, dc221622. Advance online publication. https://doi.org/10.2337/dc22-1622

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Article

EndoBarrier™ Implantation Rapidly Improves Insulin Sensitivity in Obese Individuals with Type 2 Diabetes Mellitus

Anna Obermayer [1], Norbert J. Tripolt [1], Faisal Aziz [1], Christoph Högenauer [2], Felix Aberer [1], Florian Schreiber [2], Andreas Eherer [2], Caren Sourij [3], Vanessa Stadlbauer [2,4], Eva Svehlikova [1,5], Martina Brunner [1,5], Nandu Goswami [6], Harald Kojzar [1], Peter N. Pferschy [1,4], Thomas R. Pieber [1,4] and Harald Sourij [1,4,*]

[1] Division of Endocrinology and Diabetology, Medical University of Graz, 8010 Graz, Austria; a.obermayer@medunigraz.at (A.O.); norbert.tripolt@medunigraz.at (N.J.T.); faisal.aziz@stud.medunigraz.at (F.A.); felix.aberer@medunigraz.at (F.A.); eva.svehlikova@medunigraz.at (E.S.); martina.brunner@medunigraz.at (M.B.); harald.kojzar@medunigraz.at (H.K.); peter.pferschy@medunigraz.at (P.N.P.); thomas.pieber@medunigraz.at (T.R.P.)

[2] Division of Gastroenterology and Hepatology, Medical University of Graz, 8010 Graz, Austria; christoph.hoegenauer@medunigraz.at (C.H.); florian.schreiber@medunigraz.at (F.S.); andreas.eherer@medunigraz.at (A.E.); Vanessa.stadlbauer@medunigraz.at (V.S.)

[3] Department of Internal Medicine, Division of Cardiology, Medical University of Graz, 8010 Graz, Austria; caren.sourij@medunigraz.at

[4] CBmed—Center for Biomarker Research in Medicine, 8010 Graz, Austria

[5] CRC—Clinical Research Center, Medical University of Graz, 8010 Graz, Austria

[6] Otto Loewi Research Centre, Physiology Division, Medical University of Graz, 8010 Graz, Austria; nandu.goswami@medunigraz.at

* Correspondence: ha.sourij@medunigraz.at

Abstract: The EndoBarrier™ medical device is a duodenal-jejunal bypass liner designed to mimic the effects of gastric bypass surgery to induce weight loss and glycaemic improvement. In this study, 10 participants with type 2 diabetes mellitus (T2DM), a mean body mass index (BMI) of 43.3 ± 5.0 (kg/m^2) and a mean glycated haemoglobin A1c (HbA1c) of 60.6 ± 8.6 mmol/mol were examined at baseline (before implantation of EndoBarrier™), 4 weeks after implantation, at 36 weeks (right before explantation) and 24 weeks after the removal of the device to explore the short and long-term effects on glucose metabolism. Besides a significant reduction in body weight and fat mass, EndoBarrier™ treatment significantly improved insulin sensitivity during Botnia clamp investigations after four weeks of implantation. The beneficial effects decreased over time but remained significant 24 weeks after removal of the device.

Keywords: EndoBarrier™; obesity; duodenal-jejunal bypass liner; type 2 diabetes mellitus; Botnia clamp

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1. Introduction

The global burden of overweight and obesity has become a major health challenge over recent decades. Amongst a number of detrimental health consequences, obesity in particular, significantly increases the risk for development of type 2 diabetes mellitus (T2DM). By 2045, an estimated 700 million people will suffer from overt diabetes mellitus and a further 374 million people will live with impaired glucose tolerance [1]. T2DM is associated with a reduction in life expectancy of up to 7 years in men and women [2] and has a significant negative impact on global health care budgets.

Very low calorie diets improve glucose control and can even lead to diabetes remission in people with T2DM [3]. Besides medication therapy, bariatric surgery is another efficient option to improve glucose metabolism in morbidly obese people. Moreover, bariatric

interventions in obese individuals with T2DM reduce cardiovascular events as well as overall mortality [4]. However, bariatric surgery represents a largely irreversible anatomical change of the gastrointestinal tract, that bears a certain risk for surgical complications and can lead to chronic adverse events such as malabsorption syndromes [5].

The EndoBarrier™, a 60 cm long fluoropolymer sleeve, developed by GI Dynamics (Lexington, MA, USA) is intended to be used as a device to induce weight loss. It can be inserted endoscopically and the nitinol anchor secures the Endobarrier™ in the duodenal bulb. The device unfolds through the duodenum and the proximal part of the jejunum. It delays the absorption of nutrients by preventing the contact of chyme with the intestinal mucosa of the duodenum [6]. Due to the manufacturer's recommendations, it is designed to remain in situ for 12 months. The main effects include significant weight loss and an improvement in glucose control [7]. However, the mechanisms of the improvement in glucose metabolism have not yet been thoroughly studied.

The aim of this study was to assess glycaemic effects of the EndoBarrier™ in obese participants with T2DM by performing Botnia clamp (intravenous glucose tolerance test followed by hyperinsulinaemic euglycemic clamp) and a mixed meal tolerance test before implantation, 4 weeks after the implantation of the EndoBarrier™, at 36 weeks when it was removed, as well as 24 weeks after the removal of the device.

2. Materials and Methods

2.1. Study Design

This was an open, prospective, single-center, single-arm pilot study, serving as a basis for an adequately powered trial with the Endobarrier™ in people with diabetes and/or non-alcoholic fatty liver disease in the future. Ten obese participants with a BMI between 30.0 and 49.0 kg/m^2 and established T2DM with suboptimal glycaemic control (HbA1c $\geq$ 6.5% (48 mmol/mol)) were enrolled in this study. Inclusion, exclusion criteria and study procedures are described in detail in the study protocol [8]. The study was approved by the local ethical committee of the Medical University of Graz (EC number 26–280 ex 13/14) and the participants were recruited from the outpatient clinic at the Division of Endocrinology and Diabetology at the Department of Internal Medicine, Medical University Graz, Austria.

2.2. Endobarrier™ Device

The device was implanted and explanted under general anesthesia by trained gastroenterologists. Participants were advised to take omeprazole 40 mg twice daily starting 3 days before the implantation until 2 weeks after explantation of the EndoBarrier™ device (GI Dynamics, Lexington, MA, USA). Participants were instructed to follow a liquid diet for 2 weeks after the implantation and change slowly to a normal diet with no macronutrient restrictions over the following 10 days. Biopsies of the upper gastro-intestinal tract were taken prior to implantation and after explantation of the EndoBarrier™.

2.3. Examinations

For trial purposes, participants underwent physical examinations, blood sampling, Botnia clamps, mixed meal tolerance tests, lactulose/mannitol tests and dual-energy X-ray absorptiometry (DXA; GE Healthcare, Waukesha, WI, USA) measurements including body composition before the implantation, 4 and 36 weeks after the implantation, as well as 24 weeks after the explantation. Body composition was assessed using DXA to determine lean mass, fat mass, bone mineral content and total body composition. A self-administered, semi-quantitative FFQ (Food Frequency Questionnaire) was used at every visit to assess usual food consumption.

Changes in cardiovascular risk factors were calculated by the UKPDS (UK Prospective Diabetes Study) risk engine.

Routine parameters were determined using a Cobas analyzer (Roche Diagnostics, Mannheim, Germany).

Blood samples for glucagon like peptide 1 (GLP-1) were collected in pre-chilled tubes containing EDTA + aprotinin. After centrifugation, plasma samples were frozen at − 80 °C until analysis. For the determination of human glucagon like peptide 1 (GLP-1) a commercially available ELISA kit was used (active GLP-1 ELISA (GLP-1 (7–36) and (9–36), ALPCO Diagnostics, Salem, NH, USA)). The test was performed according the instructions provided by the distributor [8].

2.4. Statistical Analysis

As we performed a pilot trial involving Botnia clamps, we did not perform a formal sample size estimation. However, post hoc power analysis showed, that our study had more than 90% power to demonstrate the observed difference.

All data are presented as mean ± standard deviation. Data were checked for distribution normality by the Shapiro–Wilk test. The Friedman test was applied to compare parameters over time and the Durbin–Conover test with Bonferroni corrections was applied for post hoc multiple pairwise comparisons of parameters. The weight adjusted glucose infusion rate (GIR) was calculated with a multilevel non-linear mixed model with post hoc multiple comparison and Bonferroni correction. All statistical analyses were performed using SPSS 22 software for Windows (SPSS Inc., Chicago, IL, USA, 2018). A p-value of <0.05 was considered statistically significant.

3. Results

All of the 10 enrolled participants (6 female) completed the study. The mean age was 48 ± 9 years, duration of T2DM was 7 ± 6 years.

Mean body weight decreased from 121.2 ± 18.5 kg to 116.3 ± 18.2 kg (p = 0.006) after 4 weeks of EndoBarrier™ therapy and to 115.1 ± 21.4 kg (p = 0.075 vs. baseline) until explantation of the device after 36 weeks. However, there was a slight increased to 117.2 ± 20.8 kg (p = 0.117 vs. baseline) 24 weeks after explantation.

Baseline fat mass measured by DXA was 58.1 ± 12.0 kg and decreased to 55.0 ± 12.5 kg after 4 weeks (p = 0.006) and to 53.6 ± 15.2 kg after 36 weeks (p = 0.021) but increased again 24 weeks after explantation to 54.3 ± 15.2 kg and was not significantly different from baseline (p = 0.141). No changes in gut permeability were observed. Detailed results are presented in Table 1.

Table 1. Comparison of outcome parameters from baseline to 4 and 36 weeks after implantation and 24 weeks after removal of the EndoBarrier™; data are presented as mean ± SD.

	Baseline	4 Weeks	36 Weeks after Implantation	24 Weeks after Explantation	*p*-Value1	*p*-Value2	*p*-Value3
Body weight (kg)	121.2 ± 18.5	116.3 ± 18	115.1 ± 21.4	117.2 ± 20.8	0.006	0.075	0.117
Body Mass Index (kg/m^2)	43.3 ± 5.0	41.2 ± 4.8	40.6 ± 5.8	41.4 ± 6.0	0.006	0.075	0.117
Fat mass (kg)	58.1 ± 12	55.0 ± 12.5	53.6 ± 15.2	54.3 ± 15.2	0.021	0.021	0.141
C-peptide/Glucose Ratio	0.020 ± 0.015	0.019 ± 0.009	0.022 ± 0.012	0.018 ± 0.009	0.420	1.000	1.000
HbA1c (mmol/mol)	60.6 ± 8.6	57.4 ± 8.6	55.1 ± 11.7	66.1 ± 21.2	1.000	0.414	1.000
Glucose (mg/dL) AUC	440 ± 61	402 ± 107	458 ± 110	580 ± 170	0.819	1.000	0.576
C-peptide (ng/mL) AUC	11.6 ± 6.7	10.7 ± 6.3	12.5 ± 7.8	12.0 ± 3.8	1.000	1.000	0.465
Early insulin response	−0.02 ± 4.91	−1.00 ± 11.72	9.48 ± 23.50	0.63 ± 0.49	0.741	1.000	1.000
Fasting glucose (MMTT)	153 ± 28	160 ± 82	155 ± 51	170 ± 42	1.000	1.000	0.654
QUICKI	0.267 ± 0.026	0.283 ± 0.029	0.277 ± 0.038	0.290 ± 0.022	0.225	0.339	0.279
Lactulose/Mannitol Ratio	0.011 ± 0.010	0.011 ± 0.10	0.039 ± 0.072	0.005 ± 0.008	1.000	0.114	0.214
ALT	33 ± 17	32 ± 13	28 ± 10	31 ± 9	1.000	0.375	1.000
AST	26 ± 7	31 ± 14	25 ± 6	35 ± 11	0.492	1.000	0.132
GGT	49 ± 40	53 ± 60	35 ± 26	65 ± 83	1.000	0.120	1.000
UKPDS CHD	14.1 ± 17.9	14.3 ± 18.7	16.3 ± 20.4	13.6 ± 10.6	1.000	1.000	1.000
UKPDS Fatal CHD	8.4 ± 11.6	8.6 ± 12.4	9.3 ± 13.1	8.6 ± 8.6	1.000	1.000	1.000
GLP-1 (pmol/L)	27.9 ± 13.3	24.2 ± 9.2	18.2 ± 11.4	36.1 ± 56.8	1.000	0.081	1.000

AUC: Area under the Curve; ALT: Alanine transaminase; AST: Aspartate aminotransferase; GGT: Gamma-glutamyl transferase; p-value1: Comparison Baseline vs. 4 Weeks; p-value2: Comparison Baseline vs. 36 weeks; p-value3: Comparison Baseline vs. 24 weeks after explantation. UKPDS CHD: UK Prospective Diabetes Study coronary heart disease, GLP-1: Glucagon-like peptide-1, MMTT: mixed meal tolerance test, GIR: glucose infusion rate.

3.1. GIR

The mean glucose infusion rate (GIR) during Botnia clamps adjusted for weight increased from 0.50 ± 0.60 mg/kg/min at baseline to 0.86 ± 0.72 mg/kg/min after 4 weeks (p = 0.038) indicating a higher insulin sensitivity. A total of 36 weeks after insertion of the EndoBarrier™ device, the mean GIR remained significantly higher than at baseline (0.97 ± 1.36 mg/kg/min, p = 0.001) and remained significantly increased 24 weeks after explantation with 0.95 ± 1.34 mg/kg/min (p = 0.001) (Figure 1).

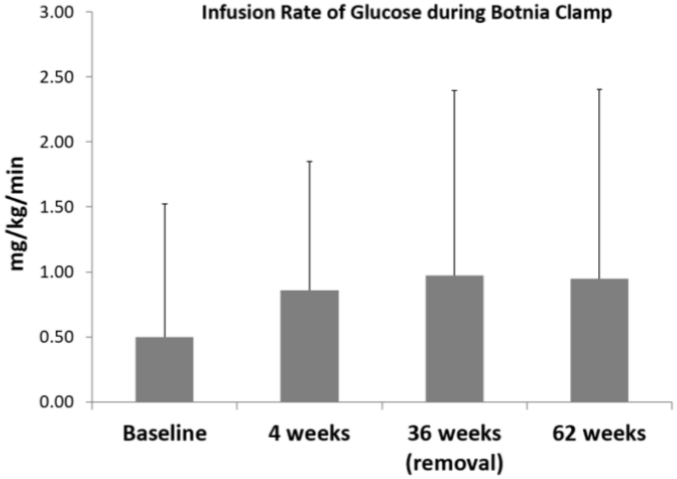

Figure 1. Infusion rate of glucose during 4 Botnia clamp investigations; data are mean ± SD, Baseline vs. 4 weeks p = 0.038; Baseline vs. 36 weeks; p = 0.001; Baseline vs. 24 weeks after explantation p = 0.001.

3.2. Diabetes Medication

36 weeks after the insertion of the device, glucose lowering treatment was reduced in five participants, one participant remained on the same treatment and four had an intensification as compared to the baseline.

3.3. FFQ

The FFQ showed a decrease in kcal (kilocalorie) consumed after the implantation of the EndoBarrier™ with a significant reduction (p = 0.013) after 36 weeks. While fat and carbohydrate intake reduced numerically over the time period, according to the FFQ, both did not meet the statistical significance level. However, protein consumption was significantly decreased at 36 weeks compared to the baseline (p = 0.009) (Figure 2).

No changes in plasma protein levels were observed throughout the study (7.25 (IQR 6.85–7.65) mg/dL at baseline versus 7.1 (IQR 6.9–7.3) mg/dL at 9 months, p = 0.763).

3.4. Biopsies

Biopsies were performed before implantation and at explantation of the EndoBarrier™ to investigate atrophic effects of the device. No signs of villous atrophy were observed in the 10 study participants.

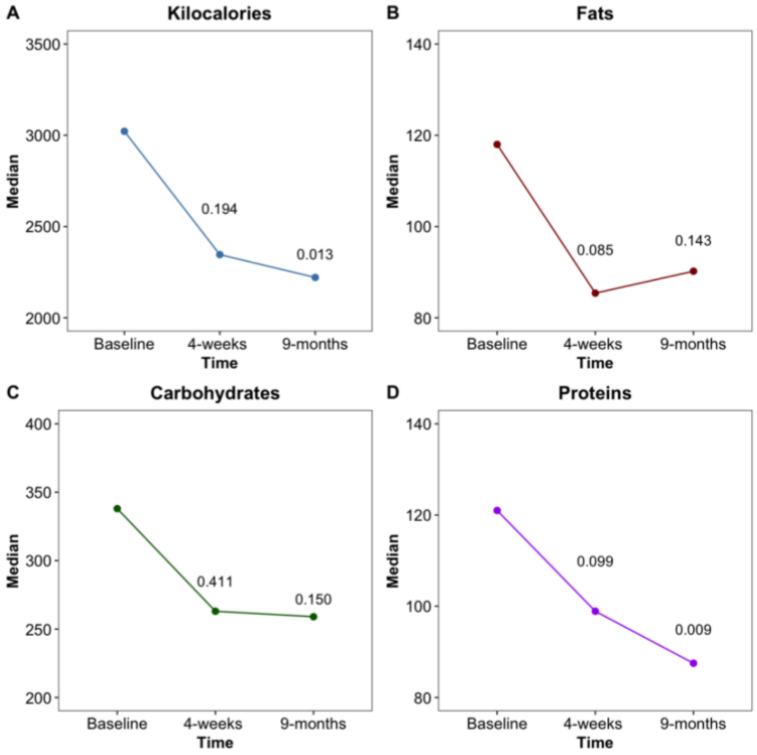

Figure 2. Changes of total kcal (**A**), as well as fat (**B**), carbohydrate (**C**) and protein (**D**) intake in grams at baseline, 4 weeks and 36 weeks after EndoBarrier™ implantation.

3.5. Adverse Events

During the study period, four serious adverse events in four participants were reported. These resulted from one case of dehydration, one case of duodenal ulcer which was treated with sucralfate, in additionally to a high dose proton pump inhibitor, one case of prolonged nausea and vomiting requiring intravenous fluid replacement and one case of haemorrhoid bleeding. Participants experiencing an adverse event did not lose more weight as compared to those without adverse events. No premature removal of the EndoBarrier™ was required.

4. Discussion and Conclusions

We observed significant changes in body fat, accompanied by rapid improvements in insulin sensitivity assessed by clamp technique in obese people with T2DM treated with the EndoBarrier™ device.

While a number of studies [7,9,10] have reported significant improvements in glucose parameters observed with the duodenal-jejunal bypass liner, reaching a maximum at 36 weeks after implantation, we were able to demonstrate a significant and sustained effect of the device on insulin sensitivity already at 4 weeks after the implantation of the device. HbA1c was reduced but this did not reach statistical significance. This could be due to adjustments in the glucose lowering medication made due to improvements in glucose levels to prevent hypoglycemia.

A significant reduction in total calories as well as a significant reduction in proteins was observed at 36 weeks after implantation. Plasma protein levels were checked at every visit and no significant change in plasma proteins was observed in our study.

Our data suggest, that the EndoBarrier™ not only reduces the contact of the chyme with the intestinal mucosa, but it also leads to reduced calorie intake. This is most likely due to the bloating or the nausea that occurs if people continue eating the usual amount of food while having the device implanted.

While we observed a partial regain in body weight and increase in HbA1c 24 weeks after the explantation of the EndoBarrier™ device, which is in line with the recent findings of Deutsch et al. [11], insulin sensitivity remained sustainably improved even 24 weeks after the explanation of the device as compared to baseline.

Previous research showed, that the Endobarrier™ device was more effective than calorie restriction only [12], however, was less effective than bariatric surgery, an intervention that can lead to a weight reduction of more than 30% [13]. The Endobarrier™ can be placed endoscopically and provides a minimally invasive, reversible option which does not change the anatomy of the digestive tract permanently as is the case with bariatric surgery. It can be used for high-risk patients as a pre-bariatric surgical intervention to lose weight before the surgery [10]. However, the Endobarrier™ device causes gastrointestinal side effects, which occurred in 40% of our participants. Similar figures have been observed in previous trials [14]. No device had to be removed prematurely in our study.

The lactulose/mannitol ratio as a measure of gut permeability remained unchanged by the implantation of the device.

While in our study no cases of hepatic abscesses occurred, within the ENDO trial (Safety and Efficacy of EndoBarrier in Subjects With Type 2 Diabetes Who Are Obese, NCT01728116), the number of abscesses in people receiving the Endobarrier™ device was higher than expected (3.5%), leading to the FDA halting the trial in March 2015 [15]. The remaining uncertainty around the causes for these adverse events led to postponement of our previously planned larger, multicenter study in people with diabetes and/or non-alcoholic fatty liver disease after the pilot phase.

We believe that the Endobarrier™ device can provide a potential temporary, reversible and minimally invasive option in people with diabetes and severe insulin resistance as well as people with non-alcoholic fatty liver disease who need to lose weight. As the device seems to be less effective than bariatric surgery, it might represent an important intermediate step between conventional medical and surgical therapy.

In conclusion, in this study we observed a rapid improvement in insulin sensitivity four weeks after the implantation of the EndoBarrier™ device assessed by Botnia clamps, an effect that was sustained and still persistent after explantation of the device.

Author Contributions: Conceptualization, H.S. and N.J.T.; methodology, E.S. and M.B.; validation, V.S.; formal analysis, A.O. and F.A. (Faisal Aziz); investigation, C.H., F.S. and A.E.; resources, F.A. (Felix Aberer), H.K. and P.N.P.; writing—original draft preparation, A.O.; writing—review and editing, N.G. and H.S.; visualization, A.O. and F.A. (Faisal Aziz); supervision, T.R.P. and H.S.; project administration, C.S.; funding acquisition, H.S. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was approved by the local ethical committee of the Medical University of Graz (EC number 26–280 ex 13/14) and conducted according to the principles of the Declaration of Helsinki (2013) and good clinical practice (GCP) guidelines. All participants gave written informed consent.

Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The dataset generated and analysed in this study is not publicably available but may be obtained from the corresponding author upon a reasonable request.

Conflicts of Interest: No conflicts related to this study are reported.

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DIABETIC Medicine

STUDY PROTOCOLS

INTERmittent FASTing in people with insulin-treated type 2 diabetes mellitus – the INTERFAST-2 study protocol

Anna Obermayer[1] | **Norbert J. Tripolt**[1] | **Peter N. Pferschy**[1,2] | **Harald Kojzar**[1] | **Angela Jacan**[2] | **Markus Schauer**[1] | **Faisal Aziz**[1] | **Abderrahim Oulhaj**[3,4] | **Felix Aberer**[1] | **Caren Sourij**[5] | **Barbara Obermayer-Pietsch**[6] | **Vanessa Stadlbauer**[2,7] | **Harald Sourij**[1]

[1]Interdisciplinary Metabolic Medicine Trials Unit, Division of Endocrinology and Diabetology, Medical University of Graz, Graz, Austria

[2]CBmed – Center for Biomarker Research in Medicine, Graz, Austria

[3]Department of Epidemiology and Public Health, College of Medicine and Health Sciences, Khalifa University, Abu Dhabi, UAE

[4]Institute of Public Health, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, UAE

[5]Division of Cardiology, Medical University of Graz, Graz, Austria

[6]Endocrinology Lab Platform, Division of Endocrinology and Diabetology, Medical University of Graz, Graz, Austria

[7]Division of Gastroenterology and Hepatology, Medical University of Graz, Graz, Austria

Correspondence
Harald Sourij, Interdisciplinary Metabolic Medicine Trials Unit, Division of Endocrinology and Diabetology, Medical University of Graz, Graz, Austria.
E-mail: ha.sourij@medunigraz.at

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Abstract

Aim: Intermittent fasting, a dietary intervention of alternate eating and fasting, has gained popularity in people trying to lose weight. Intermittent fasting could provide an alternative to classic caloric restriction in people with type 2 diabetes mellitus. The aim of the study is to determine the impact of a 12-week intermittent fasting regimen compared with usual care in people with type 2 diabetes mellitus receiving insulin therapy.

Methods: This open, single-centre, randomized controlled trial investigates participants with type 2 diabetes mellitus on insulin therapy and a glycated haemoglobin A1c (HbA1c) of ≥53 mmol/mol (≥7.0%) and a minimum insulin dose of 0.3 IU/kg body weight per day. Participants are randomized in a 1:1 ratio to either 12 weeks of intermittent fasting or the standard care group. All participants receive dietary counselling, continuous glucose monitoring, measurement of the resting metabolic rate, an oral glucose tolerance test, body composition measurement via dual-energy X-ray absorptiometry and stool samples for microbiome analyses at the beginning and at the end of the intervention. Two co-primary outcomes (analysed in hierarchical order) were chosen for the study: (i) the difference in the change of HbA1c from baseline to 12 weeks and (ii) the difference in the number of participants achieving a combined end point encompassing a body weight reduction of at least 2%, an insulin dose reduction of at least 10% and an absolute HbA1c reduction of at least 3 mmol/mol (0.3%) between the two groups.

KEYWORDS

insulin sensitivity, insulin therapy, Intermittent fasting, type 2 diabetes mellitus (T2DM), weight loss

1 | INTRODUCTION

Diabetes mellitus is characterized by chronic hyperglycaemia and impaired carbohydrate, lipid and protein metabolism caused by complete or partial insufficiency of insulin secretion and/or insulin action.[1]

Reduction of body weight has been a key strategy in mitigating the risk of developing diabetes.[2] Among obese and people with prediabetes, a weight loss of 5%–7% improves fasting glucose and insulin sensitivity.[3,4] The most commonly used approach for weight loss is daily caloric restriction. In recent years, intermittent fasting has become a prominent approach because it consists of only a restricted food intake on either specific days of the week or during a predefined period of the day.

A subtype of intermittent fasting, alternate day fasting comprises food restriction and normal food consumption on a day-to-day alternating basis, has been shown to reduce body weight by 8% in obese people after 3–24 weeks of treatment. Additionally, a reduction in triglycerides, low-density lipoprotein (LDL) cholesterol, systolic blood pressure and insulin resistance has been documented.[5–8]

When comparing caloric restriction to intermittent fasting dietary regimens in humans, both dietary regimens achieve reductions in visceral fat mass, fasting insulin and insulin resistance in overweight and obese people.[9]

So far, available human data of intermittent fasting focusing specifically on people with type 2 diabetes mellitus treated with insulin, looking into potential glycaemic, body weight and treatment improvements, are mainly limited to case series.[10,11]

Moreover, fasting and feeding schedule may interfere with the circadian rhythm and hence sleep length and quality[12] and in addition, poor sleep has been associated with insufficient glycaemic control.[13] The majority of the available data on intermittent fasting and sleep derives from Ramadan studies and were inconclusive with regard to sleep duration and sleepiness of people.[14]

People with type 2 diabetes mellitus have a distinct gut microbiome and disturbed diversity compared with healthy controls, shown to be able to affect and modulate lipogenesis, fat storage and metabolism[15–20] While dietary interventions are supposed to affect microbiome composition, research data on the effects of intermittent fasting on the microbiome in people with type 2 diabetes mellitus and insulin therapy is scarce and required.[21,22]

The aim of the current study is to investigate safety and efficacy of intermittent fasting in people with type 2 diabetes mellitus and insulin therapy compared with usual care.

What's new

- Blood sugar control and weight management can be challenging for people with type 2 diabetes mellitus.
- Intermittent fasting has become a popular option to manage weight, improve fasting glucose and insulin sensitivity.
- This study will investigate the feasibility and efficacy of intermittent fasting in people with insulin-treated type 2 diabetes mellitus.
- The information gained will enhance our understanding of fasting interventions, which can be used to improve clinical dietary recommendations.

2 | MATERIALS AND METHODS

2.1 | Study design

This was an open, single-centre, parallel, two-arm, randomized controlled trial to evaluate the effect of intermittent fasting on participants with insulin-treated type 2 diabetes mellitus over a period of 12 weeks. The investigations were performed at the Medical University of Graz, Division of Endocrinology and Diabetology, Interdisciplinary Metabolic Medicine Trials Unit. The protocol was approved by the Ethics Committee of the Medical University of Graz (EK 30-350 ex 17/18). All participants gave written consent prior to any study-related procedure. This study was conducted according to the principles of the Declaration of Helsinki, GCP-ICH and the protocol and the requirements of the concerned regulatory authorities.

2.2 | Inclusion and exclusion criteria

The study population consists of participants with type 2 diabetes mellitus, aged between 18 and 75 years (both inclusive) and a glycated haemoglobin A1c of ≥7.0% (≥53 mmol/mol). Main inclusion criteria: total daily insulin dose ≥0.3 units per kilogram of body weight and stable bodyweight in the preceding 3 months (change in weight <±3 kg). Participants who are willing to comply with study procedures, attend the study site, participate in the necessary protocols and comply with fasting protocols are included in the study. Main exclusion criteria contain active, known malignancies within the last year (excluding intraepithelial neoplasia of prostate,

gastrointestinal tract and basalioma), pregnancy or intention of becoming pregnant, breastfeeding, a history of any chronic disease process that could interfere with interpretation of study results, new hormonal supplementation or contraceptive hormonal medication changes in the last 2 months, type 1 diabetes mellitus or other forms of diabetes mellitus, an acute or chronic inflammatory disorder, alcohol abuse with more than 15 standard drinks per week, overnight shifts or intake of illicit substances.

2.3 | Sample size estimation

Sample size was estimated using the parameters of a previously published randomized controlled trial[23] that investigated the effects of intermittent fasting on glycaemic control in people with type 2 diabetes mellitus. We based our sample size estimation on the baseline HbA1c data of this study 66 ± 7 mmol/mol (8.2 ± 0.6%) and estimated that a sample size of 23 participants in each group will have a power of 85% to detect a clinically important mean difference of 6 ± 7 mmol/mol (0.5 ± 0.6%) in HbA1c between the groups at a two-sided alpha error of 5%. For the co-primary outcome a sample size of 20 participants in each group would provide a power of 0.80 to detect a difference in proportion of 40% between the intermittent fasting and the control group. This calculation assumes 10% of the control group will reach the co-primary end point (combined end point encompassing a body weight reduction of at least 2%, an insulin dose reduction of at least 10% and an absolute HbA1c reduction of at least 3 mmol/mol [0.3%]).

2.4 | Recruitment of participants

Participants with insulin-resistant type 2 diabetes mellitus are recruited from the outpatient clinics of the division of Endocrinology and Diabetology of the University Hospital Graz and form the Graz Diabetes Registry for Biomarker Research as well as via advertisement in local newspapers.

2.5 | Randomization

Participants are randomly assigned to one of the two groups, the intermittent fasting group or the control group in a 1:1 ratio. Randomization is performed using the randomizer tool 'Randomizer for Clinical Trials': http://www.randomizer.at/ (accessed 21 Oct 2019) from the Institute of Medical Informatics, Statistics and Documentation of the Medical University of Graz, Austria.

2.6 | Study hypothesis

We hypothesise that intermittent fasting over a period of 12 weeks can improve glycaemic control and body weight while being safe in people with type 2 diabetes and insulin therapy compared with usual care.

2.7 | Study outcomes

2.7.1 | Primary outcomes

- The impact of intermittent fasting on the glucose metabolism in participants with insulin-treated type 2 diabetes mellitus assessed by the difference in the change of HbA1c from baseline to 12 weeks.
- The difference in the number of participants achieving a combined end point encompassing a body weight reduction of at least 2%, an insulin dose reduction of at least 10% and an absolute HbA1c reduction of at least 3 mmol/mol (0.3%).

2.7.2 | Secondary outcomes

- Differences in the change
  a. in resting metabolic rate (RMR) from baseline to 12 weeks
  b. of glucose (area under the curve) within oral glucose tolerance test (oGTT) from baseline to 12 weeks
  c. in glycaemic pattern (continuous glucose monitoring [CGM]) from baseline to 12 weeks
  d. in body weight and body composition from baseline to 12 weeks
  e. in body composition (fat mass/lean body mass) from baseline to 12 weeks
  f. in mean 24 h blood pressure from baseline to 12 weeks
  g. in microbiome composition from baseline to 12 weeks
  h. in insulin dose from baseline to 4 weeks
  i. in insulin dose from baseline to 8 weeks
  j. in insulin dose from baseline to 12 weeks
  k. on quality of life from baseline to 12 weeks
  l. in the sleep quality and sleep disturbances from baseline to 12 weeks
  m. in the sleepiness from baseline to 12 weeks
  n. in physical activity from baseline to 12 weeks

- Differences in
  a. time spent in hypoglycaemia, hyperglycaemia and number of hypoglycaemic events with symptoms and blood glucose <3 mmol/l (<54 mg/dl)
  b. number of severe hypoglycaemic events from baseline to 12 weeks
  c. of body weight after 64 weeks
  d. of HbA1c after 64 weeks

2.7.3 | Justification of two primary outcomes

Lifestyle intervention trials in people with type 2 diabetes always face the problem that metabolic parameters, weight, and treatment can hardly be evaluated separately as they influence each other. For example, insulin dose decreases following weight loss will diminish the full impact of the weight loss on HbA1c reduction, while in contrast HbA1c reductions can simply be achieved by insulin dose increases without a contribution of the dietary intervention. Hence, as the parameters are interrelated, we believe that besides looking at the HbA1c change, it is also crucial to investigate a composite of weight, glycaemic control and insulin dose reduction. A weight loss of equal or more than 2% would indicate a weight change beyond the daily fluctuation.[24] An insulin dose reduction of 10% or more is a pragmatic estimate and indicates better insulin sensitivity[25] and an HbA1c reduction of at least 0.3% would be considered clinically meaningful.[26]

2.8 | Intervention and investigations

The flow chart of the intervention is shown in Figure 1.

46 participants (23 per group) with insulin-treated type 2 diabetes mellitus are randomly assigned to 'intermittent fasting' or 'control group', in a 1:1 ratio. Intermittent fasting comprises 3 days (Monday, Wednesday, Friday) of 75% caloric restriction (i.e. only 25% of the recommended calorie intake; this intake is only allowed as breakfast and/or lunch to maintain an 18 hours period of fasting) and 4 days (Tuesday, Thursday, Saturday and Sunday) of 0% caloric restriction (i.e. ad libitum calorie intake) in 1 week.

2.8.1 | Biobank sampling

Serum and plasma samples are pseudonymized and stored at −80°C in the Biobank of the Medical University of Graz for potential future analyses.

2.8.2 | Laboratory measurements

Blood samples are obtained at all on-site visits after a minimum of 8 h of overnight fasting (but should always take place after a normal feed day to ensure result comparability between groups) and processed by the local laboratory using standard methods for routine tests.

Insulin and c-peptide are measured by chemiluminescence on an ADVIA Centaur system (Siemens Healthcare Diagnostics). Leptin is measured using an ELISA kit (DRG Instruments GmbH). Routine parameters are determined using a cobas® analyzer (Roche Diagnostics).

2.8.3 | Peripheral blood mononuclear cell analysis

In a subgroup of up to 20 participants, 32 ml of peripheral blood is collected at baseline visit and visit 10 using

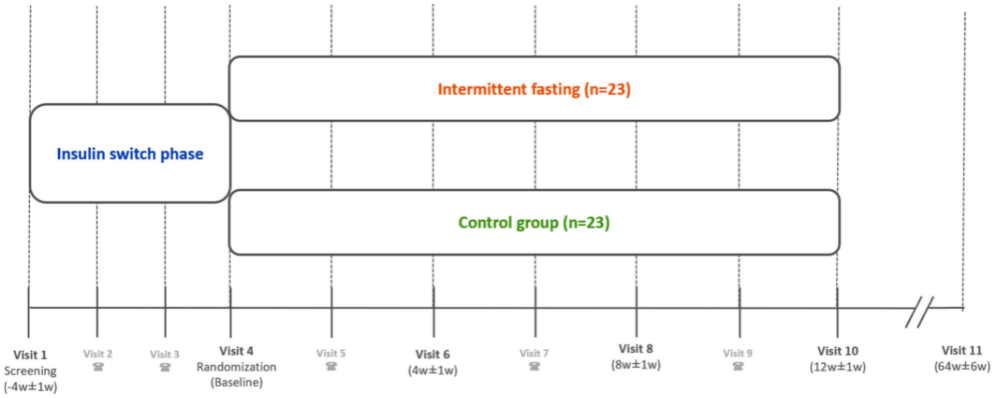

FIGURE 1 Flow chart of the intervention (an optional single follow-up after 1 year will be added)

BD Vacutainer CPT for the isolation of peripheral blood mononuclear cells. Peripheral blood mononuclear cells (PBMCs) will be further analysed using single-cell sequencing technique in collaboration with the Division of Cardiology at the Medical University of Graz, Austria.

2.8.4 | Oral glucose tolerance test

Participants are asked to postpone their insulin injection until the end of the oGTT. Insulin, C-peptide and glucose are measured before (−5), 15, 30, 60 and 120 min after ingestion of 75 g glucose (®Glucoral 75 citron, Germania Pharmazeutika). In addition, lipid parameters (total cholesterol, triglycerides, HDL, LDL, free fatty acids) and a routine safety lab are measured. The oGTT is performed after an overnight fast (apart from water) following an eating day (for the intermittent fasting group) and is evaluated by determining the area under the glucose curve.[27]

2.8.5 | Continuous glucose monitoring

Minimally invasive CGM measurement is performed using the Abbott FreeStyle Libre glucose monitoring device (Abbott Diabetes care). A sensor is introduced through the skin into the subcutaneous fat tissue. The upper arm is the main body location to applicate the sensors which are worn for 16 weeks (4 weeks insulin switch phase and 12 weeks intervention phase) by the participants. The Abbott sensor is factory calibrated and therefore does not require calibration by capillary prick blood. However, participants are instructed to scan the sensor with the corresponding recorder at least every 8 h. CGM data will be collected at on-site visits.

2.8.6 | Non-invasive 24h ambulatory blood pressure monitoring

Participants wear the blood pressure device for a 24-h period. During this time, the device is programmed to inflate and record blood pressure at pre-specified intervals (every 15 min during daytime hours and every 30 min during night-time hours), which provides approximately 50–75 blood pressure recordings during the 24-h period.

2.8.7 | Bone densitometry and body composition

Bone density scanning by dual-energy X-ray absorptiometry (DXA) is assessed with the GE Lunar iDEXA (GE Healthcare), estimating simultaneously body composition, for example, fat percentage according to the departmental Standard Operating Procedure. Body regions are defined using standard anatomical partitions. Scan areas are analysed to determine lean mass, fat mass, bone mineral density and total body composition.

2.8.8 | Bioelectrical impedance analysis

The BIACORPUS RX 4000 (Medical Healthcare GmbH) is used to assess body composition. It is a non-invasive way to examine total body water and body composition by using eight adhesive electrodes and a frequency of 50 kHz.

2.8.9 | Activity measurement

The MoviSens-device (Movisens GmbH) consists of a 5.3 × 3 × 2-cm-sized body and is fixed with a clip to the right hip. The three-axial acceleration sensor has a range of ±8 g, a resolution of 12 bit and a sampling rate of 128 Hz. The recognition of different activities is based on the extraction of mathematical and statistical features of the raw acceleration signal. Calculated features are maximum frequency, step count, and the number of mean crossings.

2.8.10 | Faeces sampling and microbiome analysis

Sampling is performed with two stool collection tubes (Sarstedt), using a stool collector (Süsse Stuhlfänger). Directions for safe and hygienic faecal collection are provided to the participants of the study in words and in pictograms.

Bacterial DNA is extracted from stool samples using the MagNA Pure LC DNA Isolation Kit III (Roche). 16S rRNA is sequenced with next-generation sequencing technology (Illumina MiSeq) and interpreted by the respective software analysers.

2.8.11 | Resting metabolic rate

The RMR is a component of energy expenditure that is measured by indirect calorimetry (IC). Participants must rest at least 30-min after at least 8 h of sleep and after at least 3 h of fasting. The measurements take place for at least 30 min and are performed in standard neutral hospital room temperature. A breath mask is placed over the

head of the participant. Oxygen consumption and carbon dioxide production are measured and energy expenditure is calculated by the Weir formula.[28]

2.8.12 | Sleep (sleep quality and sleep disturbances)

The Pittsburgh Sleep Quality Index (PSQI)[29] measures self-reported sleep quality and disturbances during the previous four weeks. PSQI has 19 items and measures 7 components of sleep: subjective sleep quality, sleep latency, sleep duration, sleep disturbance, use of sleeping medication, habitual sleep efficiency and daytime dysfunction.

2.8.13 | Sleep (sleepiness)

The Epworth Sleepiness Scale (ESS)[30] is a self-reported questionnaire that measures daytime sleepiness. The questionnaire consists of eight items with a respondents format 0 = would never doze, 1 = slight chance of dozing, 2 = moderate chance of dozing and 3 = high chance of dozing.

2.8.14 | Health-related quality of Life

The EuroQol Questionnaire (EQ-5D) measures health-related quality of life. The questionnaire contains five dimensions: mobility, self care, usual activities, pain/discomfort and depression/anxiety.[31]

2.8.15 | International Physical Activity Questionnaire

The International Physical Activity Questionnaire will be used to measure health-related physical activity.[32]

2.8.16 | Psychosomatic Competence Inventory

A questionnaire concerning interceptive awareness and self-regulatory behaviour in relation to psychosomatic competence.[33]

2.8.17 | Dietary counselling

Dietary counselling is performed by a professional dietician and general recommendations are given to all study participants according to the recommendations of the German Nutrition Society (DGE) and personal caloric goals based on age and sex are discussed. At baseline, all participants receive one hour of general dietary counselling by a trained dietician. Participants are encouraged to consume a diverse and balanced diet, with three portions of vegetables and two portions of fruits per day (in general and on eating days). There is no restriction on macronutrient intake, but recommendations include the limitation of sugar and salt as well as the encouragement to consume more whole grains, legumes and smaller amounts of meat, dairy and fat. Water and unsweetened coffee and/or tea are suggested as beverages. Simmering food with small amounts of water and fat is encouraged over fried food. Participants are advised to take their time and to slowly eat meals to enjoy their food and encourage satiety. Though all participants have already received nutritional counselling years prior to the study when they initially started insulin therapy, the concept of prandial insulin dose estimation according to the carbohydrate amount eaten is repeated. All participants will have the same number of contacts with the dietician at the on-site visits and phone visits for questions. 24-hour emergency support is available to ensure the participants safety.

The participants of the intermittent fasting-arm are given personal caloric goals for the fasting and eating days. The participants are presented with a couple of sample meals with a maximum of 500 kcal for the fasting days as a support to not exceed the recommended calorie maximum on those days.

2.8.18 | Insulin adaptation

To prevent hypoglycaemia caused by intermittent fasting,[23] a specific protocol for insulin dose adaptation is used, adapted from Unnikrishnan et al.[34] 4 weeks prior to the initiation of dietary intervention, participants switch to the same insulin treatment, consisting of glargine U300 as basal insulin and a short-acting insulin. Insulin U300 is administered in the morning. Participants using premixed insulin receive 70% of their daily insulin dose as glargine U300 whereas participants using basal-bolus insulin treatment continue on their original basal insulin dose but are switched to glargine U300. Participants who are allocated to the control group continue glargine U300 and prandial insulin as started in the switch phase with dose adjustments when deemed necessary by the treating physician.

Participants in the intermittent fasting group continue the same treatment as during the switch phase on the eating days and reduce the basal insulin by 20% on the fasting days with no prandial insulin. In case the blood sugar

TABLE 1 Insulin regimen

	Eating days	Fasting days
Control group	Continue insulin dose from switch phase	Not applicable
Intermittent fasting group	Use insulin dose from switch phase	20% basal insulin reduction, no prandial insulin

TABLE 2 Insulin dose adjustment on fasting days and eating days

Fasting days	
<3.9 mmol/L	Fast stopped and additional 10% reduction of glargine U300 dose (on fasting and eating days), intake of 24 g of carbohydrates
3.9–5.5 mmol/L	Regular monitoring of glucose level trend
5.6–7.2 mmol/L	Target range
7.3–13.9 mmol/L	Regular monitoring of glucose level trend
>13.9 mmol/L	4 units of rapid acting insulin bolus and contact site for further insulin adjustment
Eating days	
Pre-prandial 5.6–7.2 mmol/L	Target range – no insulin dose change required
Postprandial (2h) <11.1 mmol/L	Target glucose – increase prandial insulin by 2 IE if postprandial glucose is >11.1 mmol/l

falls below 3.9 mmol/L, the fast is broken, and the glargine U300 dose is reduced by another 10% (on fasting and eating days) for the remainder of the study. (Table 1) Within the insulin switch phase, we standardise the insulin regimen but aim to sustain glucose control from screening without significant intensification of glucose control. During the intervention phase on days with 0% calorie restriction, we aim for 5.6–7.2 mmol/L in the fasting state and <11.1 mmol/L2h postprandial (for detailed insulin dose adjustments, see Table 2). Participants already using glargine U300 are able to start the intervention 7 days ±1 after the screening visit if treatment is stable at the discretion of the treating physician.

2.8.19 | Statistical assessment

We defined two primary outcomes for the study to capture both changes in glucose control and the change of glucose control in conjunction with the insulin dose and the body weight changes. We will analyse these two primary end points in a hierarchical order, where the difference in the change of HbA1c from baseline to week 12 between the study groups is analysed first (at a significance level of 0.05). If the null hypothesis of this primary outcome (i.e. there is no difference in the change of HbA1c from baseline to week 12 between the two study groups) is rejected, the second primary outcome (i.e. the difference in the number of participants achieving a combined end point encompassing a body weight reduction of at least 2%, an insulin dose reduction of at least 10% and an absolute HbA1c reduction of at least 3 mmol/mol [0.3%]) will be tested at a significance level of 0.05. As the analysis will be performed in hierarchical order, according to the European Medical Agency (EMA) guideline on multiplicity issues in clinical trials (section 5.1.2.) no reduction or splitting of the α level is required.[35]

For the primary analysis of the primary outcomes, we will perform an intention-to-treat (ITT) analysis of all participants randomized using an unpaired t-test for the change in HbA1c (alpha = 5%), followed by a chi-square test comparing the two proportions of the co-primary outcome (alpha = 5%), if the first test rejects the null hypothesis.

We will then perform sensitivity analyses for the primary outcomes using multivariate imputation by chained equations) for missing data in the ITT cohort. Additional sensitivity analyses will be performed in the per protocol cohort (people adherent to the intervention according to section 2.8.19) without and with data imputation for missing data.

Furthermore, analysis of covariance or multiple linear regression model adjusting for baseline HbA1c and multiple logistic regression model adjusting for baseline co-primary, respectively, will be performed using the ITT population.

Finally, we will perform linear mixed-effect models without multiplicity adjustment and generalized linear mixed models, respectively, for the primary outcomes in the ITT population.

Continuous secondary outcomes will be analysed using both unpaired t-test and linear mixed-effect models.

Transformation will be considered if data are not normally distributed. For categorical data, summary tables will include frequencies and percentages. For continuous data, means, standard deviations, medians and upper and lower quartiles will be presented. Change in outcomes over time, overall and by treatment groups will be displayed in line plots/boxplots with corresponding global and pairwise p-values. All analyses will be performed in R version 4.1.0.

2.8.20 | Adherence to the protocol

Adherence to the protocol is assessed every 2 weeks with telephone calls and by discussing the food diary at the on-site visits in all study participants. Furthermore, fasting periods can be assessed by the CGM. Adherence is defined as following the caloric restrictions (i.e. at maximum 25% of recommended calorie intake on a fasting day) on more than 75% of the fasting days.

2.8.21 | Mitigation of bias

Participants will be randomly assigned either to intermittent fasting group or control group in a 1:1 ratio. Randomization will be performed at the Institute of Medical Informatics, Statistics, and Documentation of Medical University of Graz, Austria. To include a population representative, we will recruit participants from the diabetes outpatient clinics of the University hospital Graz, Austria, the Graz Diabetes Registry for Biomarker research and via advertisements. This will ensure not to include people treated at a tertiary centre in Austria only.

2.8.22 | Gender-related aspects

Data indicate that after 36 hours of fasting under the alternate day fasting dietary regimen (24 h total fast and 24 h ad libitum food consumption), men show an increased insulin sensitivity, whilst women show decreased insulin sensitivity.[36] We will consider potential gender aspect within the study and therefore we aim to recruit participants at an approximate 50:50 gender ratio.

2.8.23 | Study status

First participant visit was 16 September 2019. As of 22 December 2021, we have recruited 46 participants (100%).

2.8.24 | Study registration

The study was registered at DRKS (Deutsches Register Klinischer Studien – German Clinical Trial Register) on 03.09.2019 as DRKS00018070.

3 | STRENGTHS AND LIMITATIONS

One strength of this study is the continuous monitoring of the participants' glucose levels with CGM to minimise the risk of hypoglycaemia. Another strength of this study is the insulin dose adjustment plan for fasting participants, which can be adapted and used by the participants beyond the scope of the study.

A challenge of this study is the fact the HbA1c level cannot be evaluated separately from the insulin dose. Hence, we have introduced a composite end point that captures glucose lowering, insulin dose reduction and body weight reduction. Another limitation is the duration of 12 weeks designed to establish the safety and efficacy of the intermittent fasting intervention in this participant group, however, further research is needed to explore the effects of intermittent fasting over a longer time period. Another limitation of this study is that the CGM is not blinded. Participants in the control group are able to see the changes in glucose levels depending on their food intake, which could lead to a change in eating habits potentially lowering HbA1c levels.

4 | DISCUSSION

Previously, intermittent fasting was shown to cause weight loss of 3%–8% over 3–24 weeks and to reduce waist circumference by 4%–7%, which indicates that people lost some abdominal fat, associated with insulin resistance and related diseases.[37] Although reducing insulin resistance is beneficial in people with diabetes, it also bears the risk of hypoglycaemia in people treated with insulin. Data from Ramadan studies are available, showing mitigation of hypoglycaemia during Ramadan using the flash glucose monitoring system.[38] However, data from randomized controlled trials are largely missing.

Hence, our study aims to investigate the impact of a 12 weeks intermittent fasting intervention on glycaemic

control, weight loss, insulin dose reduction and hence a combination of those single end point parameters. Moreover, this study will provide important information regarding the safety of intermittent fasting in people with type 2 diabetes mellitus using basal bolus insulin treatment and guidance on how the insulin dose should be adjusted in this population.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

AUTHORSHIP

All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole and have given their approval for this version to be published.

AUTHOR CONTRIBUTIONS

HS conceived the trial. HS, NJT and AJ significantly contributed to development of the study protocol. NJT and AO contributed to efficient project management and day-to-day operation. HS and NJT contributed to acquiring ethical approval for the trial. AO, PNP, NJP, HK, BOP, VS, FAb, FAz and CS contribute to collection, analysis and interpretation of data. AOu contributed to the statistical analysis. MS is the dietician of the trial. AO wrote the final manuscript. All authors reviewed and contributed to the final manuscript.

COMPLIANCE WITH ETHICS GUIDELINES

All procedures performed in studies involving human participants are in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

ORCID

Anna Obermayer https://orcid.org/0000-0002-7663-0426
Norbert J. Tripolt https://orcid.org/0000-0002-7566-2047
Felix Aberer https://orcid.org/0000-0002-9947-1413

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Efficacy and Safety of Intermittent Fasting in People With Insulin-Treated Type 2 Diabetes (INTERFAST-2)—A Randomized Controlled Trial

Anna Obermayer, Norbert J. Tripolt, Peter N. Pferschy, Harald Kojzar, Faisal Aziz, Alexander Müller, Markus Schauer, Abderrahim Oulhaj, Felix Aberer, Caren Sourij, Hansjörg Habisch, Tobias Madl, Thomas Pieber, Barbara Obermayer-Pietsch, Vanessa Stadlbauer, and Harald Sourij

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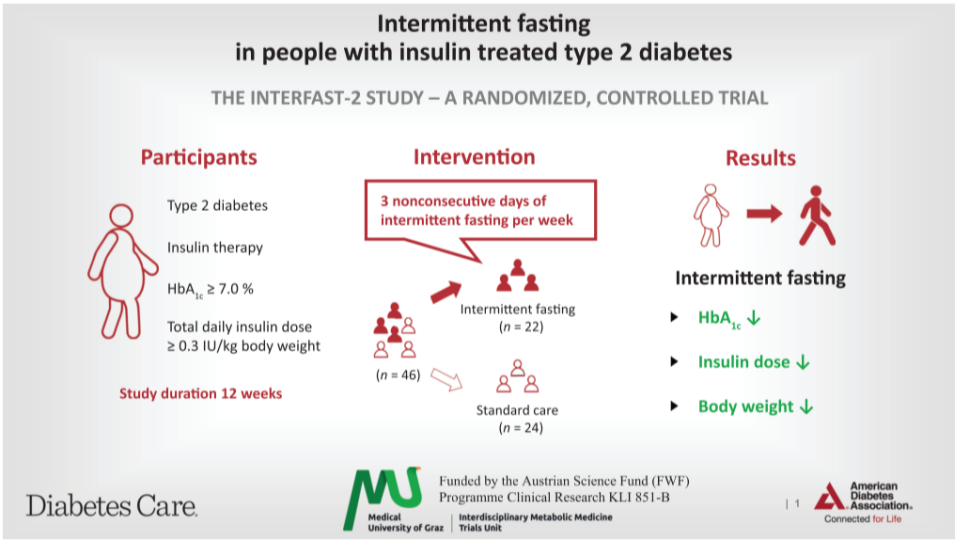

ARTICLE HIGHLIGHTS

- This study was conducted to provide robust clinical data on the effects of 12 weeks of intermittent fasting in people with insufficiently controlled insulin-treated type 2 diabetes.
- The aim of this study was to elucidate the safety and efficacy of intermittent fasting in type 2 diabetes.
- Three days of nonconsecutive intermittent fasting for 12 weeks lowered HbA_{1c}, body weight, and total daily insulin dose while improving subjective quality of life compared to a control group.
- Our findings indicate that intermittent fasting has the potential to become a promising therapy option in people with insufficiently controlled and insulin-treated type 2 diabetes.

Efficacy and Safety of Intermittent Fasting in People With Insulin-Treated Type 2 Diabetes (INTERFAST-2)—A Randomized Controlled Trial

Anna Obermayer,[1,2] Norbert J. Tripolt,[1,2] Peter N. Pferschy,[1,2,3] Harald Kojzar,[1,2] Faisal Aziz,[1,2] Alexander Müller,[1,2] Markus Schauer,[2] Abderrahim Oulhaj,[4,5] Felix Aberer,[1,2] Caren Sourij,[6] Hansjörg Habisch,[7] Tobias Madl,[7,8] Thomas Pieber,[2,3] Barbara Obermayer-Pietsch,[2,9] Vanessa Stadlbauer,[3,10] and Harald Sourij[1,2]

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OBJECTIVE

To investigate the safety and feasibility of 3 nonconsecutive days of intermittent fasting (IF) per week over 12 weeks in participants with insulin-treated type 2 diabetes.

RESEARCH DESIGN AND METHODS

Forty-six people were randomized to an IF or control group. Dietary counseling and continuous glucose monitoring was provided. Coprimary end points were the change in HbA_{1c} from baseline to 12 weeks and a composite end point (weight reduction ≥2%, insulin dose reduction ≥10%, and HbA_{1c} reduction ≥3 mmol/mol).

RESULTS

The IF group showed a significant HbA_{1c} reduction (−7.3 ± 12.0 mmol/mol) compared with the control group (0.1 ± 6.1 mmol/mol) over 12 weeks ($P = 0.012$). The coprimary end point was achieved by 8 people in the IF and none in the control group ($P < 0.001$). No severe hypoglycemia occurred.

CONCLUSIONS

IF is a safe and feasible dietary option to ameliorate glycemic control while reducing total daily insulin dose and body weight in insulin-treated people with type 2 diabetes.

With the numbers of people with type 2 diabetes rising worldwide, dietary modifications provide an essential therapeutic approach for blood glucose, weight, and cardiovascular risk-factor management (1,2). Intermittent fasting (IF) has emerged as an alternative to classic daily caloric reduction (3). The approaches to IF range from limiting food consumption to certain hours of the day to alternate-day fasting (4,5). People with insulin-treated type 2 diabetes often struggle with weight gain (6), resulting in a vicious cycle of increasing insulin doses required to overcome the insulin resistance, leading to further weight gain, and ultimately resulting in higher cardiovascular risk (7). A recent meta-analysis suggested IF as an appropriate diet strategy in people with type 2 diabetes; however, the risk of hypoglycemia during fasting states in insulin-treated individuals remains a crucial barrier to adhere to diets demanding caloric restriction and further randomized controlled trials are required to verify its feasibility and safety in this population (8).

[1]Interdisciplinary Metabolic Medicine Trials Unit, Medical University of Graz, Graz, Austria
[2]Division of Endocrinology and Diabetology, Department of Internal Medicine, Medical University of Graz, Graz, Austria
[3]CBmed – Center for Biomarker Research in Medicine, Graz, Austria
[4]College of Medicine and Health Sciences, Khalifa University, Abu Dhabi, United Arab Emirates
[5]Research and Data Intelligence Support Center, Khalifa University, Abu Dhabi, United Arab Emirates
[6]Division of Cardiology, Department of Internal Medicine, Medical University of Graz, Graz, Austria
[7]Gottfried Schatz Research Center for Cell Signaling, Metabolism and Aging, Molecular Biology and Biochemistry, Medical University of Graz, Graz, Austria
[8]BioTechMed-Graz, Graz, Austria
[9]Endocrinology Lab Platform, Division of Endocrinology and Diabetology, Department of Internal Medicine and Department of Gynecology and Obstetrics, Medical University of Graz, Graz, Austria
[10]Division of Gastroenterology and Hepatology, Medical University of Graz, Graz, Austria

Corresponding author: Harald Sourij, ha.sourij@medunigraz.at

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We hypothesized that 12 weeks of IF could improve glycemic control and decrease body weight while being safe to practice in people with insulin-treated type 2 diabetes compared with a control group.

RESEARCH DESIGN AND METHODS

This open, single-center, randomized, controlled trial, Intermittent fasting in subjects with insulin-treated type 2 diabetes mellitus (INTERFAST-2), was conducted at the University Hospital Graz, Graz, Austria, and approved by the ethics committee of the Medical University of Graz, Graz, Austria (EK 30-350 ex 17/18). The detailed study protocol was published previously (9). Briefly, this study included volunteers, aged between 18 and 75 years (both inclusive), with insulin-treated type 2 diabetes, an HbA_{1c} ≥53 mmol/mol (≥7.0%), and a daily insulin dose of ≥0.3 IU/kg body wt.

The IF group practiced IF 3 days a week, reducing their calories on these days by 75% (i.e., consuming only 25% of the recommended caloric intake). Ingestion was only allowed at breakfast and/or lunch to maintain an 18-h period of fasting. Participants were asked to keep a food diary to monitor adherence. On the remaining 4 days, participants of the IF group had no caloric restriction. There was no restriction on macronutrient composition or on the consumption of water, unsweetened coffee, and tea without milk. On eating days, participants were allowed to consume any type of food or drink without any caloric restriction. Both groups had a comparable number of interactions with the study staff.

All participants were switched to the same basal insulin (insulin glargine U300) prior to the randomization. The basal insulin was administered in the morning. For fasting days, participants were instructed to reduce basal insulin by 20% and prandial insulin was only administered for glucose correctional reasons. To

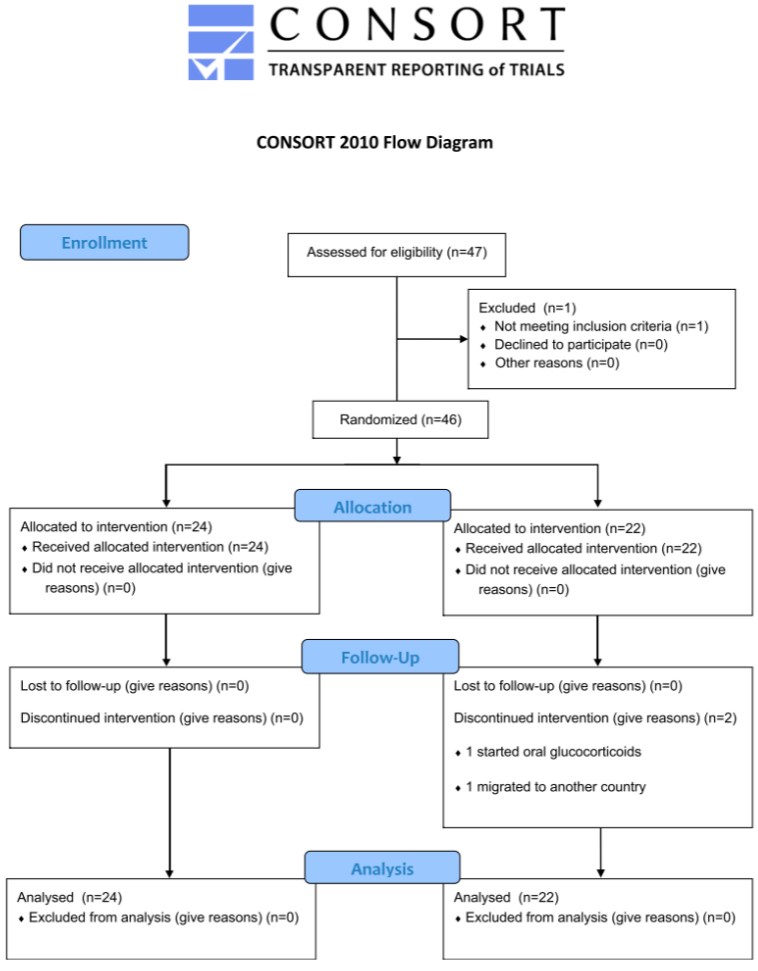

Figure 1—Trial flowchart.

reduce the risk of hypoglycemia during the IF days, participants were given an insulin dose adjustment protocol for fasting days (Supplementary Table 2). Oral nonsulfonylurea medication was continued on fasting days (9).

All participants used a FreeStyle Libre continuous glucose monitoring system (CGM; Abbott Diabetes Care, Alameda, CA) device for the 12 weeks of the study and the insulin switch phase. Data were collected using LibreView software (www.libreview.com). Lipoproteins and serum metabolites were analyzed using nuclear magnetic resonance spectroscopy (10).

We analyzed the coprimary outcomes of *1*) difference in the change in HbA_{1c} from baseline to week 12 and *2*) difference in the number of participants achieving a combined end point (weight reduction ≥2%, insulin dose reduction ≥10%, and HbA_{1c} reduction ≥3 mmol/mol) for the study in a hierarchical order (change in HbA_{1c} first) using an intention-to-treat approach. Continuous primary and secondary outcomes were analyzed using both unpaired *t* tests and linear mixed-effect models. A list of the predefined secondary outcomes are provided in Supplementary Table 3.

Data and Resource Availability

The data set generated during and analyzed in the study is available upon reasonable request from the corresponding author.

RESULTS

We screened 47 subjects, of whom 46 participants (22 women and 24 men) were randomized to the IF group (n = 22) or the control group (n = 24). Two participants of the IF group did not complete the intervention (Fig. 1). The mean age was 63 ± 7 years, diabetes duration was 21 ± 9 years, BMI was 34.3 ± 4.5 kg/m^2, HbA_{1c} was 67 ± 11 mmol/mol (8.3 ± 1.1%), and the mean total daily insulin dose was 56 ± 27 IU. The full details of the baseline characteristics are given in Table 1. After 12 weeks, HbA_{1c} in the IF group decreased by 7.3 ± 12.0 mmol/mol compared with an increase in the control group by 0.1 ± 6.1 mmol/mol (P = 0.012) (Fig. 2). The difference in the change in HbA_{1c} between the control and IF group remained statistically significant (P = 0.008) after adjusting for age, sex, diabetes duration, and baseline HbA_{1c}. The mean time above range over the entire 12 weeks was significantly lower in the IF group (30.4 ± 20.9%) than in the control group (42.1 ± 16.1%, P = 0.029). The mean time in range was significantly higher in the IF group (68.0 ± 20.2%) compared with the control group (56.6 ± 16.0%, P = 0.031), while the mean time below range over the 12 weeks was similar in the IF (1.6 ± 2.0%) and the control group (1.3 ± 2.2%, P = 0.334) (Supplementary Fig. 1).

Table 1—General characteristics at baseline

	All (n = 46)	Control (n = 24)	Fasting (n = 22)
Age (years)	63 ± 7	61 ± 7	65 ± 6*
Male sex	24 (52)	14 (58)	10 (46)
Duration of diabetes (years)	21 ± 9	18 ± 7	24 ± 10*
Weight (kg)	100 ± 15	104 ± 13	96 ± 16
Height (m)	1.71 ± 0.09	1.72 ± 0.09	1.70 ± 0.07
BMI (kg/m^2)	34.3 ± 4.5	35.0 ± 4.3	33.5 ± 4.7
HbA_{1c} (%)	8.3 ± 1.1	8.2 ± 1.0	8.5 ± 1.2
HbA_{1c} (mmol/mol)	67 ± 11	66 ± 10	69 ± 12
Total daily insulin dose (IU)	56 ± 27	59 ± 33	52 ± 19
Fasting glucose (mg/dL)	174 ± 44	176 ± 41	173 ± 47
Total cholesterol (mg/dL)	163 ± 45	164 ± 41	162 ± 51
HDL cholesterol (mg/dL)	48 ± 17	42 ± 15	57 ± 17*
LDL cholesterol (mg/dL)	81 ± 35	81 ± 32	80 ± 39
Blood pressure systolic (mmHg)	141 ± 22	145 ± 23	136 ± 19
Blood pressure diastolic (mmHg)	82 ± 10	85 ± 10	80 ± 10
Other glucose-lowering medication			
GLP-1RA	20 (43)	11 (46)	9 (41)
SGLT-2 inhibitors	20 (43)	9 (38)	11 (50)
DPP4-inhibitors	14 (30)	5 (21)	9 (41)
Metformin	34 (74)	17 (71)	17 (77)
Comorbidities			
Hypertension	39 (85)	18 (75)	21 (96)
Heart failure	5 (11)	1 (4)	4 (18)
Coronary artery disease	12 (26)	6 (25)	6 (27)
History of myocardial infarction	10 (22)	3 (13)	7 (32)
History of stroke	2 (4)	2 (8)	0 (0)
Retinopathy	10 (22)	5 (21)	5 (23)
Polyneuropathy	18 (39)	11 (46)	7 (32)
Amputation	2 (4)	1 (4)	1 (5)
Hyperlipidemia	41 (89)	21 (88)	20 (91)

Categorical data are presented as n (%), and continuous variables are presented as mean ± SD. DPP4, dipeptidyl peptidase 4; GLP1-RA, glucagon-like peptide 1 receptor agonist; SGLT-2, sodium–glucose linked transporter 2. *$P < 0.05$.

After 12 weeks, 8 participants (40%) in the IF group achieved the composite coprimary end point compared with none of the participants in the control group ($P < 0.001$) (Fig. 3). The same number of participants in the IF group (n = 8 [40%]) achieved the combined end point when higher thresholds were applied (at least 3% weight loss, at least 0.5% HbA_{1c} reduction, and at least 10% insulin dose reduction).

After 12 weeks of intervention, the IF group showed a significant reduction in weight (4.77 ± 4.99 kg) compared with the control group (+0.27 ± 1.34 kg, $P < 0.001$) and in fat mass (3.5 ± 3.3 kg in the IF group and +0.1 ± 1.3 kg in the control group, $P < 0.001$). There was no statistically significant difference in the change in lean mass or bone mass between the two groups according to the DXA measurements.

The resting metabolic rate (RMR) was not different between the IF and control group, both at baseline (IF: 2,286 ± 357 kcal, control: 2,439 ± 375 kcal) and after

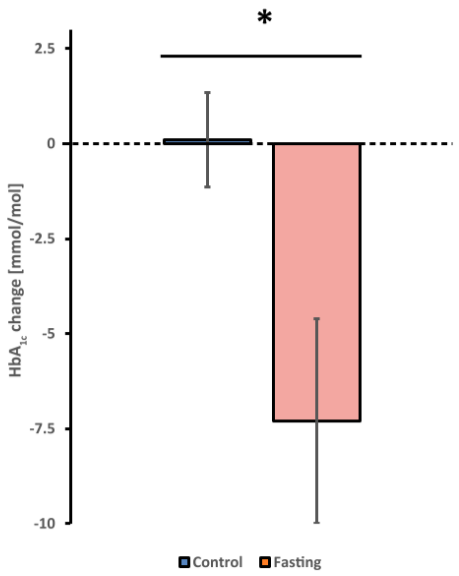

Figure 2—Change in HbA_{1c} from baseline to 12 weeks in control and IF group. Data are displayed as mean ± SEM. *P = 0.012.

12 weeks (IF: 2,248 ± 331 kcal, control: 2,429 ± 398 kcal). No difference was observed in the change of the RMR from baseline to 12 weeks between the two groups (P = 0.735). Likewise, no difference was observed in the change of the physical activity levels between the groups (P = 0.541). The mean total daily dose of insulin at baseline was 52 ± 19 IU in the IF group and 59 ± 33 IU in the control group. At 12 weeks, the IF group had an insulin dose of 45 ± 19 IU while the control group had an insulin dose of 63 ± 35 IU, resulting in a total daily insulin dose reduction in the IF group over 12 weeks by 9 ± 10 IU as opposed to the control group with an increase by 4 ± 10 IU (P = 0.008). A significant difference in the change of perceived health (EuroQol-5D visual analog scale) between the IF (from 70 ± 20 to 74 ± 21) and control group (from 70 ± 20 to 65 ± 23) was observed (P = 0.043).

Results of nuclear magnetic resonance–based metabolomics analysis showed the metabolites most contributing to the difference between fasting and control subjects included acetic acid, dimethylsulfone, and some ketone bodies (acetoacetic acid, 3-hydroxybutyric acid, and acetone). Of all 35 metabolites investigated, only acetic acid (probably derived from fatty acid metabolism) significantly increased in fasting individuals (32 ± 10 μmol/L vs. 19 ± 8 μmol/L, fold-change, 1.68; P_{adj} = 0.002) (Supplementary Fig. 2).

Of the 22 participants in the IF group, 20 (91%) achieved >75% adherence to the given fasting protocol.

During the study period, five serious adverse events leading to hospitalization were reported, two in the IF group and three in the control group. None of the serious adverse events were considered to be related to the study intervention.

CONCLUSIONS

We demonstrated that 3 days of nonconsecutive IF per week over the duration of 12 weeks improved HbA_{1c}, reduced body weight, and led to a total daily insulin dose reduction in people with insulin-treated type 2 diabetes.

Our data are in line with previous studies showing that IF was effective in HbA_{1c} reduction in people with type 2 diabetes (11). Li et al. (12) also reported data from a 7-day fasting program with a maximum intake of 300 kcal to reduce body weight in participants with type 2 diabetes; however, participants with intensified insulin treatment were excluded. Hence, our study extends previous beneficial effects on body weight and glycemic control to people with type 2 diabetes treated with insulin. Recent meta-analyses demonstrated similar HbA_{1c}-reducing potential of IF compared with continuous calorie restriction in people with type 2 diabetes, while the weight loss appeared to be more pronounced (8,13) with IF. Mechanistic studies suggest that prolonged fasting might have additional beneficial metabolic effects, independent of weight loss, by switching the metabolism to fatty acid mobilization, β-oxidation, and enhanced ketone body production or inducing autophagy (14).

From a clinical perspective, for some individuals, IF appears to be an easy to apply dietary intervention without the need for continuous caloric reductions, ultimately leading to reduced caloric intake through the time-restricted eating pattern without vigorous documentation or calorie counting (15,16). As demonstrated in our study, the risk of hypoglycemia during IF can be mitigated by reducing the insulin dose on fasting days and using a CGM system, as previously observed during Ramadan fasting (17). However, it appears critical that participants and health care personnel are instructed on insulin dose adjustments during IF.

One of the limitations of dietary studies on glycemic parameters in insulin-treated individuals with type 2 diabetes is that glucose control, body weight reduction, and insulin dose are interrelated and that changes in the insulin dose can alter the observed HbA_{1c}. For this reason, we chose a coprimary outcome besides HbA_{1c} to investigate changes in HbA_{1c} along with weight and insulin dose. A limitation of our study is the intermittently scanned (is)CGM was introduced in 16 participants in the control group and in 13 in the IF group at study start and that the isCGM use was not blinded to the participants, which might have influenced the eating behavior of the participants in both groups. Finally, participants were allowed to eat up to 25% of the recommended daily caloric intake on the fasting days as breakfast and/or lunch, which was originally introduced to reduce hypoglycemic risk and increase adherence to the study protocol in people with insulin-treated type 2 diabetes. With our study data we would feel confident to omit this caloric intake in further studies.

Strengths of the study include its randomized controlled design in people with type 2 diabetes using a basal bolus insulin regimen with a reproducible insulin

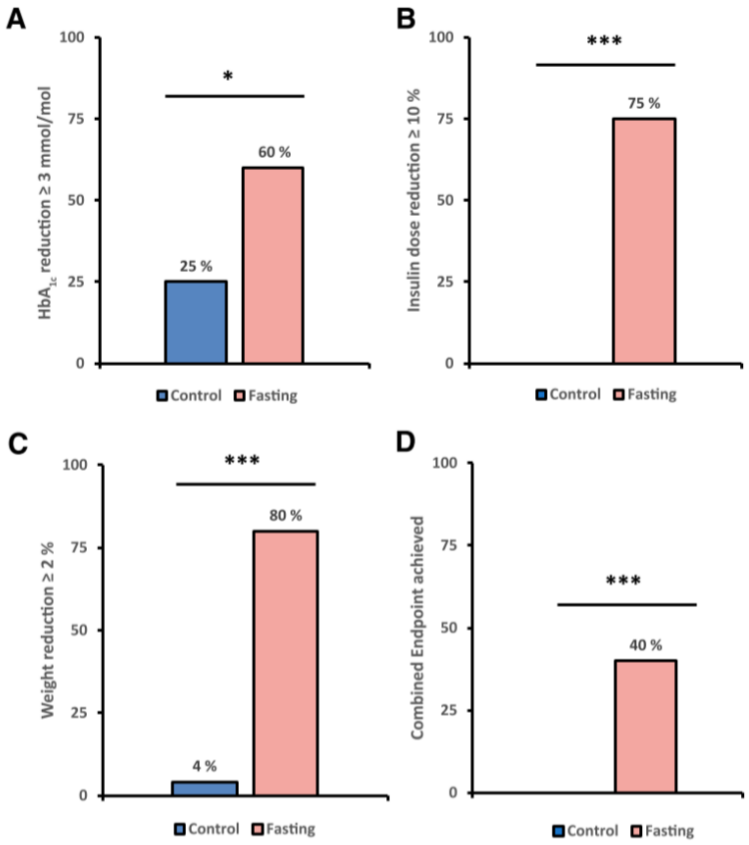

Figure 3—Coprimary end point; percentage of participants achieving each individual aspect and the combined coprimary end point. *$P < 0.05$, ***$P < 0.001$.

dosing adjustment algorithm together with isCGM data and metabolomics analysis of IF induced changes. We also monitored the RMR and physical activity throughout the study, which remained unchanged.

Our data demonstrate that IF over 12 weeks in insulin-treated people with type 2 diabetes is safe, reduces HbA_{1c}, body weight, and total daily insulin dose, while RMR and the physical activity levels remained unaltered.

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