A Low-Carbohydrate Diet in Type 2 Diabetes

Effects over six months on glycemic control, cardiovascular risk factors, vascular endothelial function and pro-inflammatory markers

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I. Preface

This thesis is based on data from an intervention study, conducted at Steno Diabetes Center Odense (SDCO) and Department of Gastroenterology and Hepatology at Odense University Hospital between November 2018 and May 2019. Grants funding this study was received from the Region of Southern Denmark, Danish Diabetes Academy (supported by the Novo Nordisk Foundation), Odense University Hospital, University of Southern Denmark, the A.P. Møller Foundation and the Christenson-Cesons Family Fund. The RedCap database and support was provided by Odense Patient Data Network (OPEN). There is no conflict of interest related to this Ph.D.

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III. Abbreviations

BP	Blood pressure
CHO	Carbohydrates
CVD	Cardiovascular disease
E%	Percentage of total energy intake
ED	Endothelial dysfunction
EE	Energy expenditure
FMD	Flow-mediated vasodilation
HDL	High density lipoprotein
HPRCD	High-protein reduced-carbohydrate diet
HsCRP	High sensitivity C-reactive protein
IL-6	Interleukin 6
Kcal	Kilocalories
LBM	Lean body mass
LCD	Low-carbohydrate diet
LDL	Low density lipoprotein
LPL	Lipoprotein lipase
MetS	Metabolic syndrome
NAFLD	Non-alcoholic fatty liver disease
NID	Nitroglycerine-induced dilation
NO	Nitrogen oxide
PCOS	Polycystic ovary syndrome
REE	Resting energy expenditure
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TEE	Total energy expenditure
TG	Triglycerides
VLCD	Very low carbohydrate diet

IV. Overview of included papers

Paper I

Gram-Kampmann EM, Hansen CD, Hugger MB, Jensen JM, Brønd JC, Hermann AP, Krag A, Olsen MH, Beck-Nielsen H, Højlund K.

Effects of a 6-month, low-carbohydrate diet on glycaemic control, body composition, and cardiovascular risk factors in patients with type 2 diabetes: An open-label randomized controlled trial.

Diabetes Obes Metab. 2022 Apr;24(4):693-703.

Paper II

Gram-Kampmann EM, Olesen TB, Hansen CD, Hugger MB, Jensen JM, Handberg A, Beck-Nielsen H, Krag A, Olsen MH, Højlund K.

A six-month low-carbohydrate diet high in fat does not adversely affect endothelial function or low-grade inflammation in patients with type 2 diabetes. An open-label randomized controlled trial.

Submitted to Cardiovasc Diabetol

V. Table of contents

1. Introduction for laymen

Reduced carbohydrate (CHO) diets and ketogenic diets have been used for at least a hundred years to treat some conditions such as type 1 diabetes and intractable epilepsy [1]. The interest for reduced CHO diets as a mean for weight-loss got a revival in 1970's, when Dr. Robert Atkins published his first book "The Diet Revolution", which recommended a ketogenic, high-fat diet to increase health and promote a weight loss. However, this diet was regarded as an extreme measure for weight-loss and was heavily debated due to the proposed high intake of saturated fats in this diet [2], in a time with increasing fat phobia and recommendations of high-fibre, low fat diets for optimal health [2]. At the beginning of the 2000s, there was a renewed and growing interest for CHO-restricted- and ketogenic diets, which also included CHO-restriction in type 2 diabetes (T2D) to enhance glycemic control. However, there was a lack of knowledge on the health effects of CHO-reduction in the diet. This included both the effects in apparently healthy persons as well as in diabetic or obese populations, in which the risk of developing cardiovascular disease are increased.

Recommendations were informal and largely based on personal opinions, where health authorities either endorsed or condemned a CHO restricted diet. However, an increasing scientific interest in CHO-restricted diets led to a growing number of studies reporting the effects of CHO-restricted diets also in patients with T2D. The studies of CHO-restriction in patients with T2D were often of short duration (days to weeks), and the reported effects on clinical outcomes such as HbA1c, weight, dyslipidemia and risk of cardiovascular disease were heterogenic. Nevertheless, the emerging scientific evidence was sufficient for the American Diabetes Association (ADA) to reconsider the reduced calorie, high-CHO and low-fat diet as a possible dietary intervention for diabetes in 2008, and accept CHO restriction to a minimum of 130 g/day. An intake of CHO lower than that was not recommended based on the notion that the central nervous system requires adequate glucose supply, as well as uncertain metabolic effects [3]. Thus, there were still insufficient knowledge on the effects of CHO-restriction on glycemic control, the metabolic effects, the impact on cardiovascular risk and the long-term effects of CHO-restricted diets in patients with diabetes.

The scientific ambiguity of the effects of CHO-restriction in T2D in the start of last decade stimulated my interest in this research field. Together with my supervisors, we, therefore, decided to design and conduct a study of the effect of CHO-restriction to a degree (10-25 E%) defined as a low-carbohydrate diet (LCD) in patients with T2D, who were instructed to maintain their caloric intake, physical activity level and non-insulin antidiabetic medication. In this PhD-thesis. We report the effect of a 6 months LCD high in fat on glycemic control, body weight, body composition and classical risk factors of cardiovascular disease including endothelial function and markers of systemic chronic low-grade inflammation in patients with T2D. The study was conducted at the Steno Diabetes Center Odense in collaboration with the Department of Gastroenterology and Hepatology and FLASH at Odense University Hospital.

2 Background

2.1 Type 2 diabetes

2.1.1 Prevalence and etiology

An estimate of global diabetes prevalence from 2019 states that diabetes is currently affecting 463 million people, or 9.3 % of the world population [4]. This staggering number encompasses the growing epidemic of a disease entity that both reduces quality-of-life [5], life-span [6] and increases economic burden on society [7].

Type 2 diabetes (T2D) has a heterogenic and multifactorial etiology, where both genetic inheritance and environmental factors influence on development. The genetic component of T2D is confirmed in family- and twin studies. Family studies have pointed to a clear genetic susceptibility in offspring if either one or both parents are affected with T2D [8], and in twin studies, with increased risk both in monozygotic twins if the other twin is affected [9, 10], as well as in dizygotic twins [11]. The understanding of the genetic contribution in T2D has increased greatly during the last 30-40 years due to developments in genetic techniques. The application of genome-wide association studies (GWAS) has made great progress in defining the complex genetic architecture of T2D, with success in identifying genetic variants that cause smaller effects than in traditional genotype-phenotype studies [12]. Around 600 gene variants/loci associated with T2D have been identified so far [13], of which the majority target different key components in T2D such as insulin secretion, insulin sensitivity and related phenotypic traits including glucose and insulin levels. These gene variants may be potential targets for future pharmacotherapy or help predict T2D risk [14].

The genetic predisposition cannot, however, explain the rapid increase in the prevalence of T2D, but here environmental factors come in play. The effects of numerous environmental factors including lifestyle components that all contribute in a synergistic manner start already in the womb with an increased risk of later T2D in infants born premature [15, 16], with low birth-weight [17, 18] or of a mother affected by gestational diabetes, which also increases risk of type 1 diabetes (T1D) [19, 20]. The upbringing may later contribute with factors such as sedentary lifestyle, child obesity and a low quality dietary pattern in addition to growing up in a stressful environment, high levels of pollutants or low accessibility to health services; all contributing to a later T2D diagnosis [21-23].

2.1.2 Pathophysiology of type 2 diabetes

T2D is a complex disease characterized by various degrees of insulin resistance, defined as a subnormal response to secreted insulin with manifesting hyperglycemia when the pancreatic β-cells no longer can compensate for the increasing insulin resistance [24]. Insulin resistance mainly occurs in skeletal muscle [25],

the liver and/or adipose tissue [26] and can be found early in the development of T2D [25]. Skeletal muscle insulin resistance may even be present in normal-weight offspring of type 2 diabetic parents [27]. Budding insulin resistance may worsen with increasing obesity and physical inactivity [28]. Basal insulin secretion rates are high to compensate for the increased insulin resistance, with blunted incretin hormone response after meals and inadequate lowering of blood glucose [29]. Despite an increased demand of insulin, patients with T2D both have decreased β-cell mass and a lower number of insulin secretory granules [30].

A growing body of evidence suggests a link between adipose tissue dysfunction and the development insulin resistance and hence the risk of T2D. Excess calorie intake and deposition of fat in adipocytes beyond its individual capacity to expand induces increased adipose cell volume (hypertrophy), which together with other mechanisms causes increased adipocyte stress and apoptosis. This, in turn activates inflammation in the adipose tissue and secretion of pro-inflammatory adipokines [31]. The resulting systemic low-grade inflammation seen in both obesity states and T2D may be a link between obesity, insulin resistance and T2D together with increased lipolysis in adipose tissue, which leads to fat overflow and ectopic fat deposition in the liver and skeletal muscle, rendering these organs insulin resistant [32]. Low-grade inflammation is further discussed below.

T2D is closely related to other insulin resistant states such as the metabolic syndrome (MetS) [33], polycystic ovary syndrome (PCOS) [34], gestational diabetes [35] and other obesity-related states associated with increased systemic low-grade inflammation such as atherosclerosis [36], low testosterone [37], hypertension [38] and some types of cancer [39]. With increased adiposity also comes an increased risk of non-alcoholic fatty liver disease (NAFLD), a pathological feature also associated with incident T2D and increased visceral adiposity [40].

2.1.3 Complications in type 2 diabetes

In long-standing T2D, there is increased risk of developing both microvascular complications [41] such as nephropathy, neuropathy, retinopathy, and macrovascular complications such as cardiovascular disease (CVD), diabetic foot and risk of amputation, as well as increased risk of all-cause mortality. The earlier the diagnosis of T2D, the higher the risk of an earlier death [42]. There is 2-3 times higher risk of CVD and cardiovascular death in diabetes compared to the general population, but mortality rates varies depending on age, glycemic control and renal complications [43, 44]. The risks of cardiovascular death and all-cause mortality in patients with T2D have decreased in high-income countries over the latter years, whereas associated morbidities such as cancer, dementia and infections (tuberculosis, pneumonia) have increased [45]. There is global heterogeneity in mortality rates, which may be attributed to differences in health-care

access, national guidelines for screening both for T2D and for complications, proactive management of hyperglycemia and risk factors for complications, race differences and socioeconomic status [46].

2.2 Weight-loss strategies in type 2 diabetes

Management of overweight and obesity is a cornerstone in the prevention and treatment of T2D [47]. Obesity is defined as having a body-mass index (BMI) higher than 30 $kg \cdot m^{-2}$. The global problem of an increasing prevalence of obesity is driven by easy access to affordable energy-dense food, combined with over-consumption [48]. The weight-loss strategies are often step-wise, divided into three phases; lifestyle intervention with focus on diet and physical activity, pharmacotherapy and bariatric surgery. In the first phase, the obese patient is advised about healthy changes in the diet and to increase the daily physical activity level [49]. The goal of these recommendations are to increase energy expenditure (EE) and decrease calorie intake. There is often need for additional strategies, such as support from healthcare staff and dietitian [47], modifying environmental factors, remedies (such as apps or food diaries) to help with calorie-counting and track weight-changes [50]. Diets may be individualized, as long as the patient manages to sustain the calorie-deficit until reached goal-weight and weight-maintenance are achieved [47]. However, it is difficult to achieve a successful weight-loss [51], which have been defined as an intentional loss of more than 10 % of bodyweight and keeping it at least one year [52]. The problems with diets, are that compliance and motivation to adhere to the diets decrease with time [53]. In addition, with increasing weight loss, appetite increases due to hormonal adaptions [54], thermogenesis is lowered and resting energy expenditure (REE) decreases [55], leading to recovering of calorie intake and weight regain. The obesity treatment may need to be intensified with pharmacotherapy, where the FDA criteria for weight-reducing drugs are BMI ≥ 30 kg/m2 or BMI ≥ 27 kg/m2 and a comorbidity associated with obesity (e.g. obstructive sleep apnea, T2D, hypertension, dyslipidemia or CVD. These medications either help suppress appetite or blocks fat-reabsorption from the small intestine by inhibiting the pancreatic lipase, such as high-dose glucagon-like peptid-1 (GLP-1)receptor agonists, phentermine (amphetamine-derivate), lorcaserin (selective 5-HT2c agonist), orlistat, bupropion or bupropion-naltrexone combination and amfepramone [56, 57]. The goal of the medical treatment is to enforce the healthy lifestyle changes and aid in adherence to lowering calorie-intake [47].

There are also surgical options if or when these measures fail. Metabolic surgeries may be considered for patients with a very high BMI ≥ 40 kg/m2 or BMI ≥ 35 with an obesity-related comorbidity as mentioned above [58]. The most common surgical options include Roux-en-Y gastric bypass, vertical sleeve gastrectomy, laparoscopic adjustable band and biliopancreatic diversion with duodenal switch [59]. Bariatric surgery is more effective than lifestyle change and medical treatment in terms of long-time weight loss and -stability,

but comes with risk of perioperative mortality and risk of complications, including nutritional deficiencies [59]. Roux-en-Y gastric bypass is superior in terms of weight-loss over 10 years than non-surgical options and superior to adjustable gastric band and sleeve gastrectomy over 4 years [60].

2.3 Carbohydrate restriction in type 2 diabetes

2.3.1 Definition of carbohydrate restricted diets

Before 2015, there was no clear definition of what defines e.g. a LCD until Feinman et al [61] proposed a definition, which later has been widely accepted and further developed (Table 1) [62]. The classic low-carbohydrate diet entails a maximum of 10-26 energy percent (E%) carbohydrates (or around 50-130 g CHO per day on a 2000-kilocalory diet), while a very-low carbohydrate diet (VLCD) limits CHO intake to 10 E%. If CHO-intake is low enough, this may result in nutritional ketosis, a state with glycogen depletion and breakdown of fatty acids from adipose tissue to produce ketone bodies. The definition of nutritional ketosis is a beta-hydroxybutyrate-concentration between 0,5-5,0 mmol/l [63]. Strict ketogenic diets may exclude cardio protective foods rich in dietary fibre and vitamins [64], while LCD or diets with a higher a CHO-intake allow some intake of vegetables, fruits and grains. Since 2008, the ADA has accepted CHO restriction to a minimum of 130 g/day as an dietary approach for weight-loss and improving glycemic control [3]. The recommended lower limit of CHO-intake was modified in 2019, where the new recommendations stated that the required amount of CHO included in the diet for optimal health is unknown [65].

Table 1: Suggestions by Feinman et al [61] on defining diets based on carbohydrate intake based on a 2000-kcal diet

Forms of carbohydrate diets	Carbohydrate intake
Very low-carbohydrate ketogenic diet (VLCKD)	20–50 g/d or <10 E% of the 2000 kcal/d diet
Low-carbohydrate diet (LCD)	<130 g/d or <26 E%
Moderate-carbohydrate diet	26 E%–45 E%
High-carbohydrate diet	>45 E%

These definitions have later been expanded to include “classic ketogenic diet” (KD) [62]. CHO restriction in diet goes hand in hand with substituting energy from carbohydrates with another energy source, and LCDs may be high in fat or high in protein, or both. Most studies compare a LCD high in fat to a high-CHO- and/or a diet with less than 30 E% fat [66].

2.3.2 Mechanism of action in carbohydrate restriction

Dietary carbohydrates serves as a main source of fuel in normal energy metabolism. The approach to lower carbohydrate intake stems from the hypothesis that all carbohydrates are converted to readily-metabolized sugars, and a minimization of ingested carbohydrates would lower insulin demand [67]. The anabolic effects of insulin are to promote energy storage by stimulating glucose transport in to metabolically active tissues, inhibiting gluconeogenesis in the liver and kidney and lipolysis in adipose tissue and favoring protein synthesis versus breakdown [68]. In conditions with a low-carbohydrate intake, lower levels of insulin shifts main fuel source to breakdown of stored glycogen and hepatic gluconeogenesis from amino acids, glycerols and lactate [61, 69]. The glycogen content in the liver is in average 80 grams [70] and around 400 grams of glycogen are stored in the muscle [71], whereof most is depleted in the first 36-48 hours of fasting or lack of carbohydrate intake [72]. With lower insulin levels, hepatic cholesterol production is also reduced, diverting free fatty acids and substrates towards hepatic ketone body production instead of producing hepatic very low density lipoprotein (VLDL) and low density lipoprotein (LDL) [71]. An increased release of free fatty acids (FFA) from adipose tissue occurs due to lower insulin levels as a result of low carbohydrate intake, and results in an increased hepatic conversion of FFA into 3-beta-hydroxybuturate (3BH), acetoacetate (ACHC) and acetone (AC) [71]. Ketone bodies may be used as a substrate for energy in the brain, heart, kidneys and muscle [73]. This metabolic flexibility allows for normal function without need for CHO to survive.

There is also an effect of reduced carbohydrate diets on lactate. Lactate as a marker of non-oxidative glycolysis produced in adipose tissue and skeletal muscle has emerged as a marker of abnormal metabolism, especially, in insulin resistance [74], where lactate is elevated before dysfunction in glucose metabolism can be measured [75]. It has been reported that a moderate-carbohydrate diet (< 40 E% CHO) reduced plasma lactate levels in non-diabetic, obese patients after five weeks [76].

It has been hypothesized that CHO-restricted diets may influence either EE or satiety, effects which both promote weight-loss. A meta-analysis of 29 studies of reduced-CHO diets found that restricting CHO in diet reduces total EE (TEE) in non-diabetics in studies of < 2.5 weeks of duration, but in studies > 2.5 weeks, TEE increases with ~50 kcal/day for every 10 % decrease in CHO intake compared to different control diets [77]. Studies report a significant increase in EE in non-diabetic, obese individuals in response to a ketogenic diet for 4 weeks [78], as well as weight loss maintenance with both a LCD and a moderate carbohydrate diet for 20 weeks [79], in all studies compared to a high carbohydrate diet. A third study found no significant difference in EE after a 6 months LCD in obese, non-diabetic women [80], but weight-loss was greater with LCD compared to high-carbohydrate diet. A short study of the effect of 14 days with LCD in patients with T2D could not demonstrate a difference in EE, but the patients reduced their calorie-intake spontaneously, which explained the observed weight-loss [81]. It may be that restriction of carbohydrates in the diet has an

influence on appetite by either stabilizing glucose excursions through lower levels of insulin, increased gastric emptying time with increased fat intake, or by appetite-regulating hormones affecting satiety, such as ghrelin, peptide YY or leptin, but results are inconsistent [62]. The previously mentioned study over 20 weeks by Ebbeling et al [79] found reductions in circulating ghrelin and leptin levels in response to a low-carbohydrate diet compared to a high-carbohydrate diet low in fat.

2.3.3 Effects of carbohydrate restriction on glycemic control

The effect on HbA1c is reported in all studies of reduced carbohydrate diets in T2D, and the literature is therefore comprehensive. Across interventions with CHO-restriction, there is a greater effectiveness on HbA1c over shorter time (< six months), whereas the effect on HbA1c compared to a control diet is often not significant after one year [62, 82-84]. Explanations for the attenuated effect on HbA1c over long term may be poor dietary compliance to the recommended CHO-reductions [85]. Another explanation may be changes in antidiabetic medications during the course of study. There is evidence in the literature, that the need for antidiabetic treatment is often reduced in T2D when following a LCD or VLCD; even if changes in HbA1c does not reach statistical difference, there are often significant reductions in both insulin use and use of other glucose-lowering drugs [62, 86, 87].

Over especially the last 20 years, there is an increasing number of studies reporting the effect of LCD or VLCD/KD compared with high-CHO diet in T2D [86]. There is a huge variation in the intervention strategies in both the reduced-CHO groups and the control groups in existing studies. This makes a comparison of the effect sizes of different LCDs and VLCDs on study outcomes difficult to evaluate, especially in reviews and meta-analyses. One important factor which makes comparisons of the effect of CHO-restricted diets difficult to interpret, is of course that terminology and definitions vary across studies, not only before but also after the definitions of CHO-restricted diets were proposed [61]. Some authors still refer to CHO-restriction ranging between 26-45 E% as a LCD [88]. This may pose a problem, as the effect on glycemic control increases with lower CHO intake [82]. Another factor is the use of caloric restriction as an additional strategy, as caloric restriction has a well documented beneficial effect on glycemic control in T2D [89], as well as on body weight, dyslipidemia and on blood pressure [90] which also often are reported in these studies. Caloric restriction has been applied in both the CHO-restricted and control group [91-96], in the CHO-restricted group only [97] or the control group only [98-101]. Two studies have used addition of weight-loss medication such as Orlistat to the low-fat diet in comparison to an adaptive VLCD over 48 weeks [100] or as in one study, were offered a prescription of Phentermine in both groups [96] to induce weight-loss.

The inclusion of exercise programs in studies comparing the effects of reduced-CHO diets with control diets may also confound the isolated effect of reduced CHO-diets as such exercise programs may increase

the effect of both diets on glycemic control and weight-loss. This has been included in previous studies, either with regular supervised training-regime [95] or as recommending a minimum of daily exercise to both groups [96, 98, 100]. Another important factor worth mentioning when evaluating the effect of a reduced CHO-diet in patients with T2D, are changes in antidiabetic treatment during the study. Reduction in glucose-lowering drugs during CHO-restricted diets may attenuate or even abolish the effect on HbA1c as compared to a control diet high in carbohydrates, in particular, if the reduction or discontinuation of glucose-lowering drugs is based on reasons other than the occurrence of hypoglycemia. Examples of this are cessation of thiazolidinediones to reduce risk of side effects such as edema and weight gain [102], if a clinician not involved in the study reduces or discontinues antidiabetic medication during the study [96, 103], increasing or decreasing glucose lowering medication as a response to changes in HbA1c during the course of study despite lack of hypoglycemic episodes [91, 104], or the lack of recording medicine changes during the study [98].

Table 2: Overview over LCDs 10-26 E% carbohydrate vs. different control diets

Study (year)	No included	Design/Duration	LCD group	Control group	Medicine changes	Other interventions	Effect on HbA1c	Weight loss/body composition	Effect on blood glucose	Effect on lipids
Ahmed (2020) [96]	49 with T2D who adhered to a LCD over 3 months	A retrospective analysis of electronic records over 3 months	CHO 5-10 E%; protein 20-25 E% and fat 65-70 E%	"Usual care diet", high-fiber, low-fat diet.	Discontinue SU and decrease insulin 30-50 % at baseline in the LCD group	Recommended > 30 min physical activity /day. Medicine was adjusted and discontinued in both groups	-1.32 % mean difference between groups	-12.3 kg mean difference between groups	Improved plasma glucose in LCD, difference between groups not stated	No difference between groups
Chen (2020) [103]	92 with T2D and HbA1c > 58 mmol/mol	18 mo	Limit CHO to 90 g/day, no energy restriction	CHO 50-60 E%, protein 1.0–1.2 g/kg body weight and ≤ 30% fat.	Medicine changes by PP. SC in MES after 18 mo in LCD.	NA	SC in HbA1c after 18 mo with LCD: MDIC – 1.63 %**	Weight MDIC – 2.76 kg and waist MDIC -5.69 cm. NSC in fat mass (%) or BMI.	NSC on f-G, SC in LCD after 2-h OGTT with LCD	NSC in TC, LDL, HDL nor TG
Daly (2006) [105]	102 obese with poorly controlled T2D (HbA1c 8-12 %)	3 mo, monthly group sessions. Both groups instructed in "healthy eating".	Up to 70 g CHO/day + > ½ pint milk + 1 fruit a day	"Healthy eating", emphasis on reducing fat intake and decrease portion sizes.	Lower insulin use in LCD group		NSC	-3.55 kg in LCD, -0.92 kg in C, p 0.001.	NA	Lower total cholesterol/HDL -ratio in LCD (p 0.011), NC in TG.
Guldbrand (2012) [106]	61 with T2D	2 year intervention based on 4 group-meetings	CHO 20 E%, protein 30 E% and fat 50 E%	55-60 E% CHO, protein 10-15 E%, fat 30 E%	Reductions of insulin significant in the LCD group after 6 months and 12 months.	Recommended caloric intake 1600 kcal/w, 1800 kcal/m. 4 group consultations during study.	Significantly lower in-group comparison at 6 month only, NSC between groups	Both groups lost weight at all time points (all p < 0.001) compared to baseline, NSC between groups.	NA	LDL lower in both groups at 24 mo, NSD between groups, HDL higher in both groups with NSD between groups. TG and TC NSC.
Han (2021) [104]	134 with T2D	6 mo, fixed exercise	CHO < 14 E% (< 50 g/day), protein 28 E% and fat 58 E%(35 %	CHO 53 E%, protein 17E% protein and fat30E% (15% MUFA, 9%	Significantly lower MES with LCD.	Limit SFA < 10 E%	HbA1c SD between groups and in groups –	SD between groups in both weight and BMI with LCD	Significantly lower f-Glu with LCD after 6 mo.	NA

			MUFA, 13 E% PUFA)	PUFA, SFA < 10 E%).			more with LCD.			
Iqbal (2010) [98]	144 obese T2D patients enrolled, 76 dropped out	24-mo, one private instructional session, hereafter group sessions every 4 weeks	CHO 30 g /day, no restrictions in fat or caloric intake	"low-fat diet" with fat ≤ 30 E%. Energy restriction 500 kcal/day.	Not stated.		Lower HbA1c at 6 months in LCD (-0.5 % vs. -0.1 %), NSD on other time points.	NSD between groups at any time point.		
Morris(2020) [107]	A RCT feasibility trial, randomized 2:1, 33 T2D.	12 weeks hypocaloric LCD (8 weeks weight-loss followed by 4 weeks weight maintenance	CHO < 26 E% CHO and minimum 60 g/protein/day . Instructed by nurses at baseline, week 2, 4 and 8.	Usual care (DiabetesUK), "balanced healthy eating". Instructed by nurses at baseline.	Diabetes medication could be adjusted in both groups if needed.	800-1000 kcal/day for 8 weeks in both groups, increase in kcal for weigh-maintenance 4 weeks.	-15.7 mmol/mol, p = 0.001	MDIC -7.5 kg, p < 0.001.	fPG – 2.3 mmol/l, p = 0.02. HOMAIR -0.8 p = 0.001	TG significantly lower, MDIC -0.58, p = 0.03. Otherwise NSD.
Nielsen (2005) [94]	31 obese (BMI ≥ 30) with T2D (LCD 16:CG 15).	6 months. 16 volunteers followed LCD, matched with 15 patients on high-CHO.	CHO 20 E%, protein 30 E% and fat 50 E%.Kcal-restriction 1800 kcal/men, 1600 kcal/women.	CHO 60 E%, protein 15 E% and fat 25 E%. Calorie restriction to 1600-1800 kcal/men, 1400-1600 kcal/women.	Lower insulin dose required (from mean 60 ± 33 to 39 ± 21 to 18 ± 11 week 0: 1: 24. 3 out of 11 discontinued insulin.	No instructions on exercise	HbA1c decreased significantly with LCD -1.4 ± 1.1 %, CG -0.6 ± 1.4 %.	BW decreased significantly -11.4 ± 4 with LCD, -1.8 ± 3.8 in CG	f-BG in LCD decreased significantly -3.4 ± 2.9 mmol/l and -0.6 ± 2.9 in CG	NA
Saslow (2018) [108]	1000 with T2D, Single-arm digital LCD self-manage-ment program	12 months, digital modules	CHO < 130 g /day	No control group	71.40 % were prescribed ≥ 1 antidiabetic treatment at baseline.	Participants divided in 3 groups: completers (COM), partial completers (PC) and non-completers (NC).	COM -1.17, PC -0.6, NC -0.16 mmol/mol, all significant from baseline.	COM -7.45 kg, PC and NC NSC.	NA	NA
Sato (2017) [109]	66 japanese patients with T2D	6 months	130 g CHO/day set as target, no	28 kcal/kg BW recommended .	6 reduced in ADM in LCD, 1 in CG, 3 reduced in		-0.65 % with LCD, 0.0 mmol/mol	-1.6 kg with LCD, -0.6 kg in CG (p = 0.02)	NA	NSC

	with HbA1c > 7.5%		specifics on protein/fat.	CHO 50-60 E%, protein 1.0-1.2 g/kg BW	insulin in LCD vs 3 in CG.		in CG ($p < 0.01$)			
Sato (2017) [110]	Continuance of the LCD intervention from 6 months to 18 months	18 months including the initial 6 month RCT.	After the six-month RCT as described above, patients were allowed to manage their own diets.	As above: participants managed their own diets with no further intervention.	Outpatient physician changed medicine as part of routine clinical practice.		NSC from baseline	Weight and BMI lower in both groups, NSD between groups	NA	NSC
Tay (2018) [95]	115 with T2D	2 years (61 completed)	CHO 14 E% (< 50 g/day), protein 28 E% and fat58 E% (< 10 E% SFA).	CHO 53 E%, protein 17 E% and 30 E% fat (< 10 E% SFA)	MES LCD -0.5, CP -0.2, $p = 0.03$	Exercise 3 x 60 min/week. 30 % of energy requirement food provided first 12 weeks. Both groups energy deficit 500-1000 kcal/day	Reductions in HbA1c comparable	Reductions in weight comparable	Fasting blood glucose, HOMA NSD.	TG decreased with LCD.
Yamada (2014) [99]	24 Japanese patients with T2D, HbA1c 6.9-8.4 %.	6 months RCT	CHO < 130 g /day; lower limit 70 g/day to prevent ketosis. Target CHO% 20-40 g/meal and 2 x 5 g sweets per day. Non-calorie restricted	CHO 50-60 E%, protein < 20 E% protein and fat < 25 E%. Target calorie intake calculated according to ideal body weight.	Medications were not changed unless hypoglycemia occurred.	No instructions on exercise.	HbA1c reduced in LCD group -0.6 %, $p = 0.03$.	Body weigh reduced in both groups, NSD.	NSD	NSD in any cholesterol parameter

ADA, American Diabetes Association. ADM, antidiabetic medication. BW, body weight. CG, control group. FG, fasting glucose. HDL, high-density lipoprotein. Kcal, kilocalories. LCD, low-carbohydrate diet. LDL, low-density lipoprotein. M, men. Mo, months. MDIC, mean difference in change. MUFA, monounsaturated fatty acids. NA, not applicable/not available. No, number. NSD, no significant difference between groups. NSC, no significant change. OGTT, oral glucose tolerance test. PP, primary physician. PUFA, polyunsaturated fatty acids. SC, significant change. SFA, saturated fatty acids. TC, total cholesterol. TG, triglycerides. W, women. * $p < 0.05$, ** $p < 0.01$.

Included studies are those which designed the LCD-intervention to 50-130 g/CHO a day, or if stated, between 10-26 E%. If both grams/day and E% is stated, the indicated E% was used. Articles was found through search on PubMed "low-carbohydrate AND Type 2 diabetes" and filter "RCT", and through articles used in reviews and meta-analyses. Studies designed to induce ketosis was excluded in this table. One recent retrospective study was included (Ahmad 2022) and another single-arm, digital trial (Saslow 2018).

2.3.4 Effects of carbohydrate-reduced high-protein diets on HbA1c

There is no definition of what constitutes a high-protein, reduced-carbohydrate diet (HPRCD). Interventions with HPRCD in T2D entails at least 25 E% protein [111], but in most studies of a HPRCD in T2D, the protein proportion is around 29-32 E% and CHO-proportion ~30 E%, which is defined as a moderate-carbohydrate diet (Table 1). HPRCDs have similar effects on HbA1c as LCD compared to various control diets. Skytte et al [112] reported a greater effect of HPRCD (CHO 30 E% and protein 30 E%) vs. control diet (CHO 50 E% and protein 17 E%) on HbA1c in patients with T2D over 6 weeks in a well-executed study, which included free provision of food in a cross-over trial designed for weight-stability. Watson et al also reported that there was no significant difference between groups in effect on HbA1c when comparing a HPRCD (CHO 33 E% and protein 30 E%) to a control diet (CHO ~51 E% and protein 22 E%) over 12 weeks in a phased study designed for weight-loss and later weight-maintenance [113]. Gannon et al [114] also compared a HPRCD (CHO 20 E% and protein 30 E%) to a high-CHO diet (CHO 55 E% and protein 15 E%) over five weeks in a cross-over design, and found that the HPRCD was more effective in lowering HbA1c. In a long-term study, Krebs et al [115] randomized 419 patients with T2D to a two-year "real-world setting" study with no effect of a HPRCD (CHO 40 E% and protein 30 E%) on HbA1c compared to control diet (CHO 55 E% and protein 15 E%). The authors describe, that the dietary compliance was a problem, especially with the protein intake in the HPRCD-group, and that the dietary composition trended back to baseline proportions between six months and 2 years in both groups, resulting in no change in HbA1c [115]. Another study by Larsen et al [116] over 12 months (also "real-world conditions") found no difference in the effect of a HPRDC (CHO 40 E% and protein 30 E%) on HbA1c, compared to a high-carbohydrate diet (CHO 55 E% and protein 15 E%). However, the authors demonstrated that the effect on HbA1c was highest in participants who adhered most to the diet and lost most weight in both groups [116]. In contrast, Sargrad et al [117] found that HbA1c improved more with low-protein high in-CHO diet (CHO 55 E% and protein 15 E%) compared with a HPRCD (CHO 40 E% and protein 30 E%) after eight weeks, even though weight-loss was equal in both groups.

2.3.5 Effects of carbohydrate-restricted diets on weight and body composition

CHO-restricted diets have been heavily debated since the 70's, where Dr. Atkins repurposed a ketogenic low-carbohydrate diet from relieving epilepsy and type 1 diabetes (T1D) to curb an increasing obesity epidemic [118, 119]. As discussed previously, the early lack of definition of CHO-restricted diets before 2015 [61] and heterogeneity in studies that may affect HbA1c may also affect the weight-loss in CHO-restricted diet studies [62, 120]. In T2D, early meta-analyses and reviews reported a large degree of heterogeneity across studies when clustering all interventions with CHO < 45 E%, and a small number of studies showed only small differences in weight-loss tipping in favor of a reduced carbohydrate diet [121] or no superiority at all [122-125]. Even with the more strict definitions of LCD and VLCD as CHO < 26 E% and < 10 E%, respectively, the effect on weight-loss was not significant with different durations up to 24 months [87, 126]. Over shorter durations (less than 12 months), LCD demonstrated superiority in terms of weight loss in patients with T2D [82-84, 127, 128], and one meta-analysis reported a greater effect on weight-loss with greater CHO-restriction up to 12 months [128]. It is currently widely accepted, that LCD and VLCD have superiority over high-carbohydrate diets with < 30 E% fat over shorter duration (up to six months), but no superiority on the long-term effect on weight [62]. LCD and VLCD are as effective as any other diet in reducing waist circumference as a measure of intra-abdominal fat in both obese inidividuals and patients with T2D [82, 124, 127, 129], although one study found a significantly larger decrease in intraabdominal fat mass in overweight/obese with moderate carbohydrate diet (43 E% CHO) compared to a lower fat diet [130].

A concern with any weight-loss intervention is an unfavorable loss of muscle, which would cause a decline in REE and counter-act desired effects [131]. Theoretically, if carbohydrate intake was low enough, the brain alone requires 110-120 grams of glucose per day, which would require breakdown of 160-200 g protein or almost 1 kilo of muscle tissue, if the sole energy source was conversion of amino acids into glucose [132]. This is, however, not the case, but both with LCD and VLCD, around 25 % of the observed weight-loss in non-diabetic individuals is attributed to loss of lean body mass (LBM) [133]. It seems as if a higher protein intake could reduce loss of LBM in low-carbohydrate diets [71, 133].

A part of the rapid decrease in body weight when adapting to a low-carbohydrate diet comes from the breakdown of stored glycogen, which releases retained water [71]. A study, which examined the weight loss in 20 obese individuals in response to a ketogenic VLCD for 4 months, compared three different methods to measure body composition [134]. They found a 15% loss of LBM at the end of study. However, most of the LBM lost represented loss of body water in the first phase of adapting to a ketogenic VLCD. When water loss was subtracted, only 5 % (one kilo) of the lost body weight was due to loss of muscle mass [134]. These findings do not directly contradict the statement of 25 % loss of fat-free mass with LCD or VLCD, but indicate that the anticipated loss of muscle mass may not be as severe as assumed.

2.3.6 Effects of low-carbohydrate diets on cardiovascular risk factors

The WHO has pointed out several markers of increased cardiovascular risk, and among these markers are hypertension, dyslipidemia, overweight/obesity and increased blood glucose – markers that very often are present in MetS and T2D [135]. Dyslipidemia is defined as low high-density cholesterol (HDL), high low-density cholesterol (LDL) and high triglycerides (TG). In T2D, carbohydrate restriction compared to diets with a higher CHO-content may have none or a positive impact on total cholesterol [62, 136] and HDL [62, 83, 84, 102, 120, 127, 137, 138], while the effect on LDL is small or none, as reported in several reviews and meta-analyses [83, 126, 127, 136, 138]. One review article states that the chronic exposure to high blood glucose levels drives the atherogenic profile, and therefore, the lowering of blood glucose with carbohydrate restriction will improve CVD risk markers [139]. There may be a dose-dependent effect of CHO-restriction on TG and LDL, with a greater carbohydrate restriction leading to a greater decrease of TG, and a U-shaped association with LDL [128]. One meta-analysis reported an increase in LDL in response to LCDs with < 20 E% CHO compared to high-carbohydrate, low fat diet [120], and only one study has found a positive impact on TG with a prescribed VLCD (prescribed CHO < 30 g/day) compared to a low-fat diet in obese (39 % had T2D) [140]. However, the compliance with diet after 6 months in this study deviated from the prescribed, as the LCD group reported an actual intake of 37 E% CHO [140].

CHO-reduced diets may have a positive impact on blood pressure in T2D compared to a variety of control diets with higher CHO-content, where both lower systolic blood pressure [87, 128] and diastolic blood pressure [62, 141] have been reported. While previous reviews and meta-analyses have reported conflicting results regarding the blood pressure lowering effect of CHO-reduced diets compared with different control diets in T2D [62, 83, 84], a recent review and meta-analysis of multiple studies reported a dose-dependent beneficial effect of CHO-restriction on systolic blood pressure [128].

Both blood lipids and blood pressure are, however, positively affected by weight-loss in T2D [142], and as discussed previously, LCD seems to have a greater impact on weight compared to control diet over shorter terms. Interventions differing in caloric content, exercise intervention, weight-loss and other factors makes it difficult to evaluate the “pure” effect of a reduced carbohydrate content in the diet [84].

Population-based studies of CHO-intake scores (based on E% of daily CHO-intake) have differed in results. In epidemiological studies, CHO-scores do not go as low as 26 E% as in a LCD, but with a lowest CHO-intake (or the “highest LCD-score” according to the authors) of ~ 43 E% [143]. A recent study from Japan found that reduced CHO-intake had a non-linear association with all-cause mortality if the diet was based on animal protein and -fat, but that all-cause mortality was inversely associated with higher LCD-score and plant-based protein and fat [143], confirming results from a US-based study [144]. However, a Japanese

study over 29 years found no associations with type of protein in LCD, only that LCD is inversely correlated with CVD and total mortality in non-diabetic women [145].

2.3.7 Effects of low-carbohydrate diets on endothelial dysfunction

The endothelium lines the inside of arteries and arterioles, providing a single-layer sheet of smooth muscle vascular cells in contact with the blood. A normal endothelium regulates vascular tone, potency of circulating inflammatory cells to adhere to the surface, coagulation and inflammation in the vessel wall [146]. The vascular tonus changes in response to various stimuli through a balanced release of endothelial-derived relaxing and contracting factors, mainly nitrogenoxide (NO). If the balance between relaxing factors (NO, prostacyclin) and contracting factors (angiotensin II, endothelin-1 and prostaglandins) is disrupted, this causes reduced ability to dilate and hence endothelial dysfunction (ED) [147]. Endothelial function may be measured both invasively in the coronary arteries or non-invasively through ultrasound of a peripheral artery after obstructing blood flow for a period of time, which causes shear stress and transient ischemia leading to flow-mediated vasodilation (FMD). An attenuated response is termed endothelial dysfunction (ED), but there is no clear definition of how much the endothelial response after stimulus must be blunted to define it as ED [148]. A proposed cut-off value for ED is a FMD-value below 7.1 %, and for smooth-vascular cell dysfunction, a nitroglycerine-stimulated dilation below 15.6 % in the brachial artery [149]. ED is a known risk marker of CVD; a decrease of 1 standard deviation (SD) in endothelial function is associated with a 50 % increase in the relative risk of a future cardiovascular event [150]. Factors that affect the endothelium occurs throughout life and are both non-modifiable and modifiable: increasing chronological age, male sex, menopausal status and previous CVD are non-modifiable, while modifiable risk factors include obesity, MetS/T2D, smoking, dyslipidemia and hypertension [151]. Strategies for improving endothelial function include reducing risk factors of CVD, such as smoking cessation, exercise, weight-loss, statin treatment, treatment of hypertension, antioxidants (Vitamin C), oral estrogens and treatment with angiotensin-converting enzyme inhibitors (ACE-I) [146, 152]. Most oral antidiabetic medications, as well as GLP-1 receptor agonists, also have a positive effect on endothelial dysfunction, with or without their glucose-lowering effect [153]. Effective improvement of risk factors comply with an early effect on FMD, which makes FMD a useful clinical marker [154]. However, in longstanding diabetes, the endothelium undergoes vascular structural changes such as proliferation, hypertrophy and remodeling which leads to irreversible ED [155].

FMD in the brachial artery is significantly lower in T2D with hypertension than in age-matched healthy individuals [156]. In T2D, in addition to many of the risk factors already described, there are direct effects of hyperglycemia on the endothelium, which alter the endothelial function as well as indirect effects of hyperglycemia which impact the synthesis of growth factors and vasoactive mediators [157]. The Hoorn

study found that hyperglycemia and impaired glucose tolerance work synergistically, and presence of both enhanced the risk of developing CVD if ED was present [158].

T2D is a state with chronic low-grade inflammation, with two-to threefold increase in circulating inflammatory cytokines [159]. Increased systemic pro-inflammation poses a link between obesity, T2D and ED [160] (Fig. 1). A dysfunctional endothelium loses its vascular integrity which exposes sub-endothelium and leakage of fluids; it expresses more leucocyte adhesion molecules and HLA molecules, becomes pro-thrombotic instead of anti-thrombotic and produces inflammatory cytokines [161].

It is generally accepted that diet affects risk factors of developing ED and CVD [162]. There has been concerns of a negative impact of LCD on ED in T2D, especially if saturated fat intake is increased. Weight-loss is known to have a positive effect on the endothelium and improve ED (measured as FMD), in both healthy, obese, patients with CVD and those in risk of diabetes, regardless of method used for weight-loss [152]. However, the improvement may be more pronounced with a low-fat diet (< 30 E% fat) than a CHO-reduced diet (< 45 E% CHO) [152]. In patients with T2D, there are only a few studies on the effect of LCD on endothelial function. Wycherley et al [163] examined the effect of a hypocaloric LCD (CHO 14 E%, fat 58 E%) compared to a hypocaloric high-CHO diet (CHO 53 E%, fat 30 E%) and found no difference between groups over 6 months, when diet change was combined with an exercise intervention. This group also reported the results after 24 months, where the FMD in the two groups still was comparable [95]. Meanwhile, Barbosa-Yañes [164] found a significant increase in brachial FMD over three weeks in the hypocaloric high-CHO group (CHO 50 E%, fat < 30 E%) compared to a hypocaloric VLCD (CHO < 40 g, fat 60-70 E%) and found improved brachial FMD in the high-CHO group but not in the VLCD group, which was observed despite comparable weight-loss but larger reduction in HbA1c in the VLCD group. There may be acute harmful effects of CHO-restriction over shorter time, which may ameliorates with continued duration.

2.3.8 Effects of LCD on selected markers of systemic low-grade inflammation

Mounting evidence points to adipose tissue not being an inert fat storage, but to have active endocrine, paracrine and autocrine functions which affects the whole organism [165]. Healthy adipose tissue produces multiple cytokines termed adipokines, which are biological mediators of cross-talk between different tissues to signal a change in energy demand such as observed after an exercise bout or food intake [166]. To date, more than 600 adipokines are identified [167]. In addition, about 5-15 % of healthy, lean adipose tissue consists of immune cells from the innate immune system, which perform biological functions such as clearing dead adipocytes, inhibiting proliferation of adipocyte progenitor cells and secreting pro- and anti-inflammatory cytokines [168]. Some of the most important adipokines produced by adipocytes are leptin and adiponectin, while immune cells produce interleukins such as IL-6. Leptin is a pro-inflammatory adipokine

produced in proportion to the individual fat mass to regulate appetite and affect multiple aspects of immune function [169, 170]. Reduced leptin signals nutritional deprivation, stimulates appetite and decreases EE in both humans and rodents [171, 172]. Adiponectin is an insulin-sensitizing adipokine, which has anti-inflammatory and anti-atherosclerotic properties as well [173]. It is inversely correlated to BMI, age and insulin resistance [174]. IL-6 holds a pleiotropic array of metabolic and inflammatory effects and is secreted by adipose tissue in response to inflammation and sepsis, but also by skeletal muscle in response to acute exercise. IL-6 has pro-inflammatory effects and increases lipolysis and whole body fat oxidation [175].

C-reactive Protein (CRP) is one of the most used markers of both inflammation and infection, produced by hepatocytes. CRP is traditionally used as a marker of infection [176], inflammation or sometimes cancerous disease, and returns to baseline values after treating the condition. CRP is typically low in healthy individuals with healthy adipose tissue, but in acute inflammatory or infectious conditions, it may increase up to 200-fold [177]. In patients with obesity, insulin resistance and T2D, CRP is typically elevated [178]. Beside CRP, many inflammatory proteins are often elevated in T2D, including fibrinogen, interleukin-1 (IL-1), IL-6 and tumour necrosis factor-alpha (TNFα) [179, 180]. In conditions where CRP is very low, high-sensitive C-reactive protein (hsCRP) may be used as a marker of changes in in conditions with higher levels of circulating pro-inflammatory cytokines such as obesity and T2D. HsCRP is elevated in T2D [181]. HsCRP is also reported to be an independent risk marker for CVD and may predict CVD events as well as microvascular complications in T2D [182].

Increasing obesity due to excessive caloric intake results in the expansion of especially visceral adipose tissue (VAT), which generates an unhealthy metabolic and atherogenic profile [183]. Expansion of fat mass disrupts normal adipokine secretion and causes a shift to a more pro-inflammatory profile (Fig. 1) [159]. Leptin levels increase, which increases proliferation of monocytes, T-cells and eosinophils in adipose tissue, which in turn expresses inflammatory cytokines such as TNF-α and IL-6 [168]. IL-6 promotes differentiation of T-helper cells into Th-17 cells, which are involved in auto-inflammatory diseases; dysregulation and overproduction of IL-6 leads to autoimmune disease [184]. Some anti-inflammatory drugs targets these signaling pathways to treat auto-immune diseases such as rheumatoid arthritis [185]. Leptin target cells become leptin resistant, which further contributes to increased obesity [186]. Levels of adiponectin decrease and this reduces insulin sensitivity and causes less differentiation of young subcutaneous adipocytes [173]. The increasing inflammatory state and down-regulation of the circulating levels of the anti-inflammatory adiponectin negatively affects vascular endothelial cells by increasing their expression of adhesion molecules and chemotactic factors, which leads to increased local inflammation and with ED as a consequence and this further increases adipose tissue inflammation in a vicious cycle [187]. Inflammatory cytokines, but foremost IL-6, induces the expression of CRP-genes in hepatocytes [188], but studies have suggested that adipocytes

may be stimulated to produce CRP by inflammatory cytokines [189]. CRP may not be limited to be only a marker of inflammation, but may have an active role in development of insulin resistance, development of hepatic steatosis and influence energy balance [190]. Studies report a high association between CRP-levels and the risk of incident T2D, but there are heterogeneity among studies, while increased hsCRP levels in patients with T2D may be associated with diabetic complications, such as diabetic retinopathy, nephropathy and neuropathy [191]. Increased levels of IL-6 in diabetes is associated with increased risk of a cardiovascular event, such as stroke, myocardial infarction or cardiovascular death [192-194]

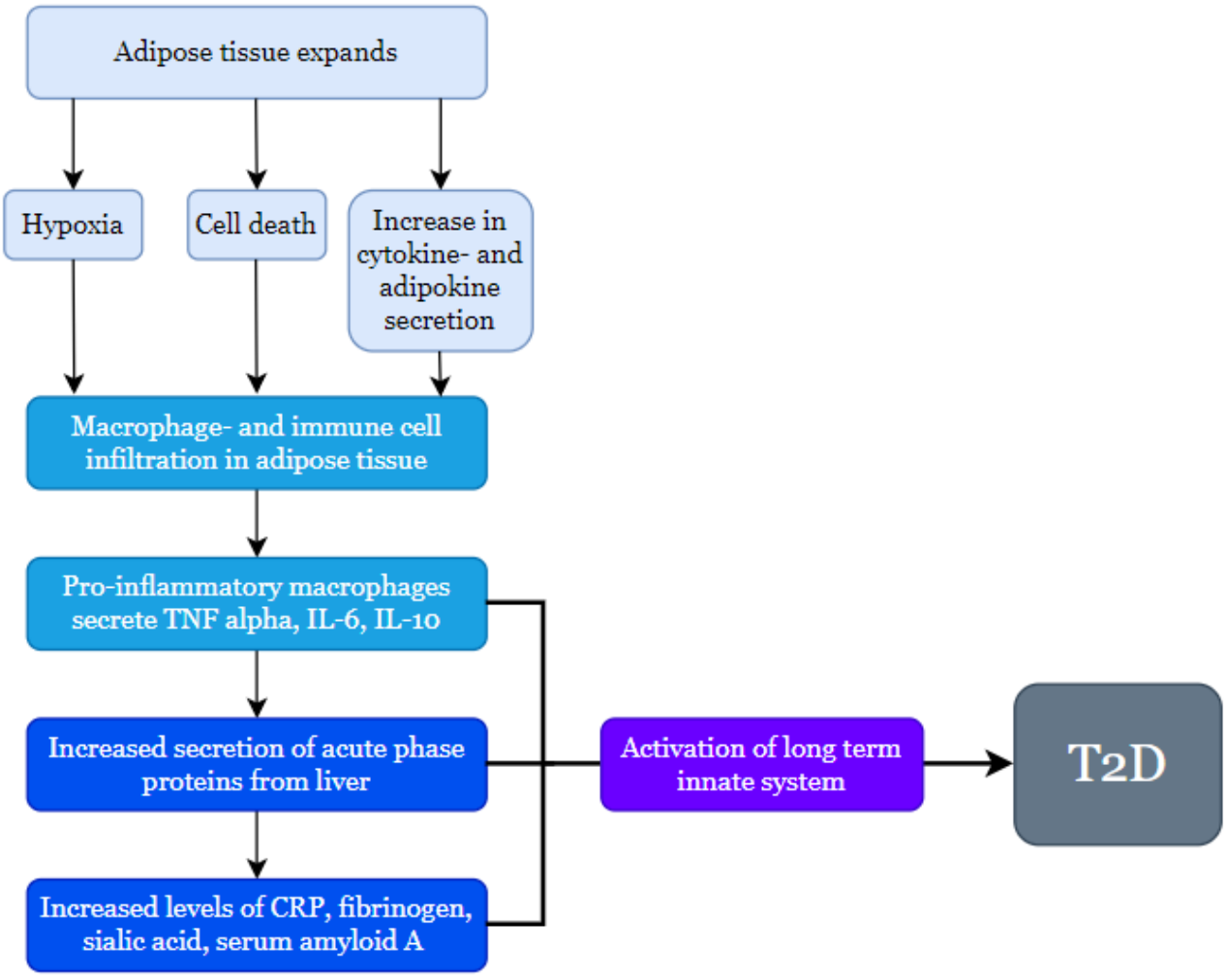


Fig 1. Adipose tissue inflammation and pro-inflammatory mechanisms. IL-6, interleukin-6. IL-10, interleukin-10. CRP, C-reactive protein. T2D, type 2 diabetes. Adapted from Stanirovic et al, 2022 [191]

Weight loss, even to a modest degree, causes positive changes in the circulating levels of adiponectin, leptin and IL-6 [195]. In healthy individuals, a VLCD combined with an exercise program resulted in a weight-loss as

well as an increase in plasma adiponectin and a reduction in plasma leptin over 12 weeks compared to control diet [196], and in young, healthy males, a VLCD (CHO < 50 g/day) without an exercise program over four weeks, there are moderate evidence of decreases in leptin might with a VLCD [197]. Another exercise study (high-intensity interval training, HIIT) over 24 weeks combined four different diets (LCD included) in patients with T2D showed a larger reduction of circulating IL-6- and leptin levels in the LCD-group, while plasma adiponectin improved most in the high CHO low fat diet group [198]. A study on knock-out mice fed a high-fat diet to induce weight-loss showed, that levels of CRP decreased along with reduced insulin resistance [199]. In healthy obese individuals, a LCD for 12 weeks lowered hsCRP compared to a high-carbohydrate low fat diet [200], as well as over 6 months [201].

There are very few studies that compare the effect of a reduced-CHO diet to a control diet on pro-inflammatory markers in T2D. Skytte et al [202], who compared a HPRCD (CHO 30 E% and protein 30 E%) vs. control diet (CHO 50 E% and protein 17 E%) in patients with T2D over 6 weeks in their cross-over trial designed for weight-stability, reported that the two diets did not affect the examined markers differently (IL6, hsCRP and TNF-α). Jonasson et al [203] compared the effect on pro-inflammatory markers of a LCD (CHO 20 E%, fat 50 E%) vs. high-CHO diet (CHO 55-60 E%, fat 30 E%) with caloric restriction in both groups, and found that IL-6 increased with the control diet, while there were no significant effect of these diets on CRP between groups after 6 months. In contrast, Davis et al compared an CHO-restricted diet, with increments in carbohydrates after two weeks with ketosis-induction, to a low-fat control diet (< 25 E% fat) and found no significant changes over time between groups in neither fasting-CRP nor –IL-6 [204].

2.4 Safety issues and adverse effects

In addition to the above-mentioned effect on glycogen storage, LCDs and KDs require a shift to higher protein- and fat utilization. The concentrations of ketones reach steady state after two to three weeks if ketosis occurs. This requires adaptions by both muscles, liver, brain and other tissues, which may last several weeks [77]. During these adaptions, it may include physical complaints such as fatigue, headache, reduced physical performance, constipation or nausea [205, 206]. If nutritional ketosis occurs, a fruity sweet breath may be noticeable. Loss of fluids with glycogen degradation and increased uric acid may lead to dehydration [206]. Patients with T2D are in higher risk of hypoglycemia if treated with insulin or sulphonylurea (SU), and treatment with sodium-glucose co transporter 2 (SGLT2) inhibitors may increase the risk of normoglycemic diabetic ketoacidosis [207]. There have been case reports of this serious adverse event occurring during LCD or KD even in the absence of SGLT2 inhibitor-treatment [208, 209].

Other adverse effects may arise due to restrictions in a variety of foods with reduced fiber intake. Dietary fibers are abundant in grains, whole-wheat products, legumes, fruits and vegetables, with intake decreasing with a higher degree of carbohydrate restriction. This may affect microflora and bowel function, with an increased risk of constipation and in the long-run, potentially colorectal cancer [210]. Reduced dietary fiber have an negative impact on cardiovascular health [211], since dietary fiber benefits glycemic control [212] and improve dyslipidemia in T2D [213]. Restrictions in fruits and some vegetables high in vitamin C and polyphenols, both important antioxidants, could worsen oxidative stress in T2D [214]. Nevertheless, a study over 2 years in patients with T2D has not been able to confirm concerns of vitamin deficits when comparing CHO-restricted diets with high-carbohydrate diet low in fat [215].

The effect of a LCD on physical performance in patients with T2D is largely unknown. LCD may affect physical performance in athletes, and adaptation to fat metabolism occurs over 5-10 days, with maximal adaption within 3-4 weeks [216]. Another concern during LCD is that of increased excretion of calcium in urine, in addition to that nutritional ketosis may increase bone loss through change in blood acidity [71]. This is rejected in a two-year study, where bone mass density (BMD) in hip and spine was unchanged after an LCD (Atkins-style, CHO < 20 g/day with increments each week) compared to a calorie-restricted high-carbohydrate, low-fat diet [217]. With respect to kidney function, there have been concerns regarding potential long-term adverse effects related to an increased protein intake in some CHO-restricted diets. The recommended protein intake for persons with pre-existing kidney disease is below the recommendations for the general population, as protein-rich food causes glomerular hyperfiltration and increases in urinary albumin excretion [218]. However, while persons with pre-existing kidney disease benefit from low-protein diet [218], there is no evidence that a high-protein diet impact kidney health of healthy persons or T2D patients without kidney disease [219].

3. Thesis hypotheses and aims

The aims of this thesis were to evaluate the effects of a 6 month, non-calorie-restricted LCD compared to a non-calorie restricted high-carbohydrate, fiber-rich control diet in patients with T2D. The two diets were evaluated under free-living conditions, and the patients were instructed to maintain their non-insulin glucose-lowering medication and level of physical activity.

More specifically we aimed to test the hypotheses that:

I. A non-calorie–restricted LCD high in fat Improves HbA1c, body weight, body composition and is safe with respect to classical risk factors of CVD such as blood lipid and blood pressure compared to a control diet in patients with T2D.

II. A non-calorie–restricted LCD high in fat adversely affects vascular function, measured as FMD and NID and selected markers of systemic chronic low-grade inflammation compared to a control diet in patients with T2D.

The mean difference in change between groups in HbA1c from baseline to 6 months was the primary outcome of the study, while changes in HbA1c from baseline to 3 months, and changes body weight, body composition and classical cardiovascular risk factors (blood pressure and lipids) from baseline to 3 and 6 months were secondary outcomes. Furthermore, the mean difference in change between groups FMD and NID as well as selected pro-inflammatory biomarkers from baseline to 6 month were prespecified secondary outcomes.

4. Methods

4.1 Study design

This study was designed as an opel-label, parallel-group, randomized controlled trial (RCT) in patients with T2D. They were enrolled between November 2016 until December 2018 at Odense University hospital, and randomized 2:1 to either follow a LCD high in fat, or a control diet high in carbohydrate, that comply with the official dietary guidelines for patients with diabetes in Denmark [220]. The duration of diet change was six months and the study included three visits (baseline, 3 months and 6 months ± 1-2 weeks) and a telephone contact after 2 weeks of diet change. If needed, additional contacts were scheduled.

The participants were randomized through an automatic, internet-based tool (RedCap Randomization module, OPEN, Odense Patient data Exploration Network, Odense, Danmark). Participants were stratified on gender (male/female) and current number of antidiabetic treatments (0-1 or ≥ 2) to avoid gender differences and differences in disease severity. If two family members were enrolled, they were randomized as one unit.

The study was approved by the Regional Committees on Health Research Ethics for Southern Denmark and was executed in accordance with the Declaration of Helsinki Declaration II. The study was registered at ClinicalTrials.gov (NCT03068078).

4.2 Study population

Patients with T2D were recruited through advertisements in local and national newspapers, flyers and posters posted in departments in different hospitals that treat diabetes and in the offices of general practitioners, postings on social media and websites and radio, as well as through the Danish Centre for Strategic Research in Type 2 Diabetes (DD2) cohort at OUH.

Inclusion criteria were a diagnosis of T2D [221], with HbA1c ≥ 48 mmol/mol with or without use of antidiabetic treatment, and duration of T2D from 6 months to 5 years, but up to 10 years if the patient was prescribed ≤ 2 glucose-lowering medications and no insulin. Stable use of antidiabetic treatment ≥ 3 months prior to inclusion was needed for inclusion, as well as well-treated cholesterol (total-cholesterol ≤ 4.5 and/or LDL ≤ 2.5), but this criterion was relieved if the patients could not or would not use cholesterol-lowering medication, hence, with low risk of changes in treatment during the study. The age had to be minimum 18 years, and they had to be able to understand written and spoken Danish, as well as signing an informed written consent.

Exclusion criteria were significant co-morbidities that could interfere with safety or compliance, including a history of cancer < 5 years or treatment with chemotherapy, liver-, cardiovascular- or severe gastrointestinal disease surgically corrected (including bariatric surgery), severe asthma/COPD, a history of

eating disorder or current alcohol overuse. Patients with severe micro- or macrovascular complications to T2D were excluded, e.g. nephropaty (estimated glomerular filtration rate < 30 mikromol/liter), neuropathy, manifest CVD (such as ischemic heart disease or stroke), foot ulcers, severe neuropathy or retinopathy. They were also excluded if they were currently adhering to a low-carbohydrate diet, used antibiotics or glucocorticoid treatment ≤ 2 months before inclusion, had an excessive weight-loss ≥ 10 kg within 3 months before inclusion, or were/planned to be pregnant.

All participants declared their interest in the study by completing a pre-screening questionnaire, where after they were contacted for further screening. Out of 345 screened participants, 73 were enrolled and randomized in the study (LCD n = 50, control n = 23) (Fig. 5). Two pairs of family members were enrolled as one unit (both to LCD). Two participants withdrew consent to participate before the baseline visit, leaving 49 participants in the LCD group and 22 in the control group. Seven participants dropped out during study (LCD n = 5, control n = 2). Thus, 64 participants completed the six months of diet change (LCD n =44, control n = 20).

4.3 Diet interventions

4.3.1 Dietetic intervention

This study was a free-living study, where no food was provided to the participants. The participants were informed about their randomization allocation during the baseline visit, where they all were thoroughly instructed by a clinical dietitian (minimum 1 hour) until they were familiar with- and understood the diet principles. They had access to additional meetings with the dietician if needed.

The dietitian contacted every participant by telephone after one week of diet change, to assess compliance and address challenges, and hereafter once a month. These contacts were performed to ensure compliance with diet, that the participants were making recommended food choices, assessing their calorie intake, evaluating weight stability, current motivation and if there were challenges with the diet. If needed and regardless of group allocation, the participants could receive extra counselling on-demand by either the participant or dietitian. All participants were invited to a 1.5 hour group specific discussion meetings every second month, where participants could exchange recipes or practical tips. A group-specific news letter was mailed every month with updates on new recipes in the data-base, food-items on discount and tips and tricks.

4.3.2 Diet recommendations in the LCD group

The LCD group were instructed to follow a diet composed of 20 E%, 50-60 E% fat and 25-30 E% protein. They were encouraged to increase intake of foods high in monounsaturated fats, such as vegetable oils, nuts,

seeds, fatty fish, avocado and olives and to keep the intake of saturated fatty acids (SFA) as low as possible. CHO-rich foods such as all potatoes, starchy vegetables and –root vegetables, various products from potatoes, rice, pasta, fast-food, cereals, fruits, low-fat products and bread was discouraged, as well as sugary soft-drinks or sweet alcohol.

4.3.3 Diet recommendations in the control group

The control group were instructed to follow the official Danish dietary guidelines, consisting of 50-60 E% carbohydrates, 20-30 E% fat, where less than 10 E% should be from SFA, and 20-25 E% protein [220]. The recommended diet was high-fiber, low-sugar foods such as wholegrain products, vegetables, root vegetables, whole-meal- and rye bread, brown rice and whole meal pasta, but also fish, lean meat, low-fat products, non- or low-fat dairy, light soft drinks and plenty of water was recommended. Intake of alcohol, sugars, sodium rich products, red meat and fast food were recommended to be limited.

Processed carbohydrates and sugar were discouraged in both groups.

4.3.4. Estimation of caloric need

The estimated energy requirement for each participant at baseline was calculated based on sex, height, weight, and level of physical activity, using the Harris Benedict formula [222]. This was used as guideline for a non-calorie–restricted intake during the entire intervention period [223]. The Harris-Benedict formula is widely used in clinical practice and takes basal metabolic rate in to account, based on sex, height, weight and physical activity level [223], with an accuracy of 63 % in overweight people [224]. Physical activity level was the self-reported activity level of the participants, with a score of 1.2 for sedentary activity, 1.4 for light activity, 1.5 for moderate activity, 1.7 for high activity and 1.9 for extremely high activity. Participants were asked not to restrict calories, but to eat until comfortable satiety and maintain weight stability during the intervention period, and the estimates of caloric need from the Harris-Benedict formula were used as a guideline for caloric intake equal to energy expenditure.

4.3.5 Information and control of dietary adherence

Written information was handed out, which included a five-day start-up plan with recipes, folders and pie charts visualizing recommended macronutrient distribution, along with a “quick-list” of recommended foods. The participants were instructed to keep track of their dietary intake through an internet-based food diary (MadLog Aps, Kolding, Danmark), with a recipe database. They were asked to fill out the food diary for four days before start of the diet and three days every month. The dietitian based their recommendations on the data entered in to the food diary. Data was collected at the end of the study.

4.4 Physical activity and analysis of accelerometer data

All participants were instructed to maintain their current level of physical activity thorough out the duration of study. Physical activity was recorded with two 3-axis logging accelerometres (Axivity AX3, Axivity Ltd, Newcastle upon Tyne, UK) attached to the skin on the thigh and lower back during seven consecutive days at baseline and after six months, with 24-hours recording. Participants were reminded of maintaining physical activity level at every visit.

The analysis of data was restricted to the period from 6.00 AM and the following 16, 17 or 18 hours. Wear time was scored through both activity- and temperature monitoring, and with the algorithm described by Skotte et al [225]. The activity types included everyday activities such as sitting, walking, running, going up stairs, cycling and standing, and the different types of activities were divided in to four categories based on activity intensity: 1) 1) sedentary (lying, standing, sitting), 2) light, 3) moderate (time spent in the moderate intensity domain (>light and <vigorous) and 4) vigorous. However, the volumes of moderate and vigorous activity were very low and were combined in the analysis.

4.5 Biochemical analysis and collection of data

Table 3: Overview over visits

Investigations and measurements	*Baseline, Visit 1* T = 0	*Telephone* T = 2 weeks after start of diet	*"On demand"-visit*	*Visit 2* T= 3 months	*End of study* T= 6 months
Antropometrics	●			●	●
DXA-scan	●				●
Dietetic consultation	●	●	●	●	●
Antidiabetic treatment adjustments**	●	●	●	●	●
FMD/NID	●				●
Standard blood test	●			●	●
Venous acid-base status + ketones	●			●	●
Compliance and adverse events		●	●	●	●

* Randomization result only applies on Visit 1

** Insulin and SU adjusted before start of diet in LCD-group and in case of reported significant symptomatic hypoglycemia

4.4.1 Anthropometric data and blood pressure

As shown (Table 3), anthropometric data (waist- and hip circumference and weight) was measured in all attended visits; height was measured without shoes at baseline on a stadiometer (SECA model 216, Munich, Germany), and weight was measured with light clothing without shoes. Waist was measured with a soft measuring-tape mid-abdominal (in the middle between the lowest rib and iliac crest) after expiration and

perpendicular to the floor, while hip circumference was measured over the femoral tuberculi. Blood pressure was measured with MobiloGraph® (IEM GmbH), in an upright sitting position, in total 10 times over 30 minutes. The first measurement was observed by the clinician, and the participant was left alone for the next measurements.

4.4.2 Blood sample analyses

Blood was drawn after an over-night fast and analyzed for HbA1c, plasma glucose, total cholesterol, HDL, LDL, TG, and blood beta-hydroxybutyrate. Fasting plasma glucose was analyzed on Radiometer ABL800 FLEX (Radiometer Medical ApS, Bronshoj, Denmark), while blood beta-hydroxybutyrate was measured using Abbott FreeStyle Precision Neo Blood Glucose and Ketone Monitoring System (Abbott Laboratories A/S, Abbott Diabetes Care). Plasma total cholesterol, HDL-cholesterol and -triglycerides were analyzed on heparinized plasma with absorbtion photometry on Cobas 8000 (Roche Diagnostics International Ltd., California). Plasma LDL cholesterol was calculated with Friedwalds formula. Serum insulin and C-peptide levels were analyzed on Cobas e411 (Roche Diagnostics International Ltd., California).

For analyzes of proinflammatory markers, serum hsCRP was determined by an in-house ELISA (Open Patient data Explorative Network (OPEN) Lab), measured in duplicates and by using commercially available monoclonal antibodies and reagents (Biotechne, R&D Systems, MN, USA) and according to the manufacturers instructions. The optical density was measured at 450 nm with background correction at 540 nm with a VICTOR Nivo multimode microplate reader (PerkinElmer, MA, USA). The limit of detection was 0.05 µg/L. The intra- and inter-assay CVs were below 15%.

The circulating IL-6 was measured in singlets on fasting EDTA plasma by the human high-sensitive IL-6 ELISA assay according to manufacturer's recommendations (R&D Systems, Abingdon, UK). Mean CV% between runs was 6.9% (EDTA plasma pool, level 8.1 pg/ml). CV% of assay controls were 20% (level 0.5 pg/ml), 12.6%, (level 3.2 pg/ml) and 18.5% (level 5.9 pg/ml).

4.4.3 Assessment of body composition

Total and regional body composition at baseline and after six months were assessed by a dual-energy x-ray absorptiometry (DXA) scan (Lunar Prodigy; General Electric Corporation, Madison, Wisconsin). The assessment of fat mass and lean mass in this study was evaluated through the automated estimations of the different compartments, which were estimated for each body region (left arm, right arm, trunk, left leg, right leg and head). The automated estimates of lean mass (g), fat mass (g) and fat percentage (%) were used. Mass was recalculated to kilos. Arms- and legs lean- and fat mass were calculated as left and right limb combined. As the percentage lean mass was not automatically estimated, whole-body lean mass (%) was calculated as (lean mass (g)/ whole body mass (g)) x 100.

4.5 FMD-and NID measurements

4.5.1 Participant preparation

Three days before FMD-assessment blood pressure lowering medication, sildenafil and vitamins were discontinued. Furthermore, the participants were instructed to refrain from strenuous exercise, chocolate, juice and tea for 48 hours and coffee, alcohol and nicotine for 12 hours prior to the ultrasound assessment, which was performed after minimum 8 hours of fasting. Modest intake of water was allowed up to two hours before examinations. Any ongoing cholesterol-lowering treatment was continued. If the participant failed to adhere to preparations, scans was assessed on another time-point.

4.5.2 FMD- and NID assessments

Endothelial function was assessed by a single operator with measurement of flow-mediated vasodilation and nitroglycerine dependent vasodilation in the brachial artery at baseline and after 6 months. A Phillips iE33 ultrasound machine with a linear array L15-7io probe and automated settings for FMD- and NID assessment was used. A Hokanson Rapid Cuff inflation System was used (Hokanson E20, Bellevue USA), and the cuff is attached with the proksimal edge at the elbow crease on the right arm (Fig. 2). The participant rested in a supine position for at least 15 minutes in a comfortable temperature. The brachial artery in the upper arm was scanned three times 60 seconds to measure baseline, lifting the transducer between each recording. The cuff was inflated minimum 25-50 mmHg above systolic pressure (minimum 200 mmHg) and pressure was maintained for five minutes, inducing forearm ischemia. Recording recommenced five seconds before cuff deflation and continued for five minutes. Anatomical markers in the upper arm were noted at baseline.

To assess nitroglycerine dependent dilation (NID), the participant rested for 15 minutes after FMD-measurement and resting diameter was again assessed three times (one minute each). The participant received a sublingual dose of 0.4 mg nitroglycerine, and diameter changes were recorded continuously for 9.4 minutes.

The limit for endothelial dysfunction was set to below 7.1 % for FMD and below 15.6 % for NID [149].

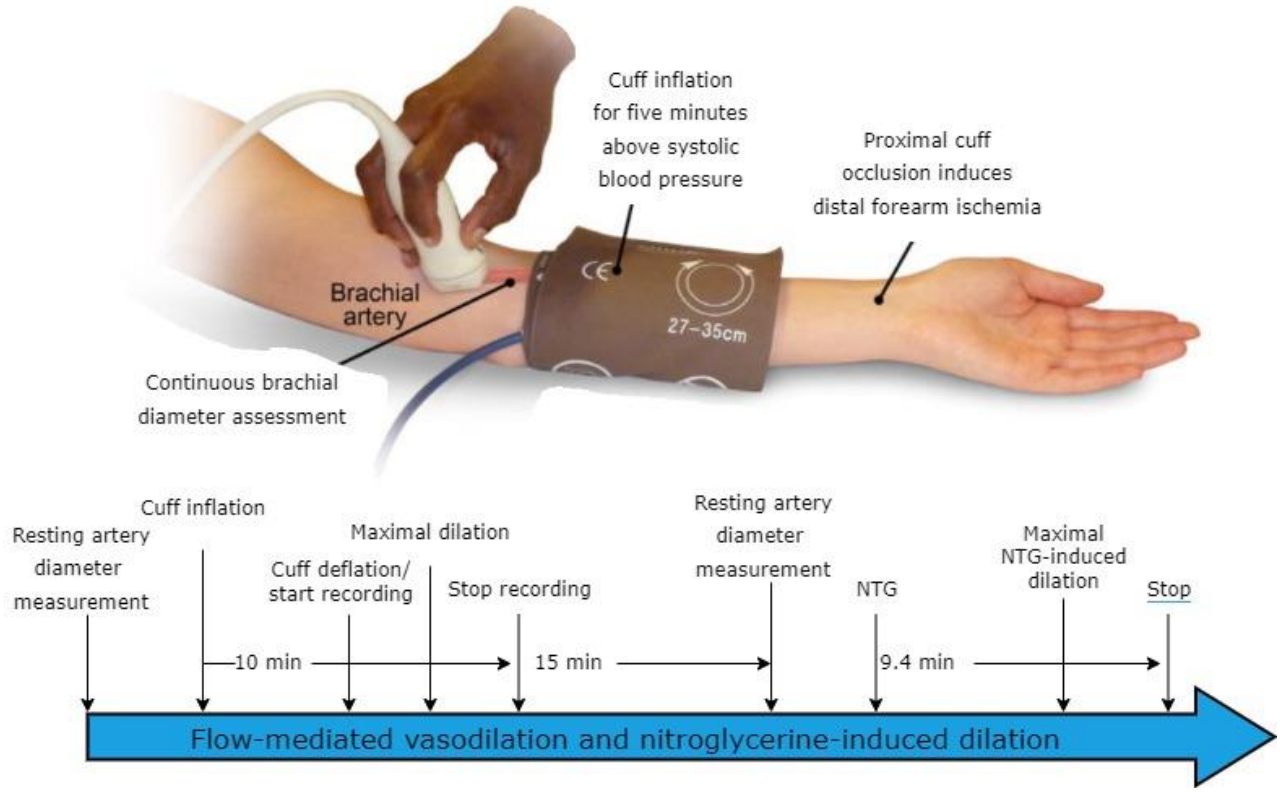


Fig 2: FMD- and NID assessment. Image modified, source *Cardiovascular regulation lab (https://cvlab-nau.mozello.com)*.

4.5.3 Post-examination analyses

Captured files were exported as DICOM or .avi-files for offline analysis with the semi-automated MIA LLC Brachial Analyzer version 6.9.1 software was used (Medical Imaging Applications LLC, Coralville, Iowa, USA), with automated edge-detection software as well as the automated end-diastolic diameters instrument. Analyzes were executed by the same operator who performed the scans, but blinded for patient ID and clinical data. FMD was calculated as the percent change from the resting artery diameter in response to reactive hyperemia after cuff deflation, and NID was calculated as the percentage change in response to nitroglycerine compared to baseline.

$$\text{FMD} = \frac{(Maximal\ diameter - Resting\ diameter)}{Resting\ diameter} * 100$$

The automated edge-detection of the near- and far wall in the MIA LLC-software was used. The region of interest (ROI), a rectangular box (Fig. 3) was defined manually on the first sequence and was placed on the part of the a. brachialis with best visualization of the edges. The ROI was sought to be kept constant through analyzes of all sequences, unless there was apparent movement. If necessary, quality control was used with confidence threshold 70 % and 2 x SD in the MIA LLC.

The mean baseline diameters was calculated from the means of end-diastolic diameters of the one-minute scans of the brachial artery (FMD RD and NID RD). FMD peak dilation (FMD max) was identified by visual inspection (Fig. 4) and calculated as a mean of minimum five consecutive end-diastolic diameters. NID peak dilation (NID max) was the mean of one recording (1 minute and 34 seconds) after reaching maximal peak nitroglycerine-stimulated dilation.

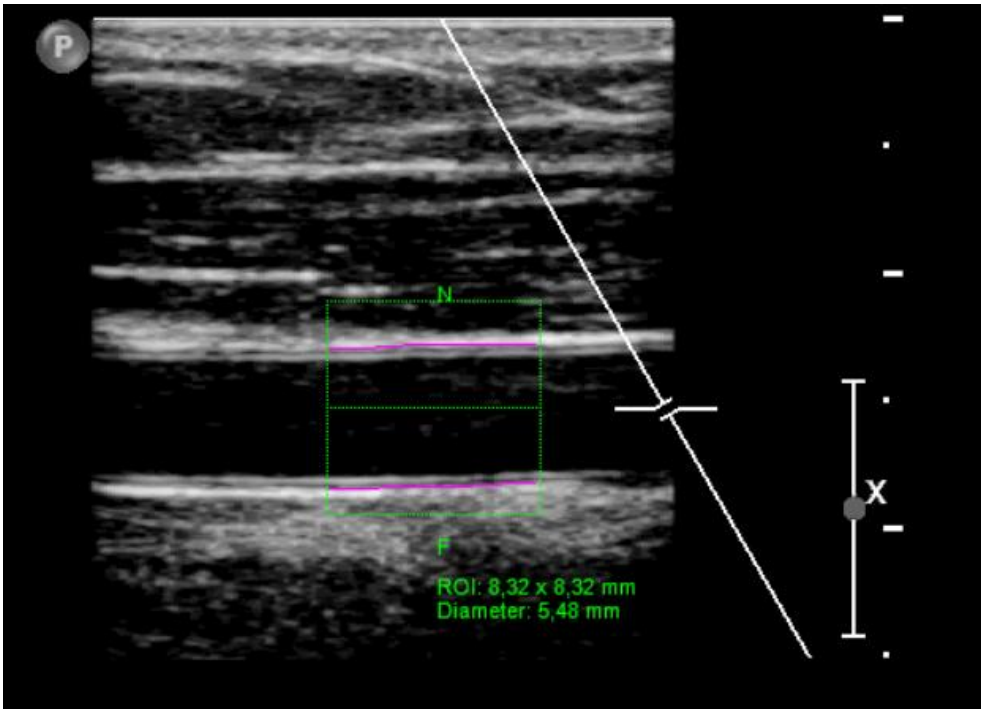


Fig. 3: ROI and edge detection in offline examinations wil MIA LLC in a recorded FMD-assessment of the brachial artery.

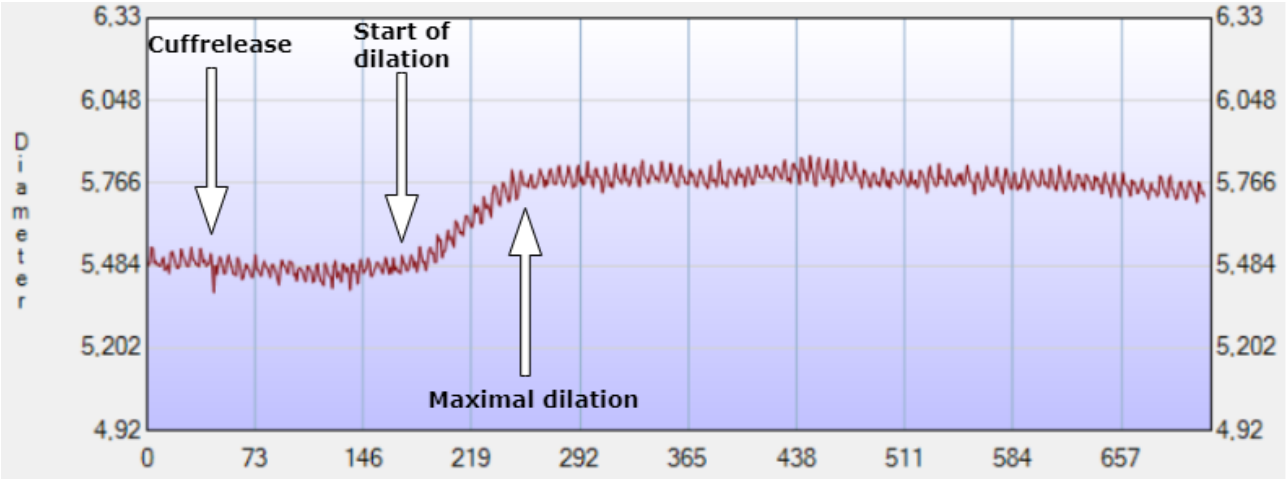


Fig. 4: Continuous measurements of the brachial artery diameter during a FMD-scan. Y-axis, diameter. X-axis, number of frames in the recording.

To ensure sufficient intra-observer reliability, FMD was assessed on 12 random volunteers on two consecutive days. The resulting intra-observer reliability coefficient was 0.9601, which was evaluated as an acceptable accuracy [226].

Table 4: FMD measurements on 12 volunteers on two consecutive days

	Day 1			Day 2		
Volunteer	FMD RD (mm)	FMD max (mm)	FMD (%)	FMD RD (mm)	FMD max (mm)	FMD (%) (%)
01	3.65	3.84	5.11	3.76	3.96	5.32
02	5.84	6.05	4.19	5.87	6.12	4.32
03	4.87	4.96	1.91	4.96	5.07	2.21
04	4.20	4.42	5.15	4.20	4.40	4.76
05	4.96	5.29	6.58	5.04	5.36	6.39
06	3.44	3.67	6.58	3.44	3.66	6.40
07	5.13	5.32	3.70	5.11	5.29	3.59
08	4.37	4.72	8.01	4.39	4.75	8.28
09	4.47	4.74	6.04	4.49	4.70	4.52
10	4.13	4.22	2.10	4.29	4.35	1.40
11	4.29	4.61	7.46	4.30	4.60	6.98
12	4.37	4.52	3.27	4.36	4.50	3.13

4.6 Glucose lowering medication and safety monitoring

All participants were instructed to measure blood glucose regularly and were informed about typical signs of hypoglycemia. If they experienced symptoms of hypoglycemia, they were asked to measure blood glucose and to contact the study staff if hypoglycemia was present to evaluate the need to reduce or discontinue glucose lowering medication to ensure glycemic safety. Documented symptomatic hypoglycemia was defined as symptoms of hypoglycemia and a plasma glucose ≤ 3.9 mmol/l. whereas clinically important hypoglycemia was defined as a plasma glucose ≤ 3.0 mmol/l. Severe hypoglycemia was defined as the need of assistance of another person or hospitalization to recover [227]. To minimize risk of hypoglycemia in the LCD group, insulin treated participants (n = 2) had their daily dose insulin lowered with 20 % at baseline. They were instructed to report plasma glucose measurements once weekly until glycemic stability without hypoglycemia was ensured. The participants treated with sulfonylureas (SU) in the LCD group (n = 4) were asked to reduce the dose if they experienced hypoglycemic events, and to report plasma glucose and/or symptoms in the same manner as the participants treated with insulin. Participants treated with SGLT-2

inhibitors with normal or low BMI were informed about symptoms of euglycemic diabetic ketoacidosis and were provided with a ketone measuring device (FreeStyle Precision, Abbott, Alameda, CA, USA) along with compatible measuring sticks.

The use of glucose-lowering drugs with a low risk of hypoglycemia (e.g. metformin, dipeptidyl peptidase-4 (DPP-4) inhibitors, GLP-1 receptor agonists and SGLT-2 inhibitors) were continued without adjustment in the majority of participants, unless their general practitioner or the participants themselves changed dose. However, there were changes in the use of glucose lowering drugs in both in the LCD group, with metformin (n = 3), GLP-1 (n = 1) or SU (n =3) as well as in the control group with DPP-4 inhibitors (n =1), insulin (n =2) and GLP-1 receptor agonists (n = 1) (the latter with a concomitant change in insulin dose). Participants were provided with extra test strips if needed, and a glucose measuring-device (Bayer Contour® Next One (Ascensia Diabetes Care Holdings, Switzerland) with test strips and instructions in correct use if needed.

The participants were also instructed to report if they had any potential adverse events as part of safety monitoring, hospital visits or consults with their general practitioner that might be related to the diet, affect well-being or compliance.

In addition, the participants were asked if they experienced symptoms in relation to the transition from their usual diet to either the LCD or the control diet. The responses are shown below (Table 5).

Table 5: Overview over experienced symptoms associated with transition to LCD or control diet after 2 weeks

Symptom	LCD (n = 48)*	Control (n = 22)	p-value
Tiredness, n (%)	11 (22.9)	1 (4.5)	0.06
Irritability, n (%)	4 (8.3)	0 (0.0)	0.16
Dizzynes, n (%)	6 (12.5)	0 (0.0)	0.08
Ortostatism, n (%)	3 (6.3)	0 (0.0)	0.23
Headache, n (%)	9 (18.8)	0 (0.0)	0.03
Trouble concentrating, n (%)	3 (6.3)	0 (0.0)	0.23
Flushing, n (%)	4 (8.3)	0 (0.0)	0.16
Palpitations, n (%)	1 (2.1)	0 (0.0)	0.50
Changed taste, n (%)	7 (14.6)	0 (0.0)	0.06
Bad breath, n (%)	4 (8.3)	0 (0.0)	0.16
Hunger, n (%)	1 (2.1)	0 (0.0)	0.50
Change in sleep pattern, n (%)	4 (8.3)	0 (0.0)	0.16

Abdominal symptoms, n (%)	10 (20.4)	0 (0.0)	
Abdominal discomfort	2 (4.1)	0 (0.0)	
Nausea	1 (2.1)	0 (0.0)	
Heartburn	3 (6.3)	0 (0.0)	
Diarea	3 (6.3)	0 (0.0)	
Obstipation	0 (0.0)	0 (0.0)	
No symptoms, n (%)	12	21	0.04

*One dropout before collection of data

4.7 Statistics

All statistical analyses were performed using STATA version 16.0 (Stata Corporation, College Station, TX).
At baseline, continuous variables were tested for normality and compared with Student's t-test for unpaired data, while categorical variables were compared by Fisher's exact test. To compare the effect of the LCD to the control diet on continuous repeated measured variables, in an intention-to-treat analysis, a linear mixed-model approach was applied with both random and fixed effects, to allow for incomplete data due to non-completers. A treatment-by-time interaction was added. Results are reported as mean difference in change (MDIC) corresponding to β-coefficient ± SE between groups from baseline to the 3- and 6 months points, respectively. The residuals of the fitted values and the best linear unbiased predictions (BLUPs) of the random effects were examined for normality. If normality was violated, variables were log transformed and median and IQR is stated. Data other than the β-coefficients are presented as means ± SEM. Significance was accepted at $p<0.05$. For the analyses of correlation between cardiovascular risk factors and FMD or NID at baseline, this was tested with univariate linear regression and the beta-coefficients ± SEM are reported.
The intra-observer reliability coefficient was computed with a two-way mixed model.
The sample size was based on the primary outcome HbA1c and was originally calculated using a SD of 13 mmol/mol. However, after enrollment of 65 participants (LCD; n=45 and control diet; n=20), it was recognized, both from previous studies and our own observations, that the SD was lower than originally assumed. The sample size was, therefore, recalculated using a SD of 10 mmol/mol. Using this new SD together with a reduction in HbA1c of 7.7 mmol/mol as the minimal clinically important difference, a 2:1 ratio of participants from the LCD versus the control group, and a maximal dropout rate of 10%, we estimated that 36 in the LCD group and 18 in the control group would be needed to obtain a power of 80 %. Another eight patients were enrolled, as they had already been invited to participate.

4.8 Methodological considerations

4.8.1 Randomization and study duration

The unequal 2:1 approach was chosen for several reasons. We expected a higher drop-out rate in the LCD group suggesting that a larger number of participants in the LCD group would be necessary, whereas it was assumed that it would be easier for participants randomized to the control diet to adhere to the diet. Another reason for choosing the 2:1 randomization was the expectation that participants, who were interested to be included in the study, would be more willing to be enrolled if the chance of allocation to the LCD diet was higher, as carbohydrate-restricted diets were frequently positively mentioned in the media in the period before study start. The six months intervention period was chosen to evaluate the full effect of the diet changes, as the participants were expected to be able adhere to the LCD for this period with the least risk of deviations from the diet instructions. As discussed above, studies with longer duration than six months may experience problems with deviations from the diet instructions.

4.8.2 Inclusion- and exclusion criteria

The criteria of T2D with a duration of maximum 5 years if insulin treated and 10 years if not was based on the assumption that loss of glycemic control and need for insulin treatment in patients with T2D indicate a severe progression of the disease with loss of beta-cell function and a higher risk of the presence of microvascular complications [228]. The cholesterol-criteria was based on the anticipation that cholesterol lowering medicine would not change during course of study if the patients cholesterol was in the range recommended for patients with T2D at inclusion, preferably with use of statins. The selected exclusion criteria were based on finding participants without diseases that could interfere with the adherence to the diets during the course of the study, as well as with the effects of the change in diet.

4.8.3 Power calculations

The power calculation was based on the primary outcome, HbA1c. Initially, this suggested that 135 participants were needed for 2:1 randomization. However, during course of study, it became evident that the power calculation were too apprehensive and these calculations were adjusted to obtain a mean difference in change of HbA1c of 7.7 mmol/mol. Using a SD of 10 mmol/mol, a maximal drop-out rate of 10 %, a 2:1 ratio of participants from the LCD versus the control group and aiming for a power of 80%, we estimated that 36 participants were needed in the LCD group and 18 in the control group.

4.8.4 Dietary considerations and collection of data on food intake

It could be argued that recommendations were in accordance with a low-carbohydrate, high-protein diet. The intention were, however, not to increase protein intake and thereby satiety, but to avoid to many restrictions on the intake of high-fat products, since a multitude of foods high in fat are also high in protein (cheeses, greek yoghurt, nuts and seeds, "paleolitic bread", eggs and fatty meat/poultry/fish). Milk products, butter, fat meat and eggs are common items in a Danish diet, but these kind of foods are also high in SFA. Although an increased intake of MUFA and a minimal intake of SFA were encouraged, the protocol did not include minimal recommendations of food items high in MUFA, such as olive- or rape seed oil, or specific recommendations on how to limit SFA intake to a minimum. It is notoriously hard to ensure compliance of diet and collecting data, which is why the dietary data should be interpreted with caution.

4.8.5 Adjustment of anti-diabetic medication

When this project was planned, there was limited knowledge on adjustments of antidiabetic treatment during CHO-restricted diets, with the first recommendations of adaption of antidiabetic medication during a LCD in T2D published in 2019 [229]. The 20 % reduction in insulin dose in the LCD group was based on a clinical estimate, but would of course have been further adjusted if necessary. The majority of the participants continued their diabetes management at the general practitioner. It could have been more convenient to advice the participants not to do so, as some participants was adjusted in anti-diabetic medication by the general practitioner during the study period. We can only speculate if this would have been an advantage for recruiting participants or the adherence to the diets, as most patients have high trust in their general practitioner and general practitioners in turn prioritize to maintain regular controls according to guidelines.

4.8.6 Collection of data

The blood pressures reported were measured in extension of the FMD- and NID measurements. The recommendations for FMD- and NID measurements states that antihypertensive medication should be withhold 24 hours or four half lives [230]. Our instruction to discontinue antihypertensive treatment for 3 days equals at least around three half-lives for the most commonly used antihypertensive drugs (calcium inhibitor, angiotensin-converting enzyme inhibitor and metoprolole) and over four half-lives for thiazide. Antihypertensive treatment may have a positive effect on FMD measurements [231]. However, the recommendations for FMD assessment published in 2019, states that when "drug-intake cannot be avoided", the timing of the FMD assessment must be consistent and standardized [232]. Three days absence of antihypertensive treatment did not put the participants at risk of symptomatic hypertension, was easy to

remember for better compliance and was repeated for the comparison six months later. However, the blood pressure measured was measured in extension of the FMD examinations both at baseline and after 6 months, and it could be argued that it was a methodological error not to instruct the participants to perform home-measurements, as this would have provided useful information.

For the FMD-measurements, the high-resolution L15-7io linear array probe was used. This probe allows for an insonation angle of 90 degrees, as it has a steerable pulse Doppler. However, due to the high-quality pictures, it only allowed for recording one minute and 34 seconds at a time, with need to record multiple sequences. It is designed for more superficial structures, but some participants brachial arteries was situated below 2-3 cm of subcutaneous fat, with the effect that the endothelium and media was harder to distinguish from each other in the automated edge detection software. However, it was sufficient for most participants arms, with high-quality, precise pictures. The pen-like design made it difficult to use a probe-holder, which may have yielded more precise results after cuff release.

The cut-off values of < 7.1 % for FMD and < 15.6 % for NID as defined for endothelial dysfunction are retrieved from a study in Japanese individuals with and without known risk factors for CVD [149]. These cut-off values may not be accurate to describe the population in this study, who were mainly Caucasian. Reference values for FMD- and NID-cut-off values were lacking [233] until Heiss et al [234] suggested cut-off values to define a FMD < 6.5 % as “impaired” and FMD < 3.1 % as “pathological” in 2022, but without a reference value to define an impaired NID. We recognize that the cut-off values stated by Maruhashi et al may not be true for this sample with T2D.

5. Results and discussion

In this section, the effects of a LCD with < 20 E% CHO and 50-60 E% fat in patients with T2D, compared to a control diet following the official Danish dietary guidelines [220] over 6 months will be presented and discussed. Our aim was to examine the effect of a LCD on glycemic control, body weight, body composition and classical cardiovascular risk factors as well as endothelial function and selected pro-inflammatory markers. The main focus in paper I of this work was the effect of a LCD on the primary outcome HbA1c, while seeking to maintain non-insulin glucose-lowering medication, physical activity level and caloric intake in both diet arms. Furthermore, in paper II, we wanted to investigate the effect of a LCD on FMD and NID as well as selected markers of low-grade inflammation, as there have been concerns that an increased fat intake may adversely affect risk factors of atherosclerosis and CVD. Our aim was to conduct the study in a manner that could be replicated in a clinic with access to a licensed dietician, and this study was free-living, where no food was provided to the participants. Both papers will be presented and discussed, and this part of the thesis is divided into sections where the results of paper I and paper II are presented and discussed separately, followed by overall conclusions.

5.1 Paper I: Effects of a 6-month, low-carbohydrate diet on glycaemic control, body composition, and cardiovascular risk factors in patients with type 2 diabetes

5.1.1 Study cohort and baseline characteristics

From November 2016 until December 2018, 347 persons were available for screening and 73 participants were enrolled and randomized in the study (Fig. 5). Two patients withdrew before commencing to the study, leaving 71 patients for inclusion in the intention-to-treat analysis, which was used to assess primary outcomes. At the baseline visit, both groups were comparable in age, sex, diabetes duration and use of glucose and blood pressure lowering medication (Table 6). The proportion of SGLT2 inhibitor treated patients was not significantly higher in the LCD group ($p = 0.15$). The majority of participants in both groups were using at least one type of anti-hyperglycemic pharmacotherapy. A high proportion of the participants in both groups were not treated with cholesterol-lowering treatment with no significant difference between groups (LCD = 44.9 %, control = 45.5 %). At baseline, there were no differences between groups in body weight, BMI and waist or hip circumference. Moreover, the fasting levels of HbA1c, plasma glucose, serum C-peptide and insulin as well as blood lipids were similar in the two groups at baseline (Table 7). Systolic blood pressure was higher in the control group compared with the LCD group.

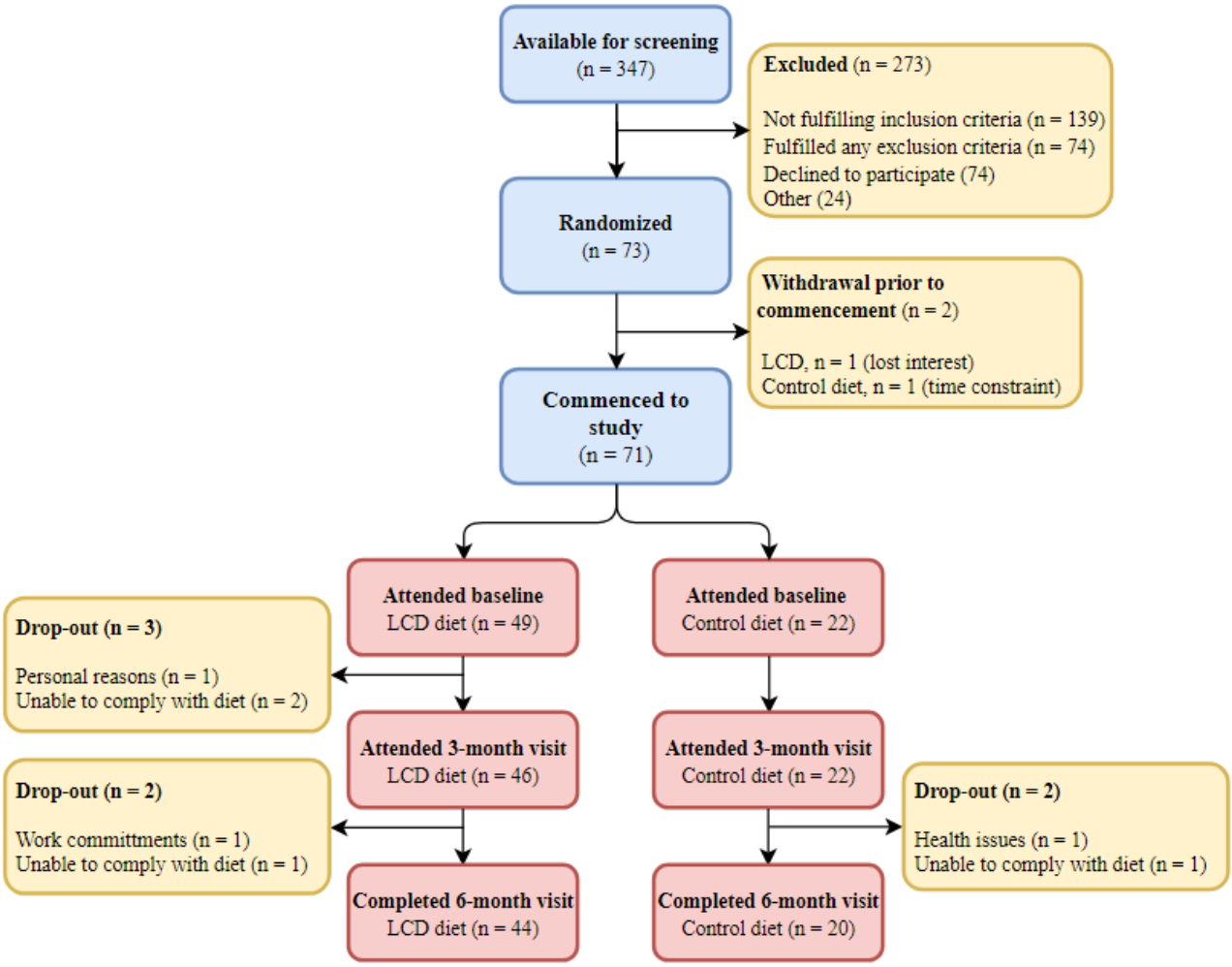


Fig. 5. Inclusion, exclusion and drop-outs flow chart

Table 6: Baseline characteristics of participants

Characteristic	LCD (n = 49)	Control (n = 22)
Age, years	57.3 ± 0.9	55.2 ± 2.7
Men (%)	22 (44.9)	9 (40.9)
Duration of diabetes, years	5.2 ± 0.5	5.0 ± 0.5
Hypertension	32 (65.3)	16 (72.7)
Smoking status		
Never smoker	24 (49.0)	11 (50.0)
Previous smoker	23 (46.9)	10 (45.5)
Current smoker	2 (4.1)	1 (4.5)
Glucose-lowering therapy		
Metformin	40 (81.6)	16 (72.7)
Sulfonylurea	4 (8.2)	0 (0.0)
DPP-4 inhibitor	6 (12.2)	3 (13.6)
GLP-1 receptor agonists	9 (18.4)	6 (27.3)
SGLT-2 inhibitor	10 (20.4)	1 (4.5)
Insulin	2 (4.1)	3 (13.6)
No. of glucose-lowering agents		
0	5 (10.2)	4 (18.2)

1	23 (46.9)	11(50.0)
2	16 (32.7)	4 (18.2)
≥ 3	5 (10.2)	3 (13.6)
Blood pressure-lowering therapy		
ACE inhibitor/ Angiotensin receptor blocker	28 (52.1)	14 (63.7)
Calcium-channel blockers	10 (20.4)	6 (27.3)
Thiazides	16 (32.7)	4 (18.2)
Beta-blockers	6 (12.2)	2 (9.1)
Cholesterol-lowering treatment	27 (55.1)	12 (54.5)

Abbrevations: DPP-4, dipeptidyl peptidase-4. GLP-1, glucagon-like peptid-1. SGLT-2, selective sodium glucose co transporter-2. ACE, angiotensin-converting enzyme. *p<0.05 and **p<0.01

5.1.2 Glycemic variables

The LCD diet over 6 months [235] induced a larger reduction in HbA1c already after 3 months of diet change compared to the control group (MDIC= -8.9 ± 1.7 mmol/mol, p < 0.001) and this effect was sustained after 6 months (MDIC = -7.5 ± 1.7, p < 0.001), (Fig. 6). This effect on HbA1c was not attenuated after adjustments for coexisting covariates such as medication, age, sex, diabetes duration and smoking, either after 3 months (β = -9.7 ± 1.7 mmol/mol, p < 0.001) or 6 months (MDIC = -7.7 ± 1.8 mmol/mol, p < 0.001. When excluding patients treated with SU or insulin in the analysis, the larger effect of LCD on HbA1c also persisted (MDIC = -9.6 ± 1.6 mmol/mol; P ≤ .0001) after 3 months and (MDIC = -8.7 ± 1.7 mmol/mol; P ≤ .0001) after 6 months. The effect of LCD versus control diet on HbA1c was still highly pronounced when excluding patients, who were not taking antidiabetic medication (β = -8.3 ± 1.8 mmol/mol; P ≤ .0001 after 3 months and MDIC = -7.6 ± 1.8 mmol/mol; P ≤ .0001 after 6 months).

The fasting plasma glucose levels measured on-site were not more improved in the LCD group compared to the control group after 3 or 6 months (p > 0.05 at both time points) (Table 7). However, self-reported blood glucose levels before meals and night improved in the LCD group compared with the control group at almost all time points after both 3 and 6 months. The levels of blood ketones measured on-site increased in the LCD group compared with the control group after 3 months (MDIC = 0.27 ± 0.09 mmol/l, p = 0.002), but this effect was transient and not sustained at 6 months (MDIC = 0.13 ± 0.08 mmol/l, p = 0.150). The change in HOMA-IR in response to the diets did not differ between groups, neither after 3 months (MDIC = -1.7 ± 1.6, p = 0.272) nor after 6 months (MDIC = -2.0 ± 1.6, p = 0.228). In line, fasting serum C-peptid levels did not respond differently to the diet, neither after 3 months (MDIC = -112 ± 89 pmol/l, p = 0.206) nor 6 months (MDIC = -93 ± 91 pmol/l, p=0.305).

Table 7: Glycemic control, cardiovascular risk factors and body composition [235]

Variable	Baseline		3 months			6 months		
	LCD (n = 49)	Control (n = 22)	LCD (n = 46)	Control (n = 22)	Mean-difference in change	LCD (n = 44)	Control (n = 20)	Mean-difference in change
HbA1c (mmol/mol)	54.3 ± 1.4	56.1 ± 1.5	43.7 ± 1.0	54.3 ± 1.8	-8.9 ± 1.7***	43.6 ± 1.0	53.2 ± 2.1	-7.5 ± 1.8***
Fasting plasma glucose (mmol/l)	8.6 ± 0.3	9.3 ± 0.4	7.2 ± 0.2	8.6 ± 0.4	-0.6 ± 0.4	7.2 ± 0.3	8.7 ± 0.5	-0.7 ± 0.4
Serum insulin (pmol/l)	173 ± 38	156 ± 19	148 ± 30	165 ± 21	32 ± 22	136 ± 30	158 ± 25	- 35 ± 23
HOMA-IR	9.7 ± 2.4	9.4 ± 1.4	7.1 ± 1.6	8.7± 1.1	-1.7 ± 1.6	6.8 ± 1.7	8.8 ± 1.9	-2.0 ± 1.6
Serum C-peptide (pmol/l)	1251 ± 88	1285 ± 77	1147 ± 93	1322 ± 96	-112 ± 89	1070 ± 75	1218 ± 77	-93 ± 91
Blood ketones (mmol/l)	0.25 ± 0.02	0.23 ± 0.02	0.48 ± 0.07	0.19 ± 0.27	0.27 ± 0.09**	0.32 ± 0.03	0.18 ± 0.02	0.13 ± 0.08
Systolic Blood Pressure (mmHg)	134 ± 2*	142 ± 3	133 ± 2	137 ± 3	3.2 ± 3.1	131 ± 2	136 ± 3	0.6 ± 3.2
Diastolic Blood Pressure (mmHg)	85 ± 1	86 ± 2	86 ± 1	84 ± 2	2.9 ± 2.0	84 ± 1	84 ± 2	1.5 ± 2.1
Heart rate (beats/min)	77 ± 2	82 ± 4	71 ± 2	74 ± 3	0.0 ± 2.2	71 ± 2	78 ± 3	-1.1 ± 2.3
Serum LDL (mmol/l)	2.3 ± 0.1	2.4 ± 0.2	2.3± 0.1	2.2 ± 0.2	0.2 ± 0.1	2.4 ± 0.1	2.2 ± 0.2	0.3 ± 0.2
Serum HDL (mmol/l)	1.2 ± 0.04	1.1 ± 0.06	1.3 ± 0.04	1.1 ± 0.05	0.1 ± 0.4*	1.3 ± 0.04	1.1 ± 0.07	0.1 ± 0.0
Serum triglycerides (mmol/l)	1.91 ± 0.17	2.12 ± 0.25	1.42 ± 0.11	1.93 ± 0.22	-0.28 ± 0.22	1.42 ± 0.11	1.66 ± 0.15	-0.05 ± 0.23
Serum total cholesterol (mmol/l)	4.3 ± 0.1	4.4 ± 0.2	4.2 ± 0.1	4.1 ± 0.2	0.2 ± 0.2	4.4 ± 0.1	4.1 ± 0.2	0.3 ± 0.2
BMI ($kg \cdot m^2$)	32.5 ± 0.9	35.2 ± 1.4	30.8 ± 0.9	34.5 ± 1.4	-1.2 ± 0.3***	30.7 ± 0.9	34.9 ± 1.5	-1.4 ± 0.4***
Weight (kg)	97.7 ± 3.2	102.1 ± 4.4	92.7 ± 3.3	99.8 ± 4.1	-3.3 ± 1.0***	92.6 ± 3.5	101.9 ± 4.4	-3.9 ± 1.0***
Waist circumference (cm)	110.9 ± 2.1	114.8 ± 3.0	103.7 ± 2.2	112.0 ± 3.6	-3.5 ± 1.2**	103.2 ± 2.3	114.0 ± 3.3	-4.9 ± 1.3***
Hip circumference (cm)	108.4± 1.7	113.4 ± 2.9	107.5 ± 1.9	113.4 ± 3.2	-1.4 ± 1.2	104.9 ± 1.8	112.2 ± 3.2	-2.0 ± 1.2

* $p < 0.05$, **$p < 0.01$, ***$p < 0.001$. BMI, body mass index.

5.1.3 Cardiovascular risk factors

The LCD in patients with T2D over 6 months did not produce clinical relevant changes in either systolic blood pressure, diastolic blood pressure, LDL, TG or heart rate compared to the control diet (all $p > 0.05$) (Table 7, Fig. 6). A small but significant increase on HDL was observed after three months in the LCD group compared with the control group (MDIC = 0.1 ± 0.4 mmol/l, $p = 0.031$), but this effect of LCD was not sustained after six months.

5.1.4 Body composition

The LCD diet induced a significant weight-loss after both 3 months (MDIC = -3.3 ± 1.0 kg, $p = 0.004$) and 6 months (MDIC = -3.9 ± 1.0 kg, $p < 0.001$) compared to the control diet [235], as well as in waist circumference at both time points (both $p < 0.004$) (Table 7). BMI decreased in response to LCD after both 3 months (MDIC = -1.2 ± 0.3 kg/m^2, $p = 0.001$) and after 6 months (MDIC = -1.4 ± 0.4 kg/m^2, $p < 0.001$) compared to the control diet. The LCD reduced both total fat mass of (MDIC =-2.2 ± 1.0 kg, $p = 0.027$) as well as total lean mass (MDIC = -1.3 ± 0.6 kg, $p = 0.017$) compared with the control diet after 6 months. These changes in body composition were partly explained by a larger decrease in the abdominal fat mass (MDIC = -1.5 ± 0.6 kg, $p = 0.022$) after 6 months and the lean abdominal mass (MDIC= -1.1 ± 0.04 kg, $p = 0.013$ after 6 months) in the LCD group versus the control group. The body fat percentage decreased significantly with the LCD compared with the control diet (MDIC =-1.2± 0.6, $p = 0.043$).

5.1.5 Dietary data, physical activity and hypoglycemia

Participants on the LCD reduced their self-reported carbohydrate intake to ~ 14 E% at both time points and increased their fat intake to ~63 E%. Thus, the LCD group markedly reduced their carbohydrate intake and increased their fat intake compared to the LCD group after both 3 and 6 months (all $p<0.001$). This resulted in a ~2.6 fold higher intake of SFA in the LCD Group than the control group after 6 month (Table 8). There was also a significant increase in the intake of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) as well as a reduction in the intake of dietary fibers in the LCD group compared with the control group after 3 and 6 months ($p < 0.001$). Both groups maintained their level of physical activity in all categories of activity intensity (sedentary, light and moderate/ vigorous) (all $p > 0.27$) (Suppl. tables and figures, Paper I). One participant from each group had documented symptomatic hypoglycemia and one patient in the LCD group had an episode with clinically important hypoglycemia, but none of the participants experienced severe hypoglycemia with need for medical assistance.

Table 8: Nutritional intake in the groups

	Baseline		0-3 months		3-6 months	
Variable	LCD (n = 36)	Control (n = 19)	LCD (n = 45)	Control (n = 21)	LCD (n = 39)	Control (n = 19)
Kcal per day	1805 ± 77	1840 ± 97	1701 ± 52	1664 ± 104	1642 ± 62	1600 ± 119
Carbohydrates (E%)	42.1 ± 1.2	45.9 ± 1.7	12.5 ± 1.0***	48.5 ± 0.7	13.4 ± 1.2***	48.4 ± 1.0
Protein (E%)	19.4 ± 0.8	18.9 ± 0.8	22.9 ± 0.6	21.4 ± 0.8	23.4 ± 0.7	22.5 ± 1.0
Fat (E%)	38.2 ± 1.2	34.5 ± 1.9	64.5 ± 1.0***	29.5 ± 1.0	63.2 ± 1.2***	28.3 ± 1.0
Saturated fat (g/day)	24.8 ± 1.7	21.7 ± 3.0	44.2 ± 1.8***	16.1 ± 1.5	40.7 ± 2.2***	15.6 ± 1.3
Monounsaturated fat (g/day)	20.8 ± 1.6	18.5 ± 2.7	36.1 ± 1.7***	13.6 ± 1.3	33.0 ± 1.8***	12.5 ± 1.1
Polyunsaturated fat (g/day)	8.6 ± 0.7	8.2 ± 0.8	16.5 ± 0.9***	7.6 ± 0.6	15.8 ± 1.2***	7.0 ± 0.7
Dietary fibres (g/day)	21.8 ± 1.1	24.3 ± 1.4	16.0 ± 0.9***	31.2 ± 2.2	15.9 ± 1.1***	30.1 ± 2.1

***p < 0.001 for mean difference in change between groups

5.1.6 Discussion

This study [235] demonstrates that a non-calorie restricted LCD high in fat with a recommended CHO-intake < 20 E% has significant clinical benefits over a non-calorie restricted high-carbohydrate diet low in fat over 6 months in patients with T2D. The main finding is that a LCD causes a major and clinically meaningful improvement in HbA1c of -8.9 mmol/mol compared with a control diet already after 3 months, and that this effect is largely sustained up to 6 months. Even though the HbA1c was reduced to well below the 48 mmol/mol, there were only a minimal risk of hypoglycemia. There was a desirable reduction in body weight, total fat mass, abdominal fat mass and waist circumference, and the LCD did not cause undesirable changes in cardiovascular risk factors, such as HDL, LDL, triglycerides or blood pressure.

As previously discussed, nutritional therapy is a cornerstone in the treatment of T2D. The traditional, high-carbohydrate diet low in fat has been challenged ever since the 1970s, with rigorous documentation of benefits of CHO-restriction over shorter term [62]. Previous reviews and meta-analyses have drawn different conclusions regarding the efficacy of LCD on glycemic control and CVD risk factors [84]. These discrepancies may be explained by differences in the definition of a low-carbohydrate diet [61], as well as addition of other interventions in the included studies. These additional interventions may consist of calorie restriction in both groups or just the control group, adjustments of antidiabetic medication during the study even in the

absence of hypoglycemia or lack of reporting of medicine changes, addition of an exercise regimen or lack of adherence to the prescribed diet [236]. Turton et al [129] did a systematic review on LCD and VLCDs in T2D with CHO-restriction below 26 E%, and reported that strategies in the examined studies varies from ad-libitum intake to severe caloric restriction (≤ 800 kcal a day) to adaptive caloric restriction based on the individual participants progress. This produces differences in HbA1c reductions and causes the optimal CHO prescription to remain unclear [129]. In addition, if an ad-libitum LCD strategy is applied but combined with caloric restriction in the control group, this results in a reduced effect on HbA1c when comparing the groups [99, 105]. In extreme interventions, such as Tay et al's study [237] which compared a LCD (< 14 E% CHO, 58 E% fat) to a high-carbohydrate diet (53 E% CHO, 30 E% fat), with both hypocaloric intake of 500-1000 kcal less per day and combined with supervised exercise in both groups, the authors observed great improvements in HbA1c but without difference between groups. Changes in glucose lowering medication during the study might increase the risk of underestimating the effect of a LCD on glycemic control, especially if discontinuation of antidiabetic treatment is a goal, even if there is minimal risk of hypoglycemia. Saslow et al compared a moderate CHO-restricted, hypocaloric diet (45-50 E% CHO, < 30 E% fat, 500 kcal fewer kcal) to a VLCD (20-50 E% CHO/day and no caloric restriction), as well as reducing antidiabetic medicine during the study [238], which resulted in a small difference in HbA1c of -0.6 % after 6 months. In the study conducted by Davis et al [102], the intervention group followed an Atkins-like diet over one year, with an initial ketosis-inducing phase and an increasing amount carbohydrates after the initial two weeks, compared to a control diet recommended for T2D ("low-fat"). In this study, insulin and SU was adjusted in both groups according to measured blood sugars, in addition to discontinuance of thiazolidinedione at baseline. This resulted in only a modest difference in change between groups in HbA1c (-0.6 %) after three months, but not at subsequent time points [102]. Guldbrand et al [106] conducted a 6-months LCD-study (CHO 20 E%, fat 50 E%) vs. high-CHO diet (CHO 55-60 E%, fat 30 E%) with caloric restriction in both groups and reported that there was no significant difference in change of HbA1c between groups after two years, although the reduction of HbA1c was larger in the LCD group after 6 months.

Two recent publications have demonstrated a larger effect of a LCD on HbA1c compared with a control diet despite reductions in glucose lowering medicine in one of the studies, and exercise intervention and hypocaloric intake in the control group in the other study. Thus, in 2021, Han et al [104] compared a LCD (< 50 g CHO/day or 14 E%, 58 E% fat) to a high-CHO diet (53 E% CHO, 30 E% fat) in patients with T2D. Although the design of the study did not include calorie-restriction or an exercise program, the antidiabetic medication was adjusted according to HbA1c during the six-month study in both groups. However, although most medicine changes was applied in the LCD group, the LCD still caused a larger reduction in HbA1c compared with the high-CHO-diet low in fat after six months. Chen et al reported in 2022 [103], that a LCD (<

90 g CHO/day) compared to a control diet consisting of a high-CHO diet low in fat (50-60 E% CHO, ≤ 30 E% fat), with caloric restriction in the control group based on ideal weight, reduced HbA1c -0.65 % more over 18 months compared to the control diet. However, the study responsible increased glucose lowering medication every 6 month if HbA1c surpassed 64 mmol/mol (8 %), or decreased this if HbA1c came below 48 mmol/mol (6.5 %), regardless of hypoglycemia [103].

In our study [235], the LCD had a marked effect on HbA1c, despite maintained physical activity, continued antidiabetic treatment in the absence of hypoglycemia and/or hypoglycemic symptoms, as well as no calorie restriction in both groups [223]. We did however reduce insulin dose with 20 % in the LCD group at the randomization. The observed reduction of HbA1c in response to the LCD in our study is comparable to the improvements achieved with intensified treatment with antidiabetic medications [239]. Interestingly, the fasting levels of plasma glucose or serum C-peptide were not changed in the LCD group compared to the control group. The effect on self-reported blood glucose levels was however significant, with LCD inducing reductions in self-reported plasma glucose measurements at almost all reported time points (before breakfast, lunch, supper and before bed time) after 3 and 6 months compared with the control diet, except at lunchtime after 6 months (Supplemental table 1, paper 1).

There were, however, some potential negative effects associated with the LCD diet. Even though we recommended a diet high in MUFA and low in SFA, our data [235] showed that the LCD group increased their intake of SFA to 2.6 fold higher than the control group after 6 months, as well as decreased their intake of dietary fibers. A higher intake of SFA may predispose to CVD [240], although this association has been difficult to prove in meta-analyses [241, 242], and a decrease in dietary fibers may be unfavorable, as dietary fibers are believed to help stabilizing plasma glucose, as well as plasma lipids [213, 243]. Still, the classical risk factors for CVD such as blood lipids and blood pressure did not increase in response to the LCD in our study. Another expected negative effect of the LCD was the decrease in total lean mass in the LCD group after six months. This loss appeared to be mainly explained by the loss of abdominal lean mass. Some of the loss of lean mass was expected, and may be explained by the reduced glycogen content in muscles. The glycogen content in the body is ~ 500 grams, which in turn binds 3 grams of water per gram glycogen, leading to a reduction of 1-2 kilos of lean mass when glycogen is utilized [71].

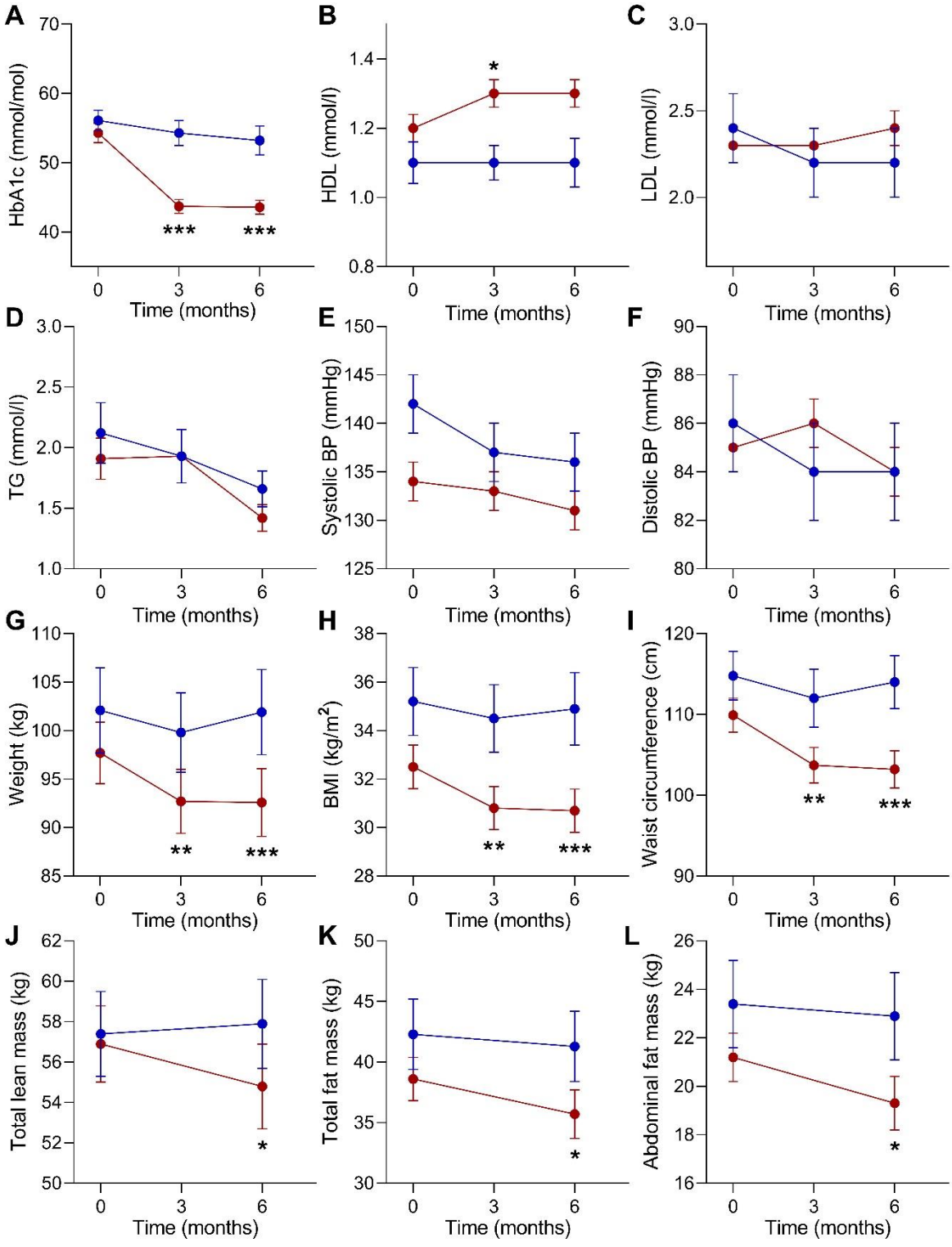


Fig. 6 [235]: Plots of selected variables presented above. Red, LCD. Blue, control. (*A*) Hba1c (mmol/mol), (*B*) HDL (mmol/l), (*C*) LDL (mmol/l), (*D*) Triglycerides (mmol/l), (*E*) systolic blood pressure (mmHg), (*F*) diastolic

blood pressure (mmHg), (*G*) Weight (kg), (*H*) BMI (kg/m2), (*I*) Waist circumference (cm), (*J*) whole body lean mass (kg), (*K*) whole body fat mass (kg), (*L*) abdominal fat mass (kg)

5.2 Paper II: A six-month low-carbohydrate diet high in fat does not adversely affect endothelial function or low-grade inflammation in patients with type 2 diabetes

5.2.1 Study cohort and baseline characteristics

The results are based on participants in which it was possible to analyze FMD- and NID-recordings. One participant was excluded due to missing data (LCD), leaving 70 patients to be analyzed. Exclusion of this participant did not affect the results on the variables presented above (glycemic variables, cardiovascular risk factors, body composition, dietary data, physical activity and hypoglycemia). For vascular measurements, there were no differences in the resting brachial artery diameter (FMD RD) (LCD 4.28 ± 0.11 mm, control 4.37 ± 0.14 mm), nor maximal dilation of the arteries (FMD max) (LCD 4.50 ± 0.11mm; control 4.55 ± 0.15 mm) between groups at baseline (both $p > 0.05$). However, the two groups differed in both FMD and NID at baseline, with higher FMD (LCD 5.19 ± 0.28 %; control 4.17 ± 0.39%, $p < 0.05$) and NID (LCD 16.66 ± 0.56 %; control 13.64 ± 1.07 %, $p < 0.05$) in the LCD group at baseline (Table 10). The ratio between FMD and NID was comparable in the two groups. There were no significant differences between groups in baseline values of plasma IL-6 or serum hsCRP levels (both $p > 0.05$) (Fig.8). At baseline, there were 61 patients in total with a FMD below the cut-off of 7.1 % for endothelial dysfunction (LCD: n=41, control; n=14), whereas 34 patients had a NID below the cut-off value of 15.6 % (LCD; n=20, control; n=14).

5.2.2 Impact of cardiovascular risk factors on baseline FMD

Univariate linear regression used to evaluate correlations between the FMD at baseline and different co-variates that are associated with increased risk of CVD, showed that FMD correlated significantly with age, systolic blood pressure (SBT) and resting diameter (Table 9). When adjusting for these covariates, the difference in FMD between groups at baseline disappeared ($p = 0.197$). NID correlated with age and resting diameter. After adjustment for NID RD and age, the difference between groups at baseline was still significant ($p = 0.007$).

Table 9: Univariate analysis of the relationships between CVD risk factors and vascular measurements

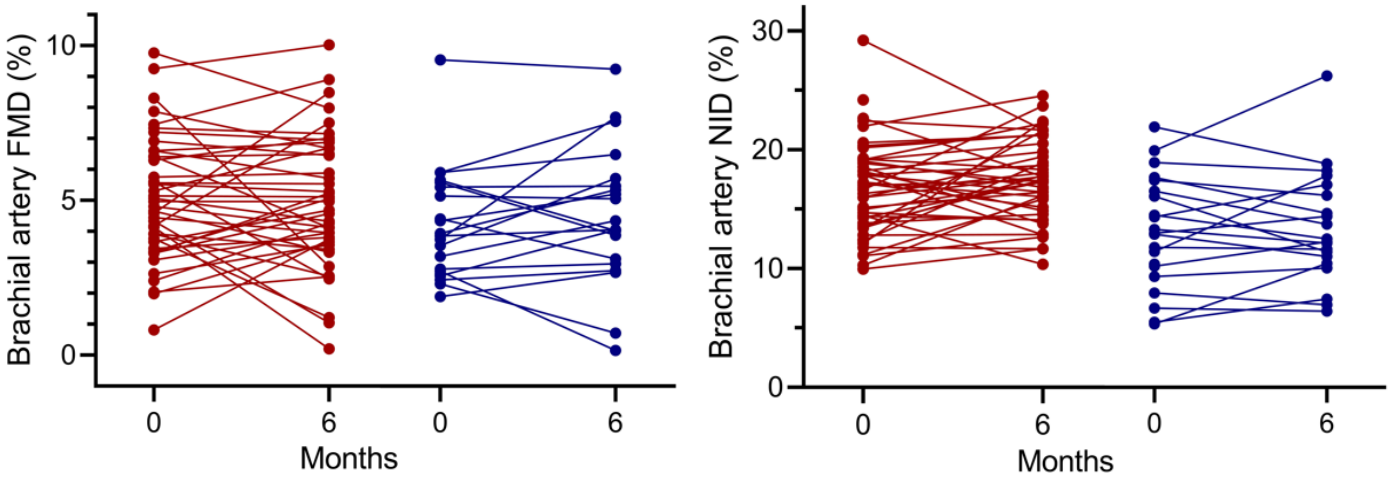
	FMD (%)	NID (%)
	Coefficients ± SE	Coefficients ± SE
Sex (female)	0.42 ± 0.47	-0.35 ± 1.07
Diabetes duration, y	-0.04 ± 0.08	-0.09 ± 0.18
Age	-0.08 ± 0.03**	-0.13 ± 0.06*
BMI	-0.01 ± 0.04	-0.07 ± 0.08
Systolic BP	-0.05 ± 0.02**	-0.06 ± 0.04
Diastolic BP	-0.04 ± 0.03	-0.04 ± 0.06
LDL-cholesterol	-0.11 ± 0.31	0.48 ± 0.70
Triglycerides	0.08 ± 0.20	0.39 ± 0.44
HbA1c	-0.01 ± 0.03	-0.00 ± 0.06
Resting diameter	-0.91 ± 0.31**	-1.89 ± 0.72*

Abbreviations: BP, blood pressure. *p<0.05 and **p<0.01

5.2.3 Changes in vascular measurements

Both FMD RD and NID RD, as well as FMD max and NID max decreased within the LCD group (all $p < 0.05$). However, there were no between-group differences in change in these parameters over time. Importantly, there were no between-group differences in change of FMD (MDIC = +0.44 ± 0.47 %, p = 0.34) or NID (MDIC =+0.59 ± 0.93 %, p = 0.53) over time (Table 10). The ratio between FMD/NID did not change with LCD diet compared to control diet after 6 months (MDIC -0.02 ± 0.04, p = 0.052). When adjusting for these covariates associated with FMD and NID at baseline, the lack of differences in change between groups for FMD (p = 0.335) and NID (p=0.598) persisted.

Fig. 7: Individual changes in FMD and NID



Colors: Red, LCD group. Blue = control group.

Table 10: Vascular measurements

	Baseline		6 months			
	LCD (n=49)	Control (n=21)	LCD (n=44)	Control (n=20)	MDIC	p-value
FMD RD (mm)	4.28 ± 0.11	4.37 ± 0.14	4.17 ± 0.11*	4.35 ± 0.15	+0.00 ± 0.04	0.93
FMD max (mm)	4.50 ± 0.11	4.55 ± 0.15	4.38 ± 0.11**	4.54 ± 0.15	-0.008 ± 0.04	0.85
FMD (%)	5.19 ± 0.28$^{\#}$	4.17 ± 0.39	5.00 ± 0.32	4.52 ± 0.50	-0.44 ± 0.47	0.34
NID RD (mm)	4.29 ± 0.11	4.39 ± 0.14	4.14 ± 0.11**	4.37 ± 0.15	-0.04 ± 0.05	0.46
NID max (mm)	5.00 ± 0.12	4.98 ± 0.15	4.86 ± 0.12*	4.97 ± 0.16	-0.02 ± 0.06	0.75
NID (%)	16.66 ± 0.56$^{\#}$	13.64 ± 1.07	17.47 ± 0.52$^{(*)}$	14.03 ± 1.10	+0.59 ± 0.93	0.53
FMD%/NID%	0.32 ± 0.02	0.33 ± 0.03	0.29 ± 0.02	0.33 ± 0.03	-0.02 ± 0.04	0.52

Data are presented as means ± SEM. Abbreviations: FMD, flow-mediated vasodilation; LCD, low carbohydrate diet; MDIC, mean difference in change; NID, nitroglycerin-induced vasodilation; RD, resting diameter. $^{\#}$p<0.05 vs control at baseline; $^{(*)}$p<0.10, *p<0.05 or **p<0.01 vs baseline *p<0.05.

5.2.4 Effect on markers of low-grade inflammation

In the LCD group, both the hsCRP (p=0.004) and IL-6 levels (p=0.013) decreased after 6 months on the diet, whereas no significant changes were seen in the control group (Fig. 8). However, when comparing changes

over time the LCD diet did not cause a significant change in either plasma IL-6 (p=0.247) or serum hsCRP (p=0.065) compared to the control diet after six months (Fig. 8)

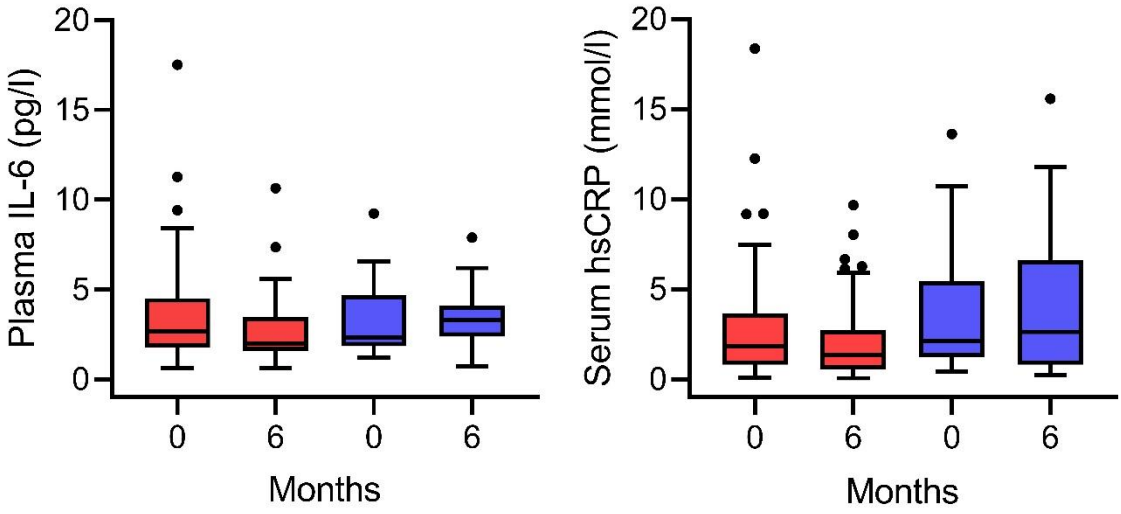


Fig. 8: Overview over P-IL-6 and S-hsCRP in the two groups at baseline and after 6 months. Red, LCD. Blue, control group.

5.2.5 Discussion paper II

Vascular function is not very well examined in T2D in LCD trials. In paper II, we report the effect of a LCD on endothelial dysfunction and selected markers of low-grade inflammation in T2D compared to a control diet over 6 months. The main findings in paper II are that 6 months of LCD with 50-60 E% fat did not cause worsening of endothelial dysfunction, measured as FMD and NID, nor did the LCD cause worsening in the selected circulating markers of low-grade inflammation compared to the control diet.

Endothelial dysfunction is partly reversible, with potential of variation during an intervention, making FMD- and NID measurements suitable to detect positive or negative effects of substantial change in the diet on the endothelium [244]. A change of diet may have impact on the risk of developing cardiovascular disease (CVD) by affecting the endothelium [245]. The results found in our study, with no differences in change between groups after 6 months of dietary change, are similar to those reported by Wycherley et al [163] after 6 months of a VLCD compared to a hypocaloric low-fat diet with a supervised exercise program, as well as the lack of an effect on FMD after a 2-year period in the extended study, described by Tay et al [95]. The authors speculated, that there may have been an influence on the results by the exercise, weight-loss, decreased intake of saturated fat and maintaining of dietary fibre-intake in their studies [163]. As described previously, in our study [235], there was no change in physical activity, the LCD group increased their intake of saturated fat significantly and there was a significant decrease in dietary fiber-intake, as well as no change in calorie

intake in the 6 months. Moreover, the LCD diet caused a decrease in weight and abdominal fat mass [235], which may have influenced the results described above. Previous studies that have examined the effect of diet-induced weight-loss on FMD have shown heterogenic results. However, a meta-analysis based on 33 studies of obese persons reported an improvement in fasting FMD by 1.11 % for each 10 kilos of weight loss, that the effect might be more pronounced if exercise was added to the intervention, and that weight-loss with a low-fat diet is more effective than a reduced-CHO diet in improving FMD [152]. In our study, it cannot be ruled out that the improved levels of HbA1c may have counteracted a negative effect of the LCD on FMD [246], but the lack of association between HbA1c and FMD and NID suggests that this is not the case.

Regarding cardiovascular risk factors and their impact on vascular function at baseline, the sample size is quite small, and potential associations with cardiovascular risk factors and impact on FMD and/or NID may not be apparent [247]. We did find a significant association between age and FMD RD with both FMD and NID, and between systolic blood pressure and FMD. It has been shown that cardiovascular risk factors also affect arterial resting diameter, with an increasing resting diameter in those with a higher risk of CVD and a higher Framingham risk score [248]. FMD and NID are calculated as the change compared to the resting diameter, which means that an increased resting diameter does have a large impact on vascular measurements [249].

Elevated circulating hsCRP and IL-6 levels are markers of low-grade inflammation and independently associated with atherosclerosis and an increased risk of cardiovascular events [250]. Moreover, circulating IL-6 levels are associated with all-cause mortality in T2D [193]. In our study, we observed no negative impact of LCD on the blood levels of IL-6 nor hsCRP compared to control diet over 6 months. It is possible, that the observed weight-loss in the LCD group may have contributed to counteract a potential negative impact of an increased fat intake, as a higher fat intake has been reported to be associated with higher IL-6 levels in both humans [251] and rats [252]. However, our results are in line with the report from Skytte et al [202], where a CHO-restricted diet high in protein and fat (CHO 30 E%, fat 40 E5 and protein 30 E%) was compared to a control diet (CHO 50 E%, fat 33 E% and protein 17 E%) in patients with T2D in a cross-over trial aiming for weight-stability. They demonstrated unchanged concentrations of inflammatory markers over 6 weeks. This also applies to the study by Davis et al. [204], in which they compared a VLCD to a control diet in T2D over 6 months with comparable weight-loss between groups. They found no significant changes in markers of low-grade inflammation after 6 months between groups. In contrast, Jonasson et al [203] demonstrated beneficial effects of the LCD diet only when compared to a high-CHO control diet in two of the five measured pro-inflammatory markers, where caloric restriction applied to both groups.

6. Overall conclusions

Our results demonstrated, that a non-calorie restricted LCD with a CHO-intake < 20 E% and increased daily energy from fat of 50-60 E% induces a significant and highly clinical relevant reduction of HbA1c, compared to a non-calorie restricted control diet with a high carbohydrate intake. These effects were observed despite sustained physical activity level. Moreover, the LCD induced a weight-loss and reduction of abdominal adiposity and total fat mass over six months compared with the control diet. However, the LCD also caused a decrease in lean mass, which may partly be explained by decreased glycogen storage and excreted water in liver and muscle. There was no negative impact on classical cardiovascular risk factors, such blood lipids or blood pressure or on the selected markers of low-grade inflammation. Importantly, the endothelial function, measured as FMD and NID was unaffected by the LCD, despite a higher intake of fat, in particular saturated fat. The risk of hypoglycemia was low, and adverse symptoms associated with the LCD was mostly minor gastrointestinal complaints.

Future studies should focus on the ability to sustain the apparent beneficial clinical effect of a LCD among patients with T2D that are motivated for this type of dietary change, as well as methods to help the patients adhere to a dietary change as extensive as a LCD. There is still a need to compare a LCD to different degrees of CHO-reduction, where the intake of high-fiber, vitamin-rich foods, such as legumes, root vegetables and non-refined grains to a higher degree are allowed. For a better understanding of the beneficial clinical and metabolic effects of a LCD, future studies could use continuous plasma-glucose measurements and repeated measurements of insulin-resistance, such as euglycemic-hyperinsulinemic clamps, to examine if there are differences in the short-term and long-term effects of a LCD, as well as including an objective measure of the effects on the cardiovascular system over long term. There is also a need for clarifying the molecular effects of LCD in insulin sensitive tissues such as skeletal muscle, liver and adipose tissue as well as the pancreatic beta-cells over time and which processes that change in response to a LCD, including how LCD affect adipose tissue inflammation in T2D. Finally, future studies should focus on finding biological markers that could help predict if a person would both benefit from and thrive on following a LCD.

7. Strengths and limitations

The strengths of this present study include the randomized design and the well-matched study groups. There was no intention to change the non-insulin antidiabetic medication during the course of study, as well as to maintain the level of physical activity, which allows for evaluating the isolated effect of a non-calorie restricted LCD compared to the control diet. The sample-size was sufficient to evaluate the effect on the primary outcome HbA1c, but also to rule out a change in FMD of 20 % according to a non-inferiority analysis. Additionally, there were very few dropouts during the course of study, even though this was designed as a free-living study with no economic support to cover increased living expenses. We showed that, even though this study implicated an extensive dietary change, it is feasible with methods that may be applied in a clinic with access to a clinical dietician. We also included objective measurements of physical activity and made dietary registrations easier for the participants in using an internet-based, easy-accessible food-diary, with a recipe database that was continuously updated to make it easier for the participants to make appropriate food choices according to randomization. We made efforts to keep the two groups completely separated to avoid dietary contamination between the groups.

There are also some important limitations to this study. This was an open-labelled study, where both the study responsible and the patients were aware of the randomization. Moreover, there was heterogeneity in dietary reporting, which ranged from daily entries to the minimum required to none and need for dietetic interviews to evaluate compliance, as well as of energy intake and macronutrient composition. The lack of strict control with dietary macronutrient composition may explain the observed increased intake of saturated fat and decreased intake of dietary fibre. Also, the study lacked a strict control of possible changes in physical activity level throughout the whole study duration. The pre-prandial diurnal glucose data relied on self-reported measurements measured on the patients own device, where lack of calibration between devices increases risk of imprecise measurements. Moreover, our sample size was sufficient to detect changes in Hba1c, but likely too small to establish change in other outcomes, such as HOMA-IR and risk factors associated with a decreased endothelial function, as well as between-group differences in changes in the pro-inflammatory markers IL-6 and hsCRP.

8. English Summary

Diabetes affects over 9.3 % of the population worldwide, where, especially, the prevalence of type 2 diabetes (T2D) is increasing. A cornerstone in the management of T2D is lifestyle changes such as physical activity and diet to facilitate a good glycemic control and reduce risk of complications. During the last 20 years, carbohydrate-restricted diets (CRD) have gained increasing popularity, in particular, low-carbohydrate diets (LCDs) with an intake of carbohydrates between 10 and 25 E% (percentage of total energy intake). Recent meta-analyses have reached conflicting results regarding the impact of CRDs on glycemic control, body weight and markers of cardiovascular risk in T2D. This may be explained by variations in the interventions, such as combining the LCD and/or the control diet with calorie-restriction, exercise programs and reduction in antidiabetic medication. However, there is evidence that the greater the carbohydrate restriction, the greater the glucose-lowering effect in patients with T2D, whereas the effect of CRDs on other risk factors for cardiovascular disease (CVD) such as blood lipids, blood pressure and body composition are uncertain. There have been concerns that LCDs high in fat may increase the risk of cardiovascular disease (CVD). This may include worsening of the endothelial function, an early marker of atherosclerosis, mediated by increased levels of markers of chronic low-grade inflammation. This, however, remains to be established.

In this thesis, we aimed to test the hypotheses that a non-calorie–restricted LCD high in fat for 6 months 1) improves glycemic control and body composition and is safe with respect to risk factors of CVD such as blood lipid and blood pressure, and 2) adversely affects CVD risk factors such as endothelial function and markers of systemic chronic low-grade inflammation compared to a control diet in patients with T2D instructed to maintain their non-insulin glucose-lowering medication and level of physical activity.

In an open-label randomized controlled trial, 71 patients with T2D were randomized 2:1, to either a LCD diet (n = 49) or a control diet (n = 22) for six months. Both diets were non-calorie restricted. The LCD group were recommended a diet with < 20 E% carbohydrates, 50-60 E% fat (mainly monounsaturated) and 20-30 E% protein, while the control group was recommended 50-60 E% carbohydrates, 15-20 E% protein and < 30 E% fat. An internet-based food log was used. They attended three visits, at baseline, after three months and six months, where fasting blood samples were drawn and anthropometric data, blood pressure and compliance were assessed. At baseline and after six months, accelerometers were applied for seven consecutive days to asses level of physical activity, a dual energy x-ray absorptiometry scan (DXA) was done to assess body composition, along with measurements of flow-mediated vasodilation (FMD) and nitroglycerine-induced vasodilation(NID) of the brachial artery. Plasma and serum were analyzed for high-sensitive CRP (hsCRP) and Interleukin 6 (IL-6) as selected markers of low-grade inflammation. The mean differences in change (MDIC) between groups for these outcomes are reported.

The LCD group reduced their carbohydrate intake to ~13 E% and increased fat intake to ~63 E% (both $p < 0.001$). HbA1c decreased significantly with LCD at both three months (-8.9 ± 1.7 mmol/mol; $P < .0001$) and at six months (-7.5 ± 1.8 mmol/mol; $P < .0001$) compared with control diet. There was a significant reduction of weight with LCD of -3.9± 1.0 kg, of BMI – 1.4 ± 0.4 kg/m2 and of waist circumference -4.9 ± 1.3 cm (all $p < 0.01$) compared with control diet. DXA-scan showed that the LCD group lost both fat mass and lean mass (both $p < 0.05$) compared with control diet. Apart for a transient improvement in HDL after three months on LCD (+0.1 ± 0.4, $p = 0.03$), there were no between-group differences in the change of blood lipids or blood pressure. The groups differed slightly at baseline, with the LCD group having slightly higher FMD and NID (both $p < 0.05$). However, there were no between-group differences in the changes of either FMD or NID. Moreover, the selected markers of systemic low-grade inflammation (hsCRP and IL-6) were not significantly changed in response to LCD compared with the control diet after six months. The LCD was generally well-tolerated, except for an increase in gastrointestinal complaints. The described changes were observed in spite of maintained level of physical activity, and there were no episodes of severe hypoglycemia.

In conclusion, in this open-label randomized controlled trial, a six-month LCD significantly reduced HbA1c and improved body composition in patients with T2D. Moreover, the LCD had no detectable negative impact on blood lipids, blood pressure, endothelial function or biomarkers of systemic low-grade inflammation, which indicate that a non-calorie-restricted LCD high in fat is safe with regard to cardiovascular risk factors. The improvements in glycemic control and body composition may have counteracted potential deleterious effects of a higher intake of saturated fat on CVD risk factors.

9. Danish summary

Diabetes rammer over 9,3 % af verdensbefolkningen og prævalensen af især type 2 diabetes (T2D) er stigende. Livsstilsændringer er en hjørnesten i behandlingen af T2D, hvor øget fysisk aktivitet og kostomlægning kan forbedre den glykæmiske kontrol og nedsætte risikoen for udvikling af sendiabetiske komplikationer. I de seneste 20 år har diæter med reduceret kulhydratindhold opnået øget i popularitet; særlig lavkulhydratdiæter (LCDs) med et kulhydratindhold på mellem 10 og 25 E% (procent af dagligt energiindtag). Tidligere meta-analyser er dog ikke helt entydige omkring de forventede effekter af kulhydratreduktion på glykæmisk kontrol, kropsvægt eller kardiovaskulære risikofaktorer. Dette kan til dels forklares af at kulhydratrestriktion i mange studier kombineres med andre interventioner så som kalorierestriktion i én eller begge grupper, træningsprogrammer eller ændringer i deltagernes diabetesmedicin. Dog foreligger der evidens for, at jo mere kulhydratindtaget begrænses, jo bedre er den blodsukkersænkende effekt hos patienter med T2D, mens effekten på kardiovaskulære risikofaktorer så som kolesterolprofil, blodtryk og kropssammensætning stadig er uklar. Dette har medført bekymring for at en kost med kulhydratrestriktion og øget fedtindtag kan bidrage til en øget risiko for hjertekarsygdom. Det inkluderer en reduceret endotelfunktion, som betragtes som en tidlig markør for begyndende arteriosklerose og som kan skyldes en stigning i cirkulerende markører for såkaldt "low-grade inflammation". Dette mangler imidlertid at blive belyst.

I denne ph.d.-afhandling, var formålet at undersøge vores hypoteser om at en 6-måneders, kalorie-ubegrænset LCD med et øget indhold af fedt 1) kan forbedre den glykæmiske kontrol og kropssammen-sætningen samt er ufarlig med henblik på risikofaktorer for hjertekarsygdom så som blodtryk og kolesterol, og 2) kan have en ugunstig virkning på endotelfunktion og markører på systemisk "low-grade inflammation" sammenlignet med en kontrol-diæt hos patienter med T2D, som i begge grupper blev instrueret i ikke at ændre i hverken deres diabetesmedicin eller fysiske aktivitetsniveau.

Studiet var et randomiseret klinisk forsøg hvor 71 patienter med T2D blev randomiseret 2:1 til enten en LCD-diæt (n = 49) eller en kontroldiæt (n = 22) i seks måneder. Begge diæter var uden kalorierestriktion. LCD-gruppen blev anbefalet en diæt med < 20 E% kulhydrat, 50-60 E% fedt (hovedsagelig monoumættet fedt) og 20-30 E% protein, mens kontrolgruppen blev anbefalet en diæt med 50-60 E% kulhydrat, < 30 E% fedt og 15-20 E% protein. Kostindtaget blev kontrolleret ed hjælp af en webbaseret kostdagbog. Data blev indsamlet under tre fremmødebesøg ved start samt efter tre og seks måneder, og én telefonkontakt efter 2 uger. Fastende blodprøver, antropometriske målinger, blodtryk og overholdelse af kosten vurderedes ved hvert besøg. Ved start og efter seks måneder blev deltagerne scannet med en dual-energy x-ray absorpiometry scanner (DXA) for at evaluere kropssammensætningen, samtidigt med målinger af endotelfunktionen i overarmsarterien (flow-medieret vasodilation (FMD) og nitroglycerin-induceret dilation

(NID)). Udvalgte markører for "low-grade inflammation" såsom high-sensitive C-reactive protein (hsCRP) og interleukin-6 (IL-6) blev analyseret i plasma og serum. Forskellene mellem grupperne rapporteres som gennemsnitsforskelle i ændringer over tid mellem grupperne (mean difference in change; MDIC).

LCD-gruppen reducerede sit kulhydratindtag til ~13 E% og forøgede sit fedtindtag til ~63 E% (begge $p < 0.001$). HbA1c blev signifikant reduceret med LCD-diæt efter tre måneder (-8.9 ± 1.7 mmol/mol; $P < .0001$) og forblev reduceret efter seks måneder (-7.5 ± 1.8 mmol/mol; $P < .0001$) sammenlignet med kontroldiæt. Vægt og BMI faldt begge signifikant med LCD-kost (-3.9± 1.0 kg i vægt hhv. – 1.4 ± 0.4 kg/m2 i BMI) og taljemål faldt med -4.9 ± 1.3 cm (all $p < 0.01$) sammenlignet med kontroldiæten. Bortset fra en forbigående forbedring i HDL efter tre måneder, observeredes ikke nogen signifikante ændringer i kolesterolprofil eller blodtryk. Der var forskel mellem grupperne ved baseline, hvor det systoliske blodtryk var højere og FMD og NID var lavere i kontrolgruppen sammenlignet med LCD-gruppen (alle $p < 0.05$). Dog observeredes ingen forskelle i ændring i hverken FMD eller NID værdier mellem grupperne efter seks måneder. Der var desuden heller ingen forskelle i ændringer over tid i de udvalgte markører på systemisk "low-grade inflammation" (hsCRP og IL-6) ved indtag af LCD-kost sammenlignet med kontroldiæt. LCD-kosten tolereredes vel, bortset fra flere klager over forbigående mavetarmgener i LCD-gruppen. Disse ovenfor beskrevne ændringer observeredes til trods af at deltagerne ikke ændrede deres fysiske aktivitetsniveau. Der var ingen alvorlige tilfælde med hypoglykæmi under studiet.

Således viser dette ikke-blindede randomiserede forsøg, at seks måneder med LCD-kost giver en signifikant reduktion i HbA1c og forbedrer kropssammensætningen hos patienter med T2D sammenlignet med kontroldiæt. Desuden observeredes ingen negative effekter på kolesterolprofil, blodtryk, endotelfunktion eller markører for "low-grade inflammation". Disse fund indikerer at en LCD uden kaloriebegrænsning, men med et øget indhold af fedt, er sikker med henblik på risikofaktorer for hjertekarsygdom. De favorable forbedringer i glykæmisk kontrol og kropssammensætning som blev observeret under studiet kan have modvirket potentielle skadevirkninger af et højere indtag af mættet fedt på kardiovaskulære risikofaktorer.

10. References

[1] PETERMAN MG. THE KETOGENIC DIET IN EPILEPSY. Journal of the American Medical Association. 1925;84:1979-83.

[2] A critique of low-carbohydrate ketogenic weight reduction regimens. A review of Dr. Atkins' diet revolution. Jama. 1973;224:1415-9.

[3] Bantle JP, Wylie-Rosett J, Albright AL, Apovian CM, Clark NG, Franz MJ, et al. Nutrition recommendations and interventions for diabetes: a position statement of the American Diabetes Association. Diabetes Care. 2008;31 Suppl 1:S61-78.

[4] Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9(th) edition. Diabetes Res Clin Pract. 2019;157:107843.

[5] Janssen LMM, Hiligsmann MJC, Elissen AMJ, Joore MA, Schaper NC, Bosma JHA, et al. Burden of disease of type 2 diabetes mellitus: cost of illness and quality of life estimated using the Maastricht Study. Diabet Med. 2020.

[6] Li G, Zhang P, Wang J, An Y, Gong Q, Gregg EW, et al. Cardiovascular mortality, all-cause mortality, and diabetes incidence after lifestyle intervention for people with impaired glucose tolerance in the Da Qing Diabetes Prevention Study: a 23-year follow-up study. Lancet Diabetes Endocrinol. 2014;2:474-80.

[7] Sortso C, Green A, Jensen PB, Emneus M. Societal costs of diabetes mellitus in Denmark. Diabet Med. 2016;33:877-85.

[8] Meigs JB, Cupples LA, Wilson PW. Parental transmission of type 2 diabetes: the Framingham Offspring Study. Diabetes. 2000;49:2201-7.

[9] Newman B, Selby JV, King MC, Slemenda C, Fabsitz R, Friedman GD. Concordance for type 2 (non-insulin-dependent) diabetes mellitus in male twins. Diabetologia. 1987;30:763-8.

[10] Willemsen G, Ward KJ, Bell CG, Christensen K, Bowden J, Dalgard C, et al. The Concordance and Heritability of Type 2 Diabetes in 34,166 Twin Pairs From International Twin Registers: The Discordant Twin (DISCOTWIN) Consortium. Twin Res Hum Genet. 2015;18:762-71.

[11] Hawkes CH. Twin studies in diabetes mellitus. Diabet Med. 1997;14:347-52.

[12] Billings LK, Florez JC. The genetics of type 2 diabetes: what have we learned from GWAS? Ann N Y Acad Sci. 2010;1212:59-77.

[13] Vujkovic M, Keaton JM, Lynch JA, Miller DR, Zhou J, Tcheandjieu C, et al. Discovery of 318 new risk loci for type 2 diabetes and related vascular outcomes among 1.4 million participants in a multi-ancestry meta-analysis. Nat Genet. 2020;52:680-91.

[14] Lyssenko V, Groop L, Prasad RB. Genetics of Type 2 Diabetes: It Matters From Which Parent We Inherit the Risk. Rev Diabet Stud. 2015;12:233-42.

[15] Crump C, Sundquist J, Sundquist K. Preterm birth and risk of type 1 and type 2 diabetes: a national cohort study. Diabetologia. 2020;63:508-18.

[16] Li S, Zhang M, Tian H, Liu Z, Yin X, Xi B. Preterm birth and risk of type 1 and type 2 diabetes: systematic review and meta-analysis. Obes Rev. 2014;15:804-11.

[17] Knop MR, Geng TT, Gorny AW, Ding R, Li C, Ley SH, et al. Birth Weight and Risk of Type 2 Diabetes Mellitus, Cardiovascular Disease, and Hypertension in Adults: A Meta-Analysis of 7 646 267 Participants From 135 Studies. J Am Heart Assoc. 2018;7:e008870.

[18] Mi D, Fang H, Zhao Y, Zhong L. Birth weight and type 2 diabetes: A meta-analysis. Exp Ther Med. 2017;14:5313-20.

[19] Reece EA. The fetal and maternal consequences of gestational diabetes mellitus. J Matern Fetal Neonatal Med. 2010;23:199-203.

[20] Blotsky AL, Rahme E, Dahhou M, Nakhla M, Dasgupta K. Gestational diabetes associated with incident diabetes in childhood and youth: a retrospective cohort study. Cmaj. 2019;191:E410-e7.

[21] Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. Lancet (London, England). 2017;389:2239-51.

[22] Dendup T, Feng X, Clingan S, Astell-Burt T. Environmental Risk Factors for Developing Type 2 Diabetes Mellitus: A Systematic Review. Int J Environ Res Public Health. 2018;15.

[23] Kolb H, Martin S. Environmental/lifestyle factors in the pathogenesis and prevention of type 2 diabetes. BMC Med. 2017;15:131.

[24] Groop LC. Insulin resistance: the fundamental trigger of type 2 diabetes. Diabetes Obes Metab. 1999;1 Suppl 1:S1-7.

[25] DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. Diabetes Care. 2009;32 Suppl 2:S157-63.

[26] Samuel VT, Shulman GI. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. J Clin Invest. 2016;126:12-22.

[27] Petersen KF, Dufour S, Shulman GI. Decreased insulin-stimulated ATP synthesis and phosphate transport in muscle of insulin-resistant offspring of type 2 diabetic parents. PLoS Med. 2005;2:e233.

[28] McCarthy MI. Genomics, type 2 diabetes, and obesity. N Engl J Med. 2010;363:2339-50.

[29] Gastaldelli A. Role of beta-cell dysfunction, ectopic fat accumulation and insulin resistance in the pathogenesis of type 2 diabetes mellitus. Diabetes Res Clin Pract. 2011;93 Suppl 1:S60-5.

[30] Marchetti P, Dotta F, Lauro D, Purrello F. An overview of pancreatic beta-cell defects in human type 2 diabetes: implications for treatment. Regul Pept. 2008;146:4-11.

[31] Zatterale F, Longo M, Naderi J, Raciti GA, Desiderio A, Miele C, et al. Chronic Adipose Tissue Inflammation Linking Obesity to Insulin Resistance and Type 2 Diabetes. Front Physiol. 2019;10:1607.

[32] Blüher M. Adipose tissue inflammation: a cause or consequence of obesity-related insulin resistance? Clin Sci (Lond). 2016;130:1603-14.

[33] Wilson PW, D'Agostino RB, Parise H, Sullivan L, Meigs JB. Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. Circulation. 2005;112:3066-72.

[34] Gambineri A, Patton L, Altieri P, Pagotto U, Pizzi C, Manzoli L, et al. Polycystic ovary syndrome is a risk factor for type 2 diabetes: results from a long-term prospective study. Diabetes. 2012;61:2369-74.

[35] Ben-Haroush A, Yogev Y, Hod M. Epidemiology of gestational diabetes mellitus and its association with Type 2 diabetes. Diabet Med. 2004;21:103-13.

[36] Sarwar N, Gao P, Seshasai SR, Gobin R, Kaptoge S, Di Angelantonio E, et al. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. Lancet (London, England). 2010;375:2215-22.

[37] Grossmann M, Thomas MC, Panagiotopoulos S, Sharpe K, Macisaac RJ, Clarke S, et al. Low testosterone levels are common and associated with insulin resistance in men with diabetes. J Clin Endocrinol Metab. 2008;93:1834-40.

[38] Kim MJ, Lim NK, Choi SJ, Park HY. Hypertension is an independent risk factor for type 2 diabetes: the Korean genome and epidemiology study. Hypertens Res. 2015;38:783-9.

[39] Kohlgruber A, Lynch L. Adipose tissue inflammation in the pathogenesis of type 2 diabetes. Curr Diab Rep. 2015;15:92.

[40] Mantovani A, Byrne CD, Bonora E, Targher G. Nonalcoholic Fatty Liver Disease and Risk of Incident Type 2 Diabetes: A Meta-analysis. Diabetes Care. 2018;41:372-82.

[41] 11. Microvascular Complications and Foot Care: Standards of Medical Care in Diabetes-2021. Diabetes Care. 2021;44:S151-s67.

[42] Sattar N, Rawshani A, Franzén S, Rawshani A, Svensson AM, Rosengren A, et al. Age at Diagnosis of Type 2 Diabetes Mellitus and Associations With Cardiovascular and Mortality Risks. Circulation. 2019;139:2228-37.

[43] Tancredi M, Rosengren A, Svensson AM, Kosiborod M, Pivodic A, Gudbjörnsdottir S, et al. Excess Mortality among Persons with Type 2 Diabetes. N Engl J Med. 2015;373:1720-32.

[44] Canoy D, Tran J, Zottoli M, Ramakrishnan R, Hassaine A, Rao S, et al. Association between cardiometabolic disease multimorbidity and all-cause mortality in 2 million women and men registered in UK general practices. BMC Med. 2021;19:258.

[45] Ali MK, Pearson-Stuttard J, Selvin E, Gregg EW. Interpreting global trends in type 2 diabetes complications and mortality. Diabetologia. 2022;65:3-13.

[46] Vetrone LM, Zaccardi F, Webb DR, Seidu S, Gholap NN, Pitocco D, et al. Cardiovascular and mortality events in type 2 diabetes cardiovascular outcomes trials: a systematic review with trend analysis. Acta Diabetol. 2019;56:331-9.

[47] American Diabetes A. 8. Obesity Management for the Treatment of Type 2 Diabetes: Standards of Medical Care in Diabetes-2019. Diabetes Care. 2019;42:S81-S9.

[48] Swinburn BA, Sacks G, Hall KD, McPherson K, Finegood DT, Moodie ML, et al. The global obesity pandemic: shaped by global drivers and local environments. Lancet (London, England). 2011;378:804-14.

[49] Kushner RF. Weight Loss Strategies for Treatment of Obesity: Lifestyle Management and Pharmacotherapy. Prog Cardiovasc Dis. 2018;61:246-52.

[50] Fruh SM. Obesity: Risk factors, complications, and strategies for sustainable long-term weight management. J Am Assoc Nurse Pract. 2017;29:S3-s14.

[51] Wing RR, Phelan S. Long-term weight loss maintenance. Am J Clin Nutr. 2005;82:222s-5s.

[52] Wing RR, Hill JO. Successful weight loss maintenance. Annu Rev Nutr. 2001;21:323-41.

[53] Greenberg I, Stampfer MJ, Schwarzfuchs D, Shai I. Adherence and success in long-term weight loss diets: the dietary intervention randomized controlled trial (DIRECT). J Am Coll Nutr. 2009;28:159-68.

[54] Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, et al. Long-term persistence of hormonal adaptations to weight loss. N Engl J Med. 2011;365:1597-604.

[55] Müller MJ, Enderle J, Bosy-Westphal A. Changes in Energy Expenditure with Weight Gain and Weight Loss in Humans. Curr Obes Rep. 2016;5:413-23.

[56] Gouni-Berthold I, Berthold HK. Current Options for the Pharmacotherapy of Obesity. Curr Pharm Des. 2019;25:2019-32.

[57] Aaseth J, Ellefsen S, Alehagen U, Sundfør TM, Alexander J. Diets and drugs for weight loss and health in obesity - An update. Biomed Pharmacother. 2021;140:111789.

[58] Gribsholt SB, Abild CB, Bruun JM, Dirksen C, Engberg AS, Juhl CB, et al. Kirurgisk behandling af svær overvægt. https://endocrinology.dk/nbv/andre-endokrinologiske-sygdomme/fedmekirurgi/2020. p. National guideline for surgical treatment of obesity.

[59] Arterburn DE, Courcoulas AP. Bariatric surgery for obesity and metabolic conditions in adults. BMJ (Clinical research ed). 2014;349:g3961.

[60] Maciejewski ML, Arterburn DE, Van Scoyoc L, Smith VA, Yancy WS, Jr., Weidenbacher HJ, et al. Bariatric Surgery and Long-term Durability of Weight Loss. JAMA Surg. 2016;151:1046-55.

[61] Feinman RD, Pogozelski WK, Astrup A, Bernstein RK, Fine EJ, Westman EC, et al. Dietary carbohydrate restriction as the first approach in diabetes management: critical review and evidence base. Nutrition (Burbank, Los Angeles County, Calif). 2015;31:1-13.

[62] Kirkpatrick CF, Bolick JP, Kris-Etherton PM, Sikand G, Aspry KE, Soffer DE, et al. Review of current evidence and clinical recommendations on the effects of low-carbohydrate and very-low-carbohydrate (including ketogenic) diets for the management of body weight and other cardiometabolic risk factors: A scientific statement from the National Lipid Association Nutrition and Lifestyle Task Force. J Clin Lipidol. 2019;13:689-711 e1.

[63] Poff AM, Koutnik AP, Egan B. Nutritional Ketosis with Ketogenic Diets or Exogenous Ketones: Features, Convergence, and Divergence. Curr Sports Med Rep. 2020;19:251-9.

[64] Mozaffarian D. Dietary and Policy Priorities for Cardiovascular Disease, Diabetes, and Obesity: A Comprehensive Review. Circulation. 2016;133:187-225.

[65] Evert AB, Dennison M, Gardner CD, Garvey WT, Lau KHK, MacLeod J, et al. Nutrition Therapy for Adults With Diabetes or Prediabetes: A Consensus Report. Diabetes Care. 2019;42:731-54.

[66] Churuangsuk C, Hall J, Reynolds A, Griffin SJ, Combet E, Lean MEJ. Diets for weight management in adults with type 2 diabetes: an umbrella review of published meta-analyses and systematic review of trials of diets for diabetes remission. Diabetologia. 2022;65:14-36.

[67] Ludwig DS, Ebbeling CB. The Carbohydrate-Insulin Model of Obesity: Beyond "Calories In, Calories Out". JAMA Intern Med. 2018;178:1098-103.

[68] Norton L, Shannon C, Gastaldelli A, DeFronzo RA. Insulin: The master regulator of glucose metabolism. Metabolism. 2022;129:155142.

[69] Hatting M, Tavares CDJ, Sharabi K, Rines AK, Puigserver P. Insulin regulation of gluconeogenesis. Ann N Y Acad Sci. 2018;1411:21-35.

[70] Murray B, Rosenbloom C. Fundamentals of glycogen metabolism for coaches and athletes. Nutr Rev. 2018;76:243-59.

[71] Adam-Perrot A, Clifton P, Brouns F. Low-carbohydrate diets: nutritional and physiological aspects. Obes Rev. 2006;7:49-58.

[72] Wahren J, Ekberg K. Splanchnic regulation of glucose production. Annu Rev Nutr. 2007;27:329-45.

[73] Laffel L. Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. Diabetes Metab Res Rev. 1999;15:412-26.

[74] Thorburn AW, Gumbiner B, Bulacan F, Wallace P, Henry RR. Intracellular glucose oxidation and glycogen synthase activity are reduced in non-insulin-dependent (type II) diabetes independent of impaired glucose uptake. J Clin Invest. 1990;85:522-9.

[75] Lovejoy J, Newby FD, Gebhart SS, DiGirolamo M. Insulin resistance in obesity is associated with elevated basal lactate levels and diminished lactate appearance following intravenous glucose and insulin. Metabolism. 1992;41:22-7.

[76] Hu JR, Wu Y, Sacks FM, Appel LJ, Miller Iii ER, Young JH, et al. Effects of carbohydrate quality and amount on plasma lactate: results from the OmniCarb trial. BMJ Open Diabetes Res Care. 2020;8.

[77] Ludwig DS, Dickinson SL, Henschel B, Ebbeling CB, Allison DB. Do Lower-Carbohydrate Diets Increase Total Energy Expenditure? An Updated and Reanalyzed Meta-Analysis of 29 Controlled-Feeding Studies. The Journal of nutrition. 2021;151:482-90.

[78] Hall KD, Chen KY, Guo J, Lam YY, Leibel RL, Mayer LE, et al. Energy expenditure and body composition changes after an isocaloric ketogenic diet in overweight and obese men. Am J Clin Nutr. 2016;104:324-33.
[79] Ebbeling CB, Feldman HA, Klein GL, Wong JMW, Bielak L, Steltz SK, et al. Effects of a low carbohydrate diet on energy expenditure during weight loss maintenance: randomized trial. BMJ (Clinical research ed). 2018;363:k4583.
[80] Brehm BJ, Spang SE, Lattin BL, Seeley RJ, Daniels SR, D'Alessio DA. The role of energy expenditure in the differential weight loss in obese women on low-fat and low-carbohydrate diets. J Clin Endocrinol Metab. 2005;90:1475-82.
[81] Boden G, Sargrad K, Homko C, Mozzoli M, Stein TP. Effect of a low-carbohydrate diet on appetite, blood glucose levels, and insulin resistance in obese patients with type 2 diabetes. Annals of internal medicine. 2005;142:403-11.
[82] Snorgaard O, Poulsen GM, Andersen HK, Astrup A. Systematic review and meta-analysis of dietary carbohydrate restriction in patients with type 2 diabetes. BMJ Open Diabetes Res Care. 2017;5:e000354.
[83] Sainsbury E, Kizirian NV, Partridge SR, Gill T, Colagiuri S, Gibson AA. Effect of dietary carbohydrate restriction on glycemic control in adults with diabetes: A systematic review and meta-analysis. Diabetes Res Clin Pract. 2018;139:239-52.
[84] McArdle PD, Greenfield SM, Rilstone SK, Narendran P, Haque MS, Gill PS. Carbohydrate restriction for glycaemic control in Type 2 diabetes: a systematic review and meta-analysis. Diabet Med. 2019;36:335-48.
[85] Bueno NB, de Melo IS, de Oliveira SL, da Rocha Ataide T. Very-low-carbohydrate ketogenic diet v. low-fat diet for long-term weight loss: a meta-analysis of randomised controlled trials. Br J Nutr. 2013;110:1178-87.
[86] Goldenberg JZ, Day A, Brinkworth GD, Sato J, Yamada S, Jönsson T, et al. Efficacy and safety of low and very low carbohydrate diets for type 2 diabetes remission: systematic review and meta-analysis of published and unpublished randomized trial data. BMJ (Clinical research ed). 2021;372:m4743.

[87] Huntriss R, Campbell M, Bedwell C. The interpretation and effect of a low-carbohydrate diet in the management of type 2 diabetes: a systematic review and meta-analysis of randomised controlled trials. Eur J Clin Nutr. 2018;72:311-25.

[88] Kumar NK, Merrill JD, Carlson S, German J, Yancy WS, Jr. Adherence to Low-Carbohydrate Diets in Patients with Diabetes: A Narrative Review. Diabetes, metabolic syndrome and obesity : targets and therapy. 2022;15:477-98.

[89] Lean ME, Leslie WS, Barnes AC, Brosnahan N, Thom G, McCombie L, et al. Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. Lancet (London, England). 2018;391:541-51.

[90] Kirkham AA, Beka V, Prado CM. The effect of caloric restriction on blood pressure and cardiovascular function: A systematic review and meta-analysis of randomized controlled trials. Clin Nutr. 2021;40:728-39.

[91] Guldbrand H, Dizdar B, Bunjaku B, Lindstrom T, Bachrach-Lindstrom M, Fredrikson M, et al. In type 2 diabetes, randomisation to advice to follow a low-carbohydrate diet transiently improves glycaemic control compared with advice to follow a low-fat diet producing a similar weight loss. Diabetologia. 2012;55:2118-27.

[92] Li S, Lin G, Chen J, Chen Z, Xu F, Zhu F, et al. The effect of periodic ketogenic diet on newly diagnosed overweight or obese patients with type 2 diabetes. BMC Endocr Disord. 2022;22:34.

[93] Morris E, Aveyard P, Dyson P, Noreik M, Bailey C, Fox R, et al. A food-based, low-energy, low-carbohydrate diet for people with type 2 diabetes in primary care: A randomized controlled feasibility trial. Diabetes Obes Metab. 2020;22:512-20.

[94] Nielsen JV, Jonsson E, Nilsson AK. Lasting improvement of hyperglycaemia and bodyweight: low-carbohydrate diet in type 2 diabetes. A brief report. Ups J Med Sci. 2005;110:179-83.

[95] Tay J, Thompson CH, Luscombe-Marsh ND, Wycherley TP, Noakes M, Buckley JD, et al. Effects of an energy-restricted low-carbohydrate, high unsaturated fat/low saturated fat diet versus a high-carbohydrate, low-fat diet in type 2 diabetes: A 2-year randomized clinical trial. Diabetes Obes Metab. 2018;20:858-71.

[96] Ahmed SR, Bellamkonda S, Zilbermint M, Wang J, Kalyani RR. Effects of the low carbohydrate, high fat diet on glycemic control and body weight in patients with type 2 diabetes: experience from a community-based cohort. BMJ Open Diabetes Res Care. 2020;8.

[97] Durrer C, McKelvey S, Singer J, Batterham AM, Johnson JD, Gudmundson K, et al. A randomized controlled trial of pharmacist-led therapeutic carbohydrate and energy restriction in type 2 diabetes. Nat Commun. 2021;12:5367.

[98] Iqbal N, Vetter ML, Moore RH, Chittams JL, Dalton-Bakes CV, Dowd M, et al. Effects of a low-intensity intervention that prescribed a low-carbohydrate vs. a low-fat diet in obese, diabetic participants. Obesity (Silver Spring). 2010;18:1733-8.

[99] Yamada Y, Uchida J, Izumi H, Tsukamoto Y, Inoue G, Watanabe Y, et al. A non-calorie-restricted low-carbohydrate diet is effective as an alternative therapy for patients with type 2 diabetes. Intern Med. 2014;53:13-9.

[100] Yancy WS, Jr., Westman EC, McDuffie JR, Grambow SC, Jeffreys AS, Bolton J, et al. A randomized trial of a low-carbohydrate diet vs orlistat plus a low-fat diet for weight loss. Arch Intern Med. 2010;170:136-45.

[101] Saslow LR, Kim S, Daubenmier JJ, Moskowitz JT, Phinney SD, Goldman V, et al. A randomized pilot trial of a moderate carbohydrate diet compared to a very low carbohydrate diet in overweight or obese individuals with type 2 diabetes mellitus or prediabetes. PLoS One. 2014;9:e91027.

[102] Davis NJ, Tomuta N, Schechter C, Isasi CR, Segal-Isaacson CJ, Stein D, et al. Comparative study of the effects of a 1-year dietary intervention of a low-carbohydrate diet versus a low-fat diet on weight and glycemic control in type 2 diabetes. Diabetes Care. 2009;32:1147-52.

[103] Chen CY, Huang WS, Chen HC, Chang CH, Lee LT, Chen HS, et al. Effect of a 90 g/day low-carbohydrate diet on glycaemic control, small, dense low-density lipoprotein and carotid intima-media thickness in type 2 diabetic patients: An 18-month randomised controlled trial. PLoS One. 2020;15:e0240158.

[104] Han Y, Cheng B, Guo Y, Wang Q, Yang N, Lin P. A Low-Carbohydrate Diet Realizes Medication Withdrawal: A Possible Opportunity for Effective Glycemic Control. Front Endocrinol (Lausanne). 2021;12:779636.

[105] Daly ME, Paisey R, Paisey R, Millward BA, Eccles C, Williams K, et al. Short-term effects of severe dietary carbohydrate-restriction advice in Type 2 diabetes--a randomized controlled trial. Diabet Med. 2006;23:15-20.

[106] Guldbrand H, Dizdar B, Bunjaku B, Lindström T, Bachrach-Lindström M, Fredrikson M, et al. In type 2 diabetes, randomisation to advice to follow a low-carbohydrate diet transiently improves glycaemic control compared with advice to follow a low-fat diet producing a similar weight loss. Diabetologia. 2012;55:2118-27.

[107] Morris E, Aveyard P, Dyson P, Noreik M, Bailey C, Fox R, et al. A food-based, low-energy, low-carbohydrate diet for people with type 2 diabetes in primary care: A randomized controlled feasibility trial. Diabetes Obes Metab. 2019.

[108] Saslow LR, Summers C, Aikens JE, Unwin DJ. Outcomes of a Digitally Delivered Low-Carbohydrate Type 2 Diabetes Self-Management Program: 1-Year Results of a Single-Arm Longitudinal Study. JMIR Diabetes. 2018;3:e12.

[109] Sato J, Kanazawa A, Makita S, Hatae C, Komiya K, Shimizu T, et al. A randomized controlled trial of 130 g/day low-carbohydrate diet in type 2 diabetes with poor glycemic control. Clin Nutr. 2017;36:992-1000.

[110] Sato J, Kanazawa A, Hatae C, Makita S, Komiya K, Shimizu T, et al. One year follow-up after a randomized controlled trial of a 130 g/day low-carbohydrate diet in patients with type 2 diabetes mellitus and poor glycemic control. PLoS One. 2017;12:e0188892.

[111] Clifton P. Effects of a high protein diet on body weight and comorbidities associated with obesity. Br J Nutr. 2012;108 Suppl 2:S122-9.

[112] Skytte MJ, Samkani A, Petersen AD, Thomsen MN, Astrup A, Chabanova E, et al. A carbohydrate-reduced high-protein diet improves HbA1c and liver fat content in weight stable participants with type 2 diabetes: a randomised controlled trial. Diabetologia. 2019;62:2066-78.

[113] Watson N, Dyer K, Buckley J, Brinkworth G, Coates A, Parfitt G, et al. Effects of Low-Fat Diets Differing in Protein and Carbohydrate Content on Cardiometabolic Risk Factors during Weight Loss and Weight Maintenance in Obese Adults with Type 2 Diabetes. Nutrients. 2016;8.

[114] Gannon MC, Nuttall FQ. Effect of a high-protein, low-carbohydrate diet on blood glucose control in people with type 2 diabetes. Diabetes. 2004;53:2375-82.

[115] Krebs JD, Elley CR, Parry-Strong A, Lunt H, Drury PL, Bell DA, et al. The Diabetes Excess Weight Loss (DEWL) Trial: a randomised controlled trial of high-protein versus high-carbohydrate diets over 2 years in type 2 diabetes. Diabetologia. 2012;55:905-14.

[116] Larsen RN, Mann NJ, Maclean E, Shaw JE. The effect of high-protein, low-carbohydrate diets in the treatment of type 2 diabetes: a 12 month randomised controlled trial. Diabetologia. 2011;54:731-40.

[117] Sargrad KR, Homko C, Mozzoli M, Boden G. Effect of high protein vs high carbohydrate intake on insulin sensitivity, body weight, hemoglobin A1c, and blood pressure in patients with type 2 diabetes mellitus. J Am Diet Assoc. 2005;105:573-80.

[118] Dr. Atkins' diet revolution. Med Lett Drugs Ther. 1973;15:41-2.

[119] Astrup A, Meinert Larsen T, Harper A. Atkins and other low-carbohydrate diets: hoax or an effective tool for weight loss? Lancet (London, England). 2004;364:897-9.

[120] Mansoor N, Vinknes KJ, Veierod MB, Retterstol K. Effects of low-carbohydrate diets v. low-fat diets on body weight and cardiovascular risk factors: a meta-analysis of randomised controlled trials. Br J Nutr. 2016;115:466-79.

[121] Dyson PA. A review of low and reduced carbohydrate diets and weight loss in type 2 diabetes. J Hum Nutr Diet. 2008;21:530-8.

[122] Ajala O, English P, Pinkney J. Systematic review and meta-analysis of different dietary approaches to the management of type 2 diabetes. Am J Clin Nutr. 2013;97:505-16.

[123] Dyson P. Low Carbohydrate Diets and Type 2 Diabetes: What is the Latest Evidence? Diabetes Ther. 2015;6:411-24.

[124] van Wyk HJ, Davis RE, Davies JS. A critical review of low-carbohydrate diets in people with Type 2 diabetes. Diabet Med. 2016;33:148-57.

[125] Castaneda-Gonzalez LM, Bacardi Gascon M, Jimenez Cruz A. Effects of low carbohydrate diets on weight and glycemic control among type 2 diabetes individuals: a systemic review of RCT greater than 12 weeks. Nutr Hosp. 2011;26:1270-6.

[126] Meng Y, Bai H, Wang S, Li Z, Wang Q, Chen L. Efficacy of low carbohydrate diet for type 2 diabetes mellitus management: A systematic review and meta-analysis of randomized controlled trials. Diabetes Res Clin Pract. 2017;131:124-31.

[127] van Zuuren EJ, Fedorowicz Z, Kuijpers T, Pijl H. Effects of low-carbohydrate- compared with low-fat-diet interventions on metabolic control in people with type 2 diabetes: a systematic review including GRADE assessments. Am J Clin Nutr. 2018;108:300-31.

[128] Jayedi A, Zeraattalab-Motlagh S, Jabbarzadeh B, Hosseini Y, Jibril AT, Shahinfar H, et al. Dose-dependent effect of carbohydrate restriction for type 2 diabetes management: a systematic review and dose-response meta-analysis of randomized controlled trials. Am J Clin Nutr. 2022.

[129] Turton J, Brinkworth GD, Field R, Parker H, Rooney K. An evidence-based approach to developing low-carbohydrate diets for type 2 diabetes management: A systematic review of interventions and methods. Diabetes Obes Metab. 2019.

[130] Gower BA, Goss AM. A lower-carbohydrate, higher-fat diet reduces abdominal and intermuscular fat and increases insulin sensitivity in adults at risk of type 2 diabetes. The Journal of nutrition. 2015;145:177s-83s.

[131] Westman EC, Feinman RD, Mavropoulos JC, Vernon MC, Volek JS, Wortman JA, et al. Low-carbohydrate nutrition and metabolism. Am J Clin Nutr. 2007;86:276-84.

[132] Manninen AH. Metabolic effects of the very-low-carbohydrate diets: misunderstood "villains" of human metabolism. J Int Soc Sports Nutr. 2004;1:7-11.

[133] Willoughby D, Hewlings S, Kalman D. Body Composition Changes in Weight Loss: Strategies and Supplementation for Maintaining Lean Body Mass, a Brief Review. Nutrients. 2018;10.

[134] Gomez-Arbelaez D, Bellido D, Castro AI, Ordonez-Mayan L, Carreira J, Galban C, et al. Body Composition Changes After Very-Low-Calorie Ketogenic Diet in Obesity Evaluated by 3 Standardized Methods. J Clin Endocrinol Metab. 2017;102:488-98.

[135] Organization WH. Cardiovascular diseases (CVDs). 2021.

[136] Singh M, Hung ES, Cullum A, Allen RE, Aggett PJ, Dyson P, et al. Lower carbohydrate diets for adults with type 2 diabetes. Diabet Med. 2022;39:e14674.

[137] Tay J, Luscombe-Marsh ND, Thompson CH, Noakes M, Buckley JD, Wittert GA, et al. Comparison of low- and high-carbohydrate diets for type 2 diabetes management: a randomized trial. Am J Clin Nutr. 2015;102:780-90.

[138] Rafiullah M, Musambil M, David SK. Effect of a very low-carbohydrate ketogenic diet vs recommended diets in patients with type 2 diabetes: a meta-analysis. Nutr Rev. 2022;80:488-502.

[139] Diamond DM, O'Neill BJ, Volek JS. Low carbohydrate diet: are concerns with saturated fat, lipids, and cardiovascular disease risk justified? Curr Opin Endocrinol Diabetes Obes. 2020;27:291-300.

[140] Samaha FF, Iqbal N, Seshadri P, Chicano KL, Daily DA, McGrory J, et al. A low-carbohydrate as compared with a low-fat diet in severe obesity. N Engl J Med. 2003;348:2074-81.

[141] Schwingshackl L, Hoffmann G. Comparison of effects of long-term low-fat vs high-fat diets on blood lipid levels in overweight or obese patients: a systematic review and meta-analysis. J Acad Nutr Diet. 2013;113:1640-61.

[142] 8. Obesity Management for the Treatment of Type 2 Diabetes: Standards of Medical Care in Diabetes-2021. Diabetes Care. 2021;44:S100-s10.

[143] Akter S, Mizoue T, Nanri A, Goto A, Noda M, Sawada N, et al. Low carbohydrate diet and all cause and cause-specific mortality. Clin Nutr. 2021;40:2016-24.

[144] Seidelmann SB, Claggett B, Cheng S, Henglin M, Shah A, Steffen LM, et al. Dietary carbohydrate intake and mortality: a prospective cohort study and meta-analysis. Lancet Public Health. 2018;3:e419-e28.

[145] Nakamura Y, Okuda N, Okamura T, Kadota A, Miyagawa N, Hayakawa T, et al. Low-carbohydrate diets and cardiovascular and total mortality in Japanese: a 29-year follow-up of NIPPON DATA80. Br J Nutr. 2014;112:916-24.

[146] Quyyumi AA. Endothelial function in health and disease: new insights into the genesis of cardiovascular disease. Am J Med. 1998;105:32S-9S.

[147] van den Oever IA, Raterman HG, Nurmohamed MT, Simsek S. Endothelial dysfunction, inflammation, and apoptosis in diabetes mellitus. Mediators Inflamm. 2010;2010:792393.

[148] Mucka S, Miodonska M, Jakubiak GK, Starzak M, Cieslar G, Stanek A. Endothelial Function Assessment by Flow-Mediated Dilation Method: A Valuable Tool in the Evaluation of the Cardiovascular System. Int J Environ Res Public Health. 2022;19.

[149] Maruhashi T, Kajikawa M, Kishimoto S, Hashimoto H, Takaeko Y, Yamaji T, et al. Diagnostic Criteria of Flow-Mediated Vasodilation for Normal Endothelial Function and Nitroglycerin-Induced Vasodilation for Normal Vascular Smooth Muscle Function of the Brachial Artery. J Am Heart Assoc. 2020;9:e013915.

[150] Matsuzawa Y, Kwon TG, Lennon RJ, Lerman LO, Lerman A. Prognostic Value of Flow-Mediated Vasodilation in Brachial Artery and Fingertip Artery for Cardiovascular Events: A Systematic Review and Meta-Analysis. J Am Heart Assoc. 2015;4.

[151] Poredos P, Poredos AV, Gregoric I. Endothelial Dysfunction and Its Clinical Implications. Angiology. 2021;72:604-15.

[152] Joris PJ, Zeegers MP, Mensink RP. Weight loss improves fasting flow-mediated vasodilation in adults: a meta-analysis of intervention studies. Atherosclerosis. 2015;239:21-30.

[153] Takeda Y, Matoba K, Sekiguchi K, Nagai Y, Yokota T, Utsunomiya K, et al. Endothelial Dysfunction in Diabetes. Biomedicines. 2020;8.

[154] Kasprzak JD, Klosinska M, Drozdz J. Clinical aspects of assessment of endothelial function. Pharmacol Rep. 2006;58 Suppl:33-40.

[155] Avogaro A, Fadini GP, Gallo A, Pagnin E, de Kreutzenberg S. Endothelial dysfunction in type 2 diabetes mellitus. Nutr Metab Cardiovasc Dis. 2006;16 Suppl 1:S39-45.

[156] Bruno RM, Penno G, Daniele G, Pucci L, Lucchesi D, Stea F, et al. Type 2 diabetes mellitus worsens arterial stiffness in hypertensive patients through endothelial dysfunction. Diabetologia. 2012;55:1847-55.

[157] Dhananjayan R, Koundinya KS, Malati T, Kutala VK. Endothelial Dysfunction in Type 2 Diabetes Mellitus. Indian J Clin Biochem. 2016;31:372-9.

[158] van Sloten TT, Henry RM, Dekker JM, Nijpels G, Unger T, Schram MT, et al. Endothelial dysfunction plays a key role in increasing cardiovascular risk in type 2 diabetes: the Hoorn study. Hypertension. 2014;64:1299-305.

[159] Calle MC, Fernandez ML. Inflammation and type 2 diabetes. Diabetes Metab. 2012;38:183-91.

[160] Caballero AE. Endothelial dysfunction in obesity and insulin resistance: a road to diabetes and heart disease. Obesity research. 2003;11:1278-89.

[161] Hunt BJ, Jurd KM. Endothelial cell activation. A central pathophysiological process. BMJ (Clinical research ed). 1998;316:1328-9.

[162] Davis N, Katz S, Wylie-Rosett J. The effect of diet on endothelial function. Cardiol Rev. 2007;15:62-6.

[163] Wycherley TP, Thompson CH, Buckley JD, Luscombe-Marsh ND, Noakes M, Wittert GA, et al. Long-term effects of weight loss with a very-low carbohydrate, low saturated fat diet on flow mediated dilatation in patients with type 2 diabetes: A randomised controlled trial. Atherosclerosis. 2016;252:28-31.

[164] Barbosa-Yañez RL, Dambeck U, Li L, Machann J, Kabisch S, Pfeiffer AFH. Acute Endothelial Benefits of Fat Restriction over Carbohydrate Restriction in Type 2 Diabetes Mellitus: Beyond Carbs and Fats. Nutrients. 2018;10.

[165] Kawai T, Autieri MV, Scalia R. Adipose tissue inflammation and metabolic dysfunction in obesity. Am J Physiol Cell Physiol. 2021;320:C375-c91.

[166] Kirk B, Feehan J, Lombardi G, Duque G. Muscle, Bone, and Fat Crosstalk: the Biological Role of Myokines, Osteokines, and Adipokines. Curr Osteoporos Rep. 2020;18:388-400.

[167] Lehr S, Hartwig S, Sell H. Adipokines: a treasure trove for the discovery of biomarkers for metabolic disorders. Proteomics Clin Appl. 2012;6:91-101.

[168] Taylor EB. The complex role of adipokines in obesity, inflammation, and autoimmunity. Clin Sci (Lond). 2021;135:731-52.

[169] Farooqi IS, O'Rahilly S. Leptin: a pivotal regulator of human energy homeostasis. Am J Clin Nutr. 2009;89:980S-4S.

[170] Obradovic M, Sudar-Milovanovic E, Soskic S, Essack M, Arya S, Stewart AJ, et al. Leptin and Obesity: Role and Clinical Implication. Front Endocrinol (Lausanne). 2021;12:585887.

[171] Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E, et al. Role of leptin in the neuroendocrine response to fasting. Nature. 1996;382:250-2.

[172] Rosenbaum M, Leibel RL. 20 years of leptin: role of leptin in energy homeostasis in humans. J Endocrinol. 2014;223:T83-96.

[173] Nguyen TMD. Adiponectin: Role in Physiology and Pathophysiology. Int J Prev Med. 2020;11:136.

[174] Mojiminiyi OA, Abdella NA, Al Arouj M, Ben Nakhi A. Adiponectin, insulin resistance and clinical expression of the metabolic syndrome in patients with Type 2 diabetes. Int J Obes (Lond). 2007;31:213-20.

[175] Hoene M, Weigert C. The role of interleukin-6 in insulin resistance, body fat distribution and energy balance. Obes Rev. 2008;9:20-9.

[176] Clyne B, Olshaker JS. The C-reactive protein. J Emerg Med. 1999;17:1019-25.

[177] Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. 1999;340:448-54.

[178] Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA. 2001;286:327-34.

[179] Grossmann V, Schmitt VH, Zeller T, Panova-Noeva M, Schulz A, Laubert-Reh D, et al. Profile of the Immune and Inflammatory Response in Individuals With Prediabetes and Type 2 Diabetes. Diabetes Care. 2015;38:1356-64.

[180] Mirza S, Hossain M, Mathews C, Martinez P, Pino P, Gay JL, et al. Type 2-diabetes is associated with elevated levels of TNF-alpha, IL-6 and adiponectin and low levels of leptin in a population of Mexican Americans: a cross-sectional study. Cytokine. 2012;57:136-42.

[181] Mahajan A, Tabassum R, Chavali S, Dwivedi OP, Bharadwaj M, Tandon N, et al. High-sensitivity C-reactive protein levels and type 2 diabetes in urban North Indians. J Clin Endocrinol Metab. 2009;94:2123-7.

[182] Aryan Z, Ghajar A, Faghihi-Kashani S, Afarideh M, Nakhjavani M, Esteghamati A. Baseline High-Sensitivity C-Reactive Protein Predicts Macrovascular and Microvascular Complications of Type 2 Diabetes: A Population-Based Study. Ann Nutr Metab. 2018;72:287-95.

[183] Fuster JJ, Ouchi N, Gokce N, Walsh K. Obesity-Induced Changes in Adipose Tissue Microenvironment and Their Impact on Cardiovascular Disease. Circ Res. 2016;118:1786-807.

[184] Kimura A, Kishimoto T. IL-6: regulator of Treg/Th17 balance. Eur J Immunol. 2010;40:1830-5.

[185] Navarro-Millan I, Singh JA, Curtis JR. Systematic review of tocilizumab for rheumatoid arthritis: a new biologic agent targeting the interleukin-6 receptor. Clin Ther. 2012;34:788-802 e3.

[186] Zhang Y, Scarpace PJ. The role of leptin in leptin resistance and obesity. Physiol Behav. 2006;88:249-56.

[187] Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol. 2011;11:85-97.

[188] Depraetere S, Willems J, Joniau M. Stimulation of CRP secretion in HepG2 cells: cooperative effect of dexamethasone and interleukin 6. Agents Actions. 1991;34:369-75.

[189] Calabro P, Chang DW, Willerson JT, Yeh ET. Release of C-reactive protein in response to inflammatory cytokines by human adipocytes: linking obesity to vascular inflammation. J Am Coll Cardiol. 2005;46:1112-3.
[190] Kaneko H, Anzai T, Nagai T, Anzai A, Takahashi T, Mano Y, et al. Human C-reactive protein exacerbates metabolic disorders in association with adipose tissue remodelling. Cardiovasc Res. 2011;91:546-55.
[191] Stanimirovic J, Radovanovic J, Banjac K, Obradovic M, Essack M, Zafirovic S, et al. Role of C-Reactive Protein in Diabetic Inflammation. Mediators Inflamm. 2022;2022:3706508.
[192] Herder C, Schöttker B, Rothenbacher D, Roden M, Kolb H, Müller H, et al. Interleukin-6 in the prediction of primary cardiovascular events in diabetes patients: results from the ESTHER study. Atherosclerosis. 2011;216:244-7.
[193] Ofstad AP, Gullestad L, Orvik E, Aakhus S, Endresen K, Ueland T, et al. Interleukin-6 and activin A are independently associated with cardiovascular events and mortality in type 2 diabetes: the prospective Asker and Bærum Cardiovascular Diabetes (ABCD) cohort study. Cardiovascular diabetology. 2013;12:126.
[194] Kaptoge S, Seshasai SR, Gao P, Freitag DF, Butterworth AS, Borglykke A, et al. Inflammatory cytokines and risk of coronary heart disease: new prospective study and updated meta-analysis. Eur Heart J. 2014;35:578-89.
[195] Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K, et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. Br J Nutr. 2011;106 Suppl 3:S5-78.
[196] Cipryan L, Dostal T, Plews DJ, Hofmann P, Laursen PB. Adiponectin/leptin ratio increases after a 12-week very low-carbohydrate, high-fat diet, and exercise training in healthy individuals: A non-randomized, parallel design study. Nutr Res. 2021;87:22-30.
[197] Cipryan L, Maffetone PB, Plews DJ, Laursen PB. Effects of a four-week very low-carbohydrate high-fat diet on biomarkers of inflammation: Non-randomised parallel-group study. Nutr Health. 2020;26:35-42.
[198] Asle Mohammadi Zadeh M, Kargarfard M, Marandi SM, Habibi A. Diets along with interval training regimes improves inflammatory & anti-inflammatory condition in obesity with type 2 diabetes subjects. J Diabetes Metab Disord. 2018;17:253-67.

[199] Yang M, Qiu S, He Y, Li L, Wu T, Ding N, et al. Genetic ablation of C-reactive protein gene confers resistance to obesity and insulin resistance in rats. Diabetologia. 2021;64:1169-83.
[200] Ruth MR, Port AM, Shah M, Bourland AC, Istfan NW, Nelson KP, et al. Consuming a hypocaloric high fat low carbohydrate diet for 12 weeks lowers C-reactive protein, and raises serum adiponectin and high density lipoprotein-cholesterol in obese subjects. Metabolism. 2013;62:1779-87.
[201] Seshadri P, Iqbal N, Stern L, Williams M, Chicano KL, Daily DA, et al. A randomized study comparing the effects of a low-carbohydrate diet and a conventional diet on lipoprotein subfractions and C-reactive protein levels in patients with severe obesity. Am J Med. 2004;117:398-405.
[202] Skytte MJ, Samkani A, Astrup A, Larsen TM, Frystyk J, Poulsen HE, et al. Effects of a highly controlled carbohydrate-reduced high-protein diet on markers of oxidatively generated nucleic acid modifications and inflammation in weight stable participants with type 2 diabetes; a randomized controlled trial. Scand J Clin Lab Invest. 2020;80:401-7.
[203] Jonasson L, Guldbrand H, Lundberg AK, Nystrom FH. Advice to follow a low-carbohydrate diet has a favourable impact on low-grade inflammation in type 2 diabetes compared with advice to follow a low-fat diet. Ann Med. 2014;46:182-7.
[204] Davis NJ, Crandall JP, Gajavelli S, Berman JW, Tomuta N, Wylie-Rosett J, et al. Differential effects of low-carbohydrate and low-fat diets on inflammation and endothelial function in diabetes. J Diabetes Complications. 2011;25:371-6.
[205] Mooradian AD. The Merits and the Pitfalls of Low Carbohydrate Diet: A Concise Review. J Nutr Health Aging. 2020;24:805-8.
[206] Schutz Y, Montani JP, Dulloo AG. Low-carbohydrate ketogenic diets in body weight control: A recurrent plaguing issue of fad diets? Obes Rev. 2021;22 Suppl 2:e13195.
[207] Cucuzzella M, Riley K, Isaacs D. Adapting Medication for Type 2 Diabetes to a Low Carbohydrate Diet. Front Nutr. 2021;8:688540.

[208] Blanco JC, Khatri A, Kifayat A, Cho R, Aronow WS. Starvation Ketoacidosis due to the Ketogenic Diet and Prolonged Fasting - A Possibly Dangerous Diet Trend. Am J Case Rep. 2019;20:1728-31.

[209] Marzban S, Arbee M, Vorajee N, Richards GA. Non-diabetic ketoacidosis associated with a low carbohydrate, high fat diet in a postpartum lactating female. Oxf Med Case Reports. 2020;2020:omz026.

[210] Ma Y, Hu M, Zhou L, Ling S, Li Y, Kong B, et al. Dietary fiber intake and risks of proximal and distal colon cancers: A meta-analysis. Medicine (Baltimore). 2018;97:e11678.

[211] Threapleton DE, Greenwood DC, Evans CE, Cleghorn CL, Nykjaer C, Woodhead C, et al. Dietary fibre intake and risk of cardiovascular disease: systematic review and meta-analysis. BMJ (Clinical research ed). 2013;347:f6879.

[212] McRae MP. Dietary Fiber Intake and Type 2 Diabetes Mellitus: An Umbrella Review of Meta-analyses. J Chiropr Med. 2018;17:44-53.

[213] Chandalia M, Garg A, Lutjohann D, von Bergmann K, Grundy SM, Brinkley LJ. Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. N Engl J Med. 2000;342:1392-8.

[214] Czyzewska-Majchrzak L, Grzelak T, Kramkowska M, Czyzewska K, Witmanowski H. The use of low-carbohydrate diet in type 2 diabetes - benefits and risks. Ann Agric Environ Med. 2014;21:320-6.

[215] Tay J, Thompson CH, Luscombe-Marsh ND, Noakes M, Buckley JD, Wittert GA, et al. Nutritional adequacy of very low- and high-carbohydrate, low saturated fat diets in adults with type 2 diabetes: A secondary analysis of a 2-year randomised controlled trial. Diabetes Res Clin Pract. 2020;170:108501.

[216] Burke LM. Ketogenic low-CHO, high-fat diet: the future of elite endurance sport? J Physiol. 2021;599:819-43.

[217] Foster GD, Wyatt HR, Hill JO, Makris AP, Rosenbaum DL, Brill C, et al. Weight and metabolic outcomes after 2 years on a low-carbohydrate versus low-fat diet: a randomized trial. Annals of internal medicine. 2010;153:147-57.

[218] Ko GJ, Obi Y, Tortorici AR, Kalantar-Zadeh K. Dietary protein intake and chronic kidney disease. Curr Opin Clin Nutr Metab Care. 2017;20:77-85.

[219] Ko GJ, Rhee CM, Kalantar-Zadeh K, Joshi S. The Effects of High-Protein Diets on Kidney Health and Longevity. J Am Soc Nephrol. 2020;31:1667-79.

[220] Fisheries MoFAa. The official dietary guidelines Food-based dietary guidelines Revision in September 2013 based on the Nordic Nutrition Recommendations 2012 ed.

[221] 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2021. Diabetes Care. 2021;44:S15-s33.

[222] Harris JA, Benedict FG. A Biometric Study of Human Basal Metabolism. Proc Natl Acad Sci U S A. 1918;4:370-3.

[223] Gerrior S, Juan W, Basiotis P. An easy approach to calculating estimated energy requirements. Prev Chronic Dis. 2006;3:A129.

[224] Madden AM, Mulrooney HM, Shah S. Estimation of energy expenditure using prediction equations in overweight and obese adults: a systematic review. J Hum Nutr Diet. 2016;29:458-76.

[225] Skotte J, Korshoj M, Kristiansen J, Hanisch C, Holtermann A. Detection of physical activity types using triaxial accelerometers. J Phys Act Health. 2014;11:76-84.

[226] Simova I, Nossikoff A, Denchev S. Interobserver and intraobserver variability of flow-mediated vasodilatation of the brachial artery. Echocardiography. 2008;25:77-83.

[227] International Hypoglycaemia Study G. Glucose Concentrations of Less Than 3.0 mmol/L (54 mg/dL) Should Be Reported in Clinical Trials: A Joint Position Statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2017;40:155-7.

[228] Fonseca VA. Defining and characterizing the progression of type 2 diabetes. Diabetes Care. 2009;32 Suppl 2:S151-6.

[229] Murdoch C, Unwin D, Cavan D, Cucuzzella M, Patel M. Adapting diabetes medication for low carbohydrate management of type 2 diabetes: a practical guide. Br J Gen Pract. 2019;69:360-1.

[230] Alley H, Owens CD, Gasper WJ, Grenon SM. Ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery in clinical research. J Vis Exp. 2014:e52070.

[231] Miyamoto M, Kotani K, Ishibashi S, Taniguchi N. The effect of antihypertensive drugs on endothelial function as assessed by flow-mediated vasodilation in hypertensive patients. Int J Vasc Med. 2012;2012:453264.

[232] Thijssen DHJ, Bruno RM, van Mil A, Holder SM, Faita F, Greyling A, et al. Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. Eur Heart J. 2019;40:2534-47.

[233] Vlachopoulos C, Xaplanteris P, Aboyans V, Brodmann M, Cífková R, Cosentino F, et al. The role of vascular biomarkers for primary and secondary prevention. A position paper from the European Society of Cardiology Working Group on peripheral circulation: Endorsed by the Association for Research into Arterial Structure and Physiology (ARTERY) Society. Atherosclerosis. 2015;241:507-32.

[234] Heiss C, Rodriguez-Mateos A, Bapir M, Skene SS, Sies H, Kelm M. Flow-mediated dilation reference values for evaluation of endothelial function and cardiovascular health. Cardiovasc Res. 2022.

[235] Gram-Kampmann EM, Hansen CD, Hugger MB, Jensen JM, Brønd JC, Hermann AP, et al. Effects of a 6-month, low-carbohydrate diet on glycaemic control, body composition, and cardiovascular risk factors in patients with type 2 diabetes: An open-label randomized controlled trial. Diabetes Obes Metab. 2022.

[236] Tay J, de Bock MI, Mayer-Davis EJ. Low-carbohydrate diets in type 2 diabetes. Lancet Diabetes Endocrinol. 2019;7:331-3.

[237] Tay J, Luscombe-Marsh ND, Thompson CH, Noakes M, Buckley JD, Wittert GA, et al. A very low-carbohydrate, low-saturated fat diet for type 2 diabetes management: a randomized trial. Diabetes Care. 2014;37:2909-18.

[238] Saslow LR, Daubenmier JJ, Moskowitz JT, Kim S, Murphy EJ, Phinney SD, et al. Twelve-month outcomes of a randomized trial of a moderate-carbohydrate versus very low-carbohydrate diet in overweight adults with type 2 diabetes mellitus or prediabetes. Nutr Diabetes. 2017;7:304.

[239] Maloney A, Rosenstock J, Fonseca V. A Model-Based Meta-Analysis of 24 Antihyperglycemic Drugs for Type 2 Diabetes: Comparison of Treatment Effects at Therapeutic Doses. Clin Pharmacol Ther. 2019;105:1213-23.

[240] Guasch-Ferre M, Babio N, Martinez-Gonzalez MA, Corella D, Ros E, Martin-Pelaez S, et al. Dietary fat intake and risk of cardiovascular disease and all-cause mortality in a population at high risk of cardiovascular disease. Am J Clin Nutr. 2015;102:1563-73.

[241] Jakobsen MU, O'Reilly EJ, Heitmann BL, Pereira MA, Bälter K, Fraser GE, et al. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. Am J Clin Nutr. 2009;89:1425-32.

[242] Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. Am J Clin Nutr. 2010;91:535-46.

[243] Evert AB, Boucher JL, Cypress M, Dunbar SA, Franz MJ, Mayer-Davis EJ, et al. Nutrition therapy recommendations for the management of adults with diabetes. Diabetes Care. 2013;36:3821-42.

[244] Celermajer DS. Endothelial dysfunction: does it matter? Is it reversible? J Am Coll Cardiol. 1997;30:325-33.

[245] Health effects of dietary risks in 195 countries, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet (London, England). 2019;393:1958-72.

[246] Yamaji T, Harada T, Hashimoto Y, Nakano Y, Kajikawa M, Yoshimura K, et al. Relationship between hemoglobin A1c level and flow-mediated vasodilation in patients with type 2 diabetes mellitus receiving antidiabetic drugs. J Diabetes Investig. 2022;13:677-86.

[247] Naka KK, Papathanassiou K, Bechlioulis A, Kazakos N, Pappas K, Tigas S, et al. Determinants of vascular function in patients with type 2 diabetes. Cardiovascular diabetology. 2012;11:127.

[248] Maruhashi T, Iwamoto Y, Kajikawa M, Oda N, Kishimoto S, Matsui S, et al. Interrelationships Among Flow-Mediated Vasodilation, Nitroglycerine-Induced Vasodilation, Baseline Brachial Artery Diameter, Hyperemic Shear Stress, and Cardiovascular Risk Factors. J Am Heart Assoc. 2017;7.

[249] Maruhashi T, Soga J, Fujimura N, Idei N, Mikami S, Iwamoto Y, et al. Relationship between flow-mediated vasodilation and cardiovascular risk factors in a large community-based study. Heart. 2013;99:1837-42.

[250] Ridker PM, MacFadyen JG, Glynn RJ, Bradwin G, Hasan AA, Rifai N. Comparison of interleukin-6, C-reactive protein, and low-density lipoprotein cholesterol as biomarkers of residual risk in contemporary practice: secondary analyses from the Cardiovascular Inflammation Reduction Trial. Eur Heart J. 2020;41:2952-61.

[251] Chmurzynska A, Muzsik A, Krzyżanowska-Jankowska P, Walkowiak J, Bajerska J. The Effect of Habitual Fat Intake, IL6 Polymorphism, and Different Diet Strategies on Inflammation in Postmenopausal Women with Central Obesity. Nutrients. 2019;11.

[252] Andrich DE, Melbouci L, Ou Y, Auclair N, Mercier J, Grenier JC, et al. A Short-Term High-Fat Diet Alters Glutathione Levels and IL-6 Gene Expression in Oxidative Skeletal Muscles of Young Rats. Front Physiol. 2019;10:372.

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ORIGINAL ARTICLE

WILEY

Effects of a 6-month, low-carbohydrate diet on glycaemic control, body composition, and cardiovascular risk factors in patients with type 2 diabetes: An open-label randomized controlled trial

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Abstract

Aim: To investigate the efficacy and safety of a non-calorie–restricted low-carbohydrate diet (LCD) on glycaemic control, body composition, and cardiovascular risk factors in patients with type 2 diabetes (T2D) instructed to maintain their non-insulin antidiabetic medication and physical activity.

Materials and Methods: In an open-label randomized controlled trial, patients with T2D were randomized 2:1 to either a LCD with a maximum of 20 E% (percentage of total energy intake) from carbohydrates (n = 49) or a control diet with 50-60 E% from carbohydrates (n = 22) for 6 months. Examinations at enrolment and after 3 and 6 months included blood sample analyses, anthropometrics, blood pressure, accelerometer-based assessment of physical activity, and food diaries. Total fat mass and lean mass were determined by dual-energy x-ray absorptiometry scan. The mean difference in change between groups from baseline are reported.

Results: The LCD group decreased carbohydrate intake to 13.4 E% and increased fat intake to 63.2 E%, which was −30.5 ± 2.2 E% lower for carbohydrates and 30.6 ± 2.2 E% higher for fat, respectively, compared with the control group (all $P < .001$). The LCD reduced HbA1c after 3 months (−8.9 ± 1.7 mmol/mol; $P < .0001$), and this was maintained after 6 months (−7.5 ± 1.8 mmol/mol; $P < .0001$) compared with the control diet. The LCD also reduced weight (−3.9 ± 1.0 kg), body mass index (−1.4 ± 0.4 kg/m^2), and waist circumference (−4.9 ± 1.3 cm) compared with the control diet (all $P < .01$), accompanied by reductions in total fat mass (−2.2 ± 1.0 kg; $P = .027$) and lean mass (−1.3 ± 0.6 kg; $P = .017$). No changes in blood lipids or blood pressure were seen after 6 months. The level of physical activity was maintained, and there were no episodes of severe hypoglycaemia.

Conclusion: A non-calorie–restricted LCD high in fat has significant beneficial effects on glycaemic control and body composition, and does not adversely affect cardiovascular risk factors in patients with T2D. Reducing carbohydrate intake to 10-25 E% appears to be an effective and safe nutritional approach with respect to classical cardiovascular risk factors and hypoglycaemia.

KEYWORDS
low-carbohydrate diet, non-calorie–restricted, physical activity, randomized controlled trial, type 2 diabetes

1 | INTRODUCTION

The prevalence of type 2 diabetes (T2D) has steadily increased over the past 40 years, with an alarming prediction of 693 million people with diabetes worldwide in 2045.[1] While good glycaemic control is of vital importance to prevent microvascular and macrovascular complications,[2] lifestyle intervention focusing on reduced caloric intake and increased physical activity remains the first-line treatment for T2D.[3] Both glycaemic control and weight loss are achievable with dietary changes in T2D.[3] Recently, the traditional diet recommendations consisting of high-carbohydrate, low-fat diets high in fibre with a focus on energy restriction[4,5] have been challenged by carbohydrate-restricted diets, which have grown increasingly popular, not only as a means to lose body weight, but also to improve glycaemic control and reduce glucose-lowering medication in patients with T2D.[6-8]

Recent reviews and meta-analyses have evaluated the effects of carbohydrate-restricted diets compared with control diets low in fat in T2D; however, these have reached somewhat conflicting conclusions regarding the effects on HbA1c, weight loss, and cardiovascular risk factors.[8-14] The mixed results reported are partly explained by the different degrees of carbohydrate restriction.[10,11] To better distinguish among different clinical trials, it was recently proposed to define an intake of less than 10 E% (percentage of total energy intake) carbohydrates as a very low-carbohydrate ketogenic diet (VLCKD), 10-25 E% as a low-carbohydrate diet (LCD), 26-45 E% as a moderate-carbohydrate diet, and more than 45 E% as a high-carbohydrate diet.[8-10] There is evidence that the greater the carbohydrate restriction, then the greater the glucose-lowering effect in T2D,[11] whereas it is uncertain if this also applies to cardiovascular risk factors, such as dyslipidaemia and blood pressure, as well as measures of body composition.[10]

There have been concerns that a higher intake of fat in carbohydrate-restricted diets may increase the risk of cardiovascular disease (CVD), especially if the intake of saturated fatty acids (SFA) is increased.[10,15] However, carbohydrate-restricted diets high in fat appear to cause small improvements in plasma triglycerides and HDL cholesterol compared with fat restriction, but with little or no effect on serum LDL cholesterol or blood pressure.[9-14] It is far less studied whether carbohydrate-restricted diets cause beneficial changes in the body composition in patients with T2D. A study comparing the effect of a 24-week hypocaloric LCD with a control diet, both combined with increased exercise, showed comparable reductions in fat mass, lean mass, and waist circumference,[16] but the isolated effect of a LCD on body composition in patients with T2D remains to be clarified.

While the majority of reviews and meta-analyses conclude that carbohydrate-restricted diets have a greater glucose-lowering effect than control diets in patients with T2D, it is clear that these results are often affected by factors other than the carbohydrate restriction alone.[8-14] Thus, the glucose-lowering effect of carbohydrate restriction is probably underestimated in studies where discontinuation of antidiabetic medication in the carbohydrate-restricted group was a goal.[17-21] Moreover, carbohydrate restriction is often combined with caloric restriction in both groups,[20,22] the control group only,[19,23] or both reduced calorie intake and increased physical activity.[16,24] It is known that weight loss because of calorie restriction decreases HbA1c.[25] Thus, these strategies could potentially confound the isolated effect of carbohydrate restriction on HbA1c, weight loss, and cardiovascular risk factors. Moreover, inclusion of these additional factors makes the intervention less feasible under free-living conditions.

In the current study, we tested the hypothesis that a non-calorie–restricted LCD, which is feasible under free-living conditions, improves HbA1c, weight, and body composition and is safe with respect to cardiovascular risk factors and hypoglycaemia in patients with T2D instructed to maintain their non-insulin glucose-lowering medication and level of physical activity.

2 | MATERIALS AND METHODS

2.1 | Study design

This study was an open-label randomized controlled trial (RCT) on outpatients diagnosed with T2D, enrolled from November 2016 to December 2018 at Odense University Hospital. The participants were randomized to either a LCD or a control diet high in carbohydrates and fibre, following the official dietary guidelines for the general population in Denmark.[4] The study duration was 6 months and included three visits, at baseline, after 3 months, and after 6 months (±1-2 weeks), as well as a telephone call 2 weeks after the onset of diet change. Additional contacts were scheduled on demand or as a safety measure for insulin- and sulphonylurea-treated participants. The mean difference in change between groups in HbA1c from baseline to 6 months was the primary outcome, while changes in Hb1Ac, body composition, and cardiovascular risk factors from baseline to 3 and 6 months were secondary outcomes.

2.2 | Study population

Participants were recruited through public advertisements and invitations to previous study participants. The inclusion criteria were a previous diagnosis of T2D,[26] an HbA1c of more than 48 mmol/mol with or without the use of glucose-lowering pharmacotherapy, and a diabetes duration of 6 months to 5 years (but up to 10 years if current

treatment consisted of ≤2 oral antidiabetic drugs without insulin). The antihyperglycaemic pharmacotherapy should be stable for at least 3 months prior to inclusion and any dyslipidaemia should be well-treated (LDL cholesterol <2.5 mmol/L and total cholesterol <4.5 mmol/L). The participants had to be older than 18 years of age and understand oral and written Danish. All participants had to sign an informed consent form after being given oral and written information on the experimental design. The cholesterol criteria were relaxed if there was a low probability of changes in cholesterol-lowering medication during the intervention (participants who refused cholesterol-lowering treatment, statin intolerance, females of child-bearing age, or treatment with potent statins).

The exclusion criteria were significant co-morbidities, including liver disease, a history of cancer of less than 5 years, or current chemotherapy. Participants with other severe co-morbidities that could interfere with study compliance or safety (e.g. previous gastrointestinal operations, liver disease, history of an eating disorder, current alcohol overuse, or hypoglycaemic unawareness) were not included. Other exclusion criteria were continuous use of steatosis-inducing drugs or glucocorticoids and treatment with antibiotics during the last 2 months before inclusion, low-carbohydrate diet prior to inclusion, excessive weight loss (>10 kg) within the last 3 months, or pregnancy/planned pregnancy.

People declared their interest in participation through completion of a prescreening questionnaire. Of 345 people eligible for screening, 73 were enrolled in the study (Figure 1). They were randomized in a 2:1 ratio to either the LCD intervention or the control diet by random computer-generated assignment using the web-based REDCap randomization module. To ensure better adherence, family members who fulfilled the criteria were randomized as one unit by only randomizing one spouse/sibling. Randomization was stratified on sex and quantity of prescribed antidiabetic agents (0-1 or ≥2 types of antidiabetic agents). Two participants withdrew consent to participate before the baseline visit, leaving 49 participants in the LCD group and 22 in the control group. After study initiation, three participants from the LCD group dropped out before the 3-month visit, and an additional two participants from each group dropped out before the 6-month visit (Figure 1).

Informed consent was obtained from all individuals before participation. The study was approved by the Regional Committees on Health Research Ethics for Southern Denmark and was performed in accordance with the Declaration of Helsinki Declaration II. The RCT was registered at ClinicalTrials.gov (NCT03068078).

2.3 | Diet interventions

This was a free-living study and no food was provided to the participants. At the 1-h baseline visit, a licensed dietitian individually instructed participants in both groups with regard to following the principles of their allocated diets, until these were fully understood. Participants assigned to the LCD were instructed to compose their diet with a maximum of 20 E% of carbohydrates (mainly complex and water-soluble), 50-60 E% fat, and 25-30 E% protein. A high intake of monounsaturated fatty acids (MUFA) and an intake of SFA as low as possible was encouraged. Participants assigned to the control diet were instructed to follow the official Danish dietary guidelines, consisting of 50-60 E% carbohydrates mainly from fruit, vegetables, and whole-grain sources, 20-30 E% fat, where less than 10 E% should be from SFA, and 20-25 E% protein.[4] Written material and a 5-day start-up diet plan based on the calculated baseline caloric intake were provided. This was based on the estimated energy requirement that was calculated based on sex, height, weight, and physical activity level,[27] and was used as a guideline for non-calorie–restricted intake during the entire intervention period. An internet-based food diary (MadLog Aps, Kolding, Denmark) was used to keep track of the participants' energy intake and diet composition. For further details, refer to the supporting information.

2.4 | Glucose-lowering medication and safety monitoring

All participants were instructed to continue their non-insulin glucose-lowering medication, unless they experienced hypoglycaemia and were advised by the study staff to reduce or discontinue medication for glycaemic safety. In insulin-treated participants (n = 2) allocated to LCD, the daily dose was reduced by 20% at baseline, and they were instructed to report blood glucose levels at least once weekly until establishment of glycaemic control without hypoglycaemia. Participants in the LCD group treated with sulphonylureas (n = 4) were instructed to report blood glucose levels in the same manner and to reduce the dose if they experienced hypoglycaemia. All participants were instructed to measure plasma glucose levels regularly on their own glucose-measuring device before meals and at night-time, and to report measurements for at least 3 separate days at each visit (Table S1). They were also instructed to measure plasma glucose levels if they experienced symptoms of hypoglycaemia, and to report any hypoglycaemic episodes as soon as possible. Symptoms of hypoglycaemia and a plasma glucose of 3.9 mmol/L or less was defined as documented symptomatic hypoglycaemia, a plasma glucose of less than 3.0 mmol/L was defined as clinically important biochemical hypoglycaemia, and severe hypoglycaemia was defined as requiring the help of another person for recovery.[28] For further details, refer to the supporting information.

2.5 | Biochemical analysis and anthropometrics

At all three visits, biochemical analysis, weight, height, waist circumference, and blood pressure were measured by standardized procedures (see the supporting information). At baseline and at the last visit (6 months), total and regional fat mass and lean mass were assessed with a dual-energy x-ray absorptiometry (DXA) scan (Hologic, Marlborough, MA).

2.6 | Physical activity

Participants were instructed to maintain their usual physical activity level throughout the study period. Accelerometer-based assessment of physical activity was recorded on 7 consecutive days at baseline

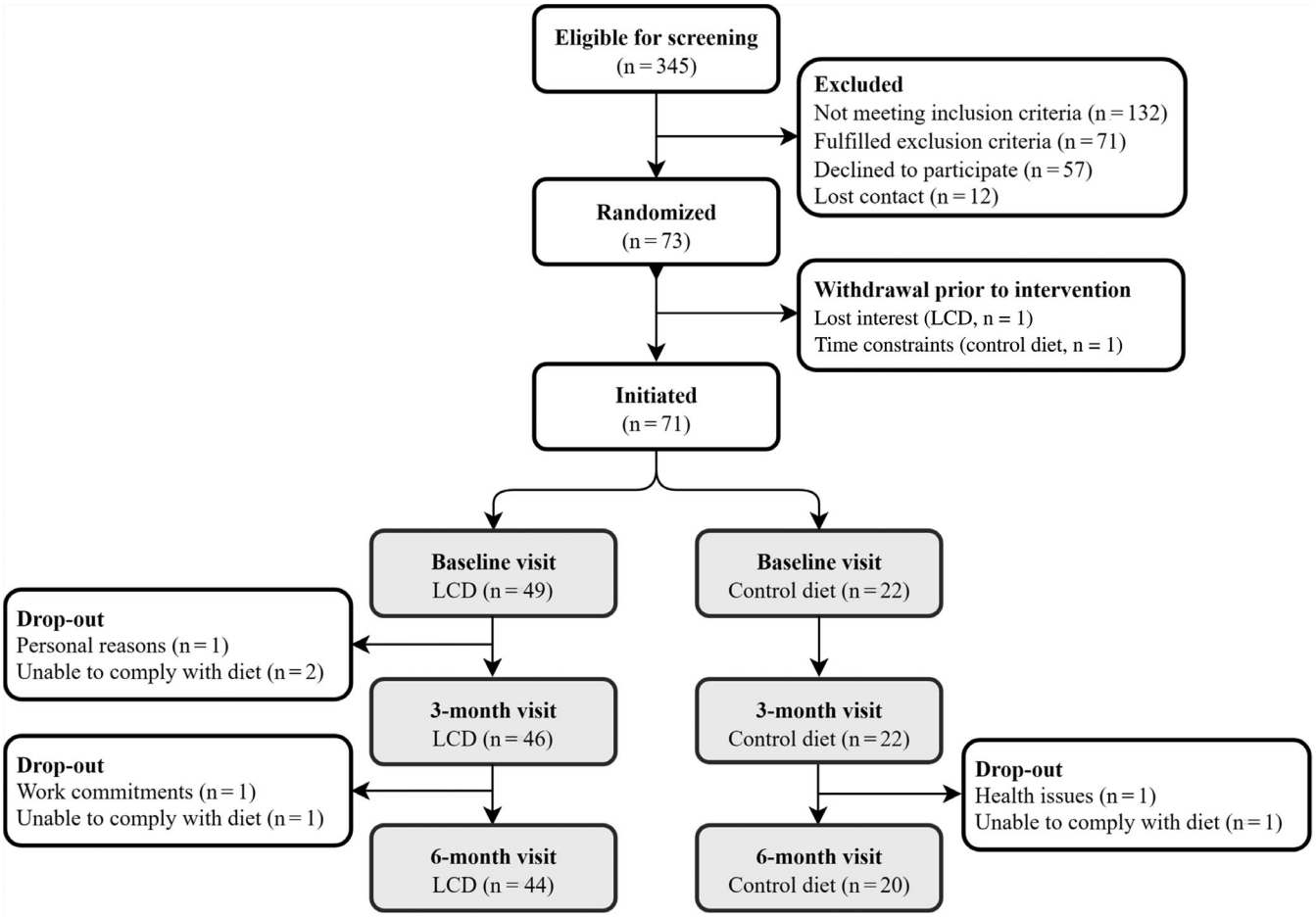

FIGURE 1 Study and participant flow diagram. LCD, low-carbohydrate diet

and after 6 months. The amounts of different activity types were estimated as described.[29] Please also see the supporting information.

2.7 | Statistics

All statistical analyses were performed using STATA version 16.0 (Stata Corporation, College Station, TX). In the first report of the primary outcome (i.e. HbA1c) from the RCT, the sample size was originally calculated using an SD of 13 mmol/mol. However, after a delayed enrolment of 45 participants allocated to LCD and 20 participants allocated to the control diet, we recognized, both from previous studies and our own observations, that the SD was lower than originally assumed. Therefore, we recalculated the sample size based on an SD of 10 mmol/mol. Using a reduction in HbA1c of 7.7 mmol/mol as the minimal clinically important difference, an SD of 10 mmol/mol, a 2:1 ratio of participants from the LCD versus the control group, and a maximal dropout rate of 10%, 36 in the LCD group and 18 in the control group, were needed to obtain a power of 80%. A few additional participants were included as they had already been invited to participate. Continuous variables at baseline were normally distributed and compared using Student's *t*-test for unpaired data. Categorical variables at baseline were compared by Fisher's exact test.

The effect of intervention on continuous variables measured repeatedly was analysed using a linear mixed-effect model approach to allow for both random and fixed effects and to allow for the presence of missing data and non-completers (intention-to-treat) considering incomplete data as missing at random. The results are reported as the mean differences in change from baseline (β-coefficient ± SE) between the LCD group and the control group after the 3- and 6-month intervention, respectively. For the mean differences in change of HbA1c between groups from baseline to 3 and 6 months, we also report estimates fully adjusted for each of the different glucose-lowering and blood pressure-lowering drugs, the use of cholesterol-lowering medication, as well as age, sex, diabetes duration, and smoking. In addition, we performed subgroup analysis leaving out either users of insulin and sulphonylureas or participants not treated with glucose-lowering drugs. Data other than the β-coefficients are presented as means ± SEM. Significance was accepted at *P* less than .05.

3 | RESULTS

3.1 | Baseline characteristics

The groups were well matched with respect to age, sex, diabetes duration, hypertension, smoking status, and medication (Table 1), as

well as glycaemic control, serum insulin, and C-peptide levels, homeostatic model assessment of insulin resistance (HOMA-IR), height, weight, body mass index (BMI), waist and hip circumferences, blood lipids, and diastolic blood pressure (Table 2). However, systolic blood pressure at baseline was higher in the control group (Table 2). The proportion of participants treated with sodium-glucose co-transporter-2 (SGLT-2) inhibitors was not significantly higher in the LCD group ($P = .15$). In both groups, 80%-90% were treated with at least one glucose-lowering drug, and 55% were taking cholesterol-lowering medication.

3.2 | Glycaemic control and related markers

The LCD caused a reduction in HbA1c after 3 months (β = −8.9 ± 1.7 mmol/mol; $P \leq .0001$), which was sustained after 6 months (β = −7.5 ± 1.7 mmol/mol; $P \leq .0001$) compared with the control diet (Table 2, Figure S1). When adjusting for medication, age, sex, diabetes duration, and smoking, the effects of the LCD on HbA1c after 3 (β = −9.1 ± 1.7 mmol/mol; $P \leq .0001$) and 6 months (β = −7.7 ± 1.8 mmol/mol; $P \leq .0001$) were not attenuated. In addition, subgroup analysis excluding users of insulin and sulphonylureas at baseline did not reduce the effect of the LCD on HbA1c after 3 (β = −9.6 ± 1.6 mmol/mol; $P \leq .0001$) or 6 months (β = −8.7 ± 1.7 mmol/mol; $P \leq .0001$). Similarly, removal of patients not taking glucose-lowering drugs did not alter the effect of the LCD on HbA1c after 3 (β = −8.3 ± 1.8 mmol/mol; $P \leq .0001$) or 6 months (β = −7.6 ± 1.8 mmol/mol; $P \leq .0001$). In line with this, the LCD was accompanied by reductions in self-reported plasma glucose measurements at almost all reported time points after 3 and 6 months compared with the control diet, except at lunchtime after 6 months (Table S1). However, the LCD showed no significant effects on fasting plasma glucose, serum insulin or C-peptide, or HOMA-IR (Table 2). In the LCD group, blood ketone levels increased after 3 months (β = 0.27 ± 0.09 mmol/L; $P = .002$) compared with the control group, but not after 6 months (Table 2).

3.3 | Cardiovascular risk factors

There were no effects of the LCD on either diastolic or systolic blood pressure, heart rate or serum levels of total cholesterol, LDL-cholesterol or triglycerides after 3 or 6 months compared with the control diet (Table 2, Figure S2). Serum-HDL increased in response to the LCD after 3 months (β = 0.1 ± 0.4 mmol/L; $P = .031$), but not after 6 months compared with the control diet ($P = .121$).

3.4 | Body composition

The LCD caused a decrease in weight after both 3 (β = −3.3 ± 1.0 kg; $P = .001$) and 6 months (β = −3.9 ± 1.0 kg; $P \leq .001$), and in BMI after both 3 (β = −1.2 ± 0.3 kg/m^2; $P = .001$) and 6 months (β = −1.4 ± 0.4 kg/m^2; $P \leq .001$), compared with the control diet (Table 2, Figure S2). Abdominal obesity measured as waist circumference also decreased in response to the LCD after both 3 (β = −3.5 ± 1.2 cm; $P = .004$) and 6 months (β = −4.9 ± 1.3 cm; $P \leq .001$) compared with the control diet.

The weight losses in the LCD group were explained by larger reductions of total fat mass (β = −2.2 ± 1.0 kg; $P = .027$), percentage body fat (β = −1.2% ± 0.6%; $P = .043$), and total lean mass (β = −1.3 ± 0.6 kg; $P = .017$) after 6 months compared with the control group, whereas percentage lean mass tended to increase in the LCD group compared with the control diet group (β = +1.2% ± 0.6%; $P = .063$)

TABLE 1 Baseline characteristics of participants

Characteristic	LCD (n = 49)	Control (n = 22)
Age, years	57.3 ± 0.9	55.2 ± 2.7
Sex		
Men	22 (44.9)	9 (40.9)
Women	27 (55.1)	13 (59.1)
Height, cm	173 ± 1	170 ± 2
Duration of diabetes, years	5.2 ± 0.5	5.0 ± 0.5
Hypertension	32 (65.3)	16 (72.7)
Smoking status		
Never smoker	24 (49.0)	11 (50.0)
Previous smoker	23 (46.9)	10 (45.5)
Current smoker	2 (4.1)	1 (4.5)
Glucose-lowering therapy		
Metformin	40 (81.6)	16 (72.7)
Sulphonylurea	4 (8.2)	0 (0.0)
DDP-4 inhibitor	6 (12.2)	3 (13.6)
GLP-1 receptor analogue	9 (18.4)	6 (27.3)
SGLT-2 inhibitor	10 (20.4)	1 (4.5)
Insulin	2 (4.1)	3 (13.6)
No. of glucose-lowering agents		
0	5 (10.2)	4 (18.2)
1	23 (46.9)	11(50.0)
2	16 (32.7)	4 (18.2)
≥3	5 (10.2)	3 (13.6)
Blood pressure-lowering therapy		
ACE inhibitor	15 (30.6)	8 (36.4)
Angiotensin receptor blocker	13 (26.5)	6 (27.3)
Calcium-channel blockers	10 (20.4)	6 (27.3)
Thiazides	16 (32.7)	4 (18.2)
Beta-blockers	6 (12.2)	2 (9.1)
Cholesterol-lowering treatment	27 (55.1)	12 (54.5)

Note: Data are means ± SEM or number (%).
Abbreviations: ACE, angiotensin-converting enzyme; DDP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; LCD, low-carbohydrate diet; SGLT-2, sodium-glucose co-transporter-2.

TABLE 2 Glycaemic control, cardiovascular risk factors, and body composition

	Baseline		3 months				6 months			
	LCD (n = 49)	Control (n = 22)	LCD (n = 46)	Control (n = 22)	Mean difference in change	*P* value	LCD (n = 44)	Control (n = 20)	Mean difference in change	*P* value
HbA1c (mmol/mol)	54.3 ± 1.4	56.1 ± 1.5	43.7 ± 1.0	54.3 ± 1.8	−8.9 ± 1.7	<.0001	43.6 ± 1.0	53.2 ± 2.1	−7.5 ± 1.8	<.0001
Fasting plasma glucose (mmol/L)	8.6 ± 0.3	9.3 ± 0.4	7.2 ± 0.2	8.6 ± 0.4	−0.6 ± 0.4	.193	7.2 ± 0.3	8.7 ± 0.5	−0.7 ± 0.4	.106
Serum insulin (pmol/L)	173 ± 38	156 ± 19	148 ± 30	165 ± 21	32 ± 22	.147	136 ± 30	158 ± 25	−35 ± 23	.127
HOMA-IR	9.7 ± 2.4	9.4 ± 1.4	7.1 ± 1.6	8.7 ± 1.1	−1.7 ± 1.6	.272	6.8 ± 1.7	8.8 ± 1.9	−2.0 ± 1.6	.228
Serum C-peptide (pmol/L)	1251 ± 88	1285 ± 77	1147 ± 93	1322 ± 96	−112 ± 89	.206	1070 ± 75	1218 ± 77	−93 ± 91	.305
Blood ketones (mmol/L)	0.25 ± 0.02	0.23 ± 0.02	0.48 ± 0.07	0.19 ± 0.27	0.27 ± 0.09	.002	0.32 ± 0.03	0.18 ± 0.02	0.13 ± 0.08	.150
Systolic blood pressure (mmHg)	134 ± 2*	142 ± 3	133 ± 2	137 ± 3	3.2 ± 3.1	.303	131 ± 2	136 ± 3	0.6 ± 3.2	.842
Diastolic blood pressure (mmHg)	85 ± 1	86 ± 2	86 ± 1	84 ± 2	2.9 ± 2.0	.163	84 ± 1	84 ± 2	1.5 ± 2.1	.464
Heart rate (beats/min)	77 ± 2	82 ± 4	71 ± 2	74 ± 3	0.0 ± 2.2	.983	71 ± 2	78 ± 3	−1.1 ± 2.3	.641
Serum LDL (mmol/L)	2.3 ± 0.1	2.4 ± 0.2	2.3 ± 0.1	2.2 ± 0.2	0.2 ± 0.1	.142	2.4 ± 0.1	2.2 ± 0.2	0.3 ± 0.2	.064
Serum HDL (mmol/L)	1.2 ± 0.04	1.1 ± 0.06	1.3 ± 0.04	1.1 ± 0.05	0.1 ± 0.4	.031	1.3 ± 0.04	1.1 ± 0.07	0.1 ± 0.0	.121
Serum triglycerides (mmol/L)	1.91 ± 0.17	2.12 ± 0.25	1.42 ± 0.11	1.93 ± 0.22	−0.28 ± 0.22	.204	1.42 ± 0.11	1.66 ± 0.15	−0.05 ± 0.23	.837
Serum total cholesterol (mmol/L)	4.3 ± 0.1	4.4 ± 0.2	4.2 ± 0.1	4.1 ± 0.2	0.2 ± 0.2	.378	4.4 ± 0.1	4.1 ± 0.2	0.3 ± 0.2	.121
BMI (kg/m^2)	32.5 ± 0.9	35.2 ± 1.4	30.8 ± 0.9	34.5 ± 1.4	−1.2 ± 0.3	.001	30.7 ± 0.9	34.9 ± 1.5	−1.4 ± 0.4	<.001
Weight (kg)	97.7 ± 3.2	102.1 ± 4.4	92.7 ± 3.3	99.8 ± 4.1	−3.3 ± 1.0	.001	92.6 ± 3.5	101.9 ± 4.4	−3.9 ± 1.0	<.001
Waist circumference (cm)	110.9 ± 2.1	114.8 ± 3.0	103.7 ± 2.2	112.0 ± 3.6	−3.5 ± 1.2	.004	103.2 ± 2.3	114.0 ± 3.3	−4.9 ± 1.3	<.001
Hip circumference (cm)	108.4 ± 1.7	113.4 ± 2.9	107.5 ± 1.9	113.4 ± 3.2	−1.4 ± 1.2	.252	104.9 ± 1.8	112.2 ± 3.2	−2.0 ± 1.2	.111

Note: The *P* values show the significance levels of the effect of intervention in the LCD group corrected for any change in the control group and baseline differences. This is given as the mean difference in change (mean ± SE). Other data are given as means ±SEM.

Abbreviations: BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; LCD, low-carbohydrate diet.

*$P \leq .05$ versus control at baseline.

TABLE 3 Body composition by DXA scan

DXA measurements	Baseline		6 months			
	LCD (n = 49)	Control (n = 22)	LCD (n = 44)	Control (n = 20)	Mean difference in change	*P* value
Total lean mass (kg)	56.9 ± 1.9	57.4 ± 2.1	54.8 ± 2.1	57.9 ± 2.2	−1.3 ± 0.6	.017
Total fat mass (kg)	38.6 ± 1.8	42.3 ± 2.9	35.7 ± 2.0	41.3 ± 2.9	−2.2 ± 1.0	.027
Total lean mass (%)	58.3 ± 1.1	56.7 ± 1.5	59.2 ± 1.2	57.4 ± 1.7	1.2 ± 0.6	.063
Total body fat (%)	38.9 ± 1.1	40.7 ± 1.6	37.9 ± 1.3	40.0 ± 1.7	−1.2 ± 0.6	.043
Abdominal lean mass (kg)	30.3 ± 0.9	30.4 ± 1.0	29.0 ± 1.1	30.9 ± 1.0	−1.1 ± 0.4	.013
Abdominal fat mass (kg)	21.2 ± 1.0	23.4 ± 1.8	19.3 ± 1.1	22.9 ± 1.8	−1.5 ± 0.6	.022
Legs lean mass (kg)	17.2 ± 0.6	17.6 ± 0.8	16.6 ± 0.7	17.6 ± 0.9	0.2 ± 0.1	.400
Legs fat mass (kg)	11.3 ± 0.7	12.3 ± 1.0	10.6 ± 0.7	11.9 ± 1.0	−0.5 ± 0.3	.081
Arms lean mass (kg)	6.3 ± 0.3	6.2 ± 0.3	6.1 ± 0.3	6.3 ± 0.4	−0.1 ± 0.1	.209
Arms fat mass (kg)	4.8 ± 0.2	5.4 ± 0.4	4.6 ± 0.3	5.28 ± 0.4	−0.2 ± 0.1	.110

Note: The *P* values show the significance levels of the effect of intervention in the LCD group corrected for any change in the control group and baseline differences. This is given as the mean difference in change (mean ± SE). Other data are given as means ±SEM.
Abbreviations: DXA, dual-energy x-ray absorptiometry; LCD, low-carbohydrate diet.

(Table 3). The LCD-induced changes in total fat and lean mass were mainly explained by reductions in abdominal fat mass (β = −1.5 ± 0.6 kg; *P* = .022) and abdominal lean mass (β = −1.1 ± 0.4 kg; *P* = .013) (Table 3).

3.5 | Dietary data and physical activity

The LCD group reduced their self-reported carbohydrate intake from 42 E% at baseline to 13 E% after 3 and 6 months. Thus, the LCD group markedly reduced their carbohydrate intake after both 3 (β = −31.9 ± 2.1 E%; *P* ≤ .001) and 6 months (β = −30.5 ± 2.2 E%; *P* ≤ .001) compared with the control diet (Table 4). Correspondingly, the LCD group increased their intake of fat after 3 (β = 31.0 ± 2.2 E%; *P* ≤ .001) and 6 months (β = 30.6 ± 2.2 E%; *P* ≤ .001) compared with the control diet. The increased intake of fat in the LCD group was explained by an increased intake of saturated fat, monounsaturated fat, and polyunsaturated fat compared with the placebo group (all *P* < .001). Protein and daily calorie intake did not change in response to the LCD. The LCD was associated with a decreased intake of dietary fibre after both 3 (β = −13.2 ± 1.8 E%; *P* ≤ .001) and 6 months (β = −11.9 ± 1.9 E%; *P* ≤ .001) compared with the control diet (Table 4). In the LCD group, one of 45 participants from baseline to 3 months, and two of 39 participants from 3 to 6 months, reported a slightly higher intake of carbohydrates (all <25 E%) than instructed, giving adherence rates of 98% and 95%, respectively. In the control group, 13 of 21 participants from baseline to 3 months, and 11 of 19 participants from 3 to 6 months, reported a lower intake of carbohydrates than instructed, giving adherence rates of 38% and 42%, respectively. The lowest reported intake was 40 E% in the control group.

The LCD was not associated with any changes in accelerometer-based measurements of physical activity intensity such as sedentary, light or moderate/vigorous activity, or types of activity like sitting, walking or steps compared with the control diet (Table S2).

3.6 | Hypoglycaemic episodes and adverse events

Documented symptomatic hypoglycaemia was reported in one participant from each group, but there were no cases of severe hypoglycaemia in the groups, and only one case of clinically important biochemical hypoglycaemia in the LCD group during the 6-month study period (Table S3).

Other self-reported potential adverse events were rare and did not differ significantly between the groups, except for an increased frequency of gastrointestinal complaints (*P* = .03) such as constipation (n = 5), diarrhoea (n = 2), and abdominal discomfort (n = 3) in the LCD group (Table S3).

4 | DISCUSSION

In this open-label RCT, we examined the effect of a 6-month non-calorie–restricted LCD (10-25 E%) on glycaemic control, body composition, and cardiovascular risk factors in patients with T2D instructed to maintain their level of physical activity and non-insulin glucose-lowering medication. Our main findings are that a non-calorie–restricted LCD compared with a control diet caused a significant reduction in HbA1c already after 3 months, and which was maintained after 6 months. Moreover, the LCD markedly reduced body weight, BMI, and abdominal adiposity, whereas no changes in blood lipids or blood pressure were seen, except for a transient increase in HDL. We observed no cases of severe hypoglycaemia or differences in non-severe hypoglycaemia or adverse events between groups, except for more gastrointestinal complaints in the LCD group.

TABLE 4 Dietary data

Variable	Baseline		0 to 3 months				3 to 6 months			
	LCD (n = 36)	Control (n = 19)	LCD (n = 45)	Control (n = 21)	Mean difference in change	*P* value	LCD (n = 39)	Control (n = 19)	Mean difference in change	*P* value
Kcal per day	1805 ± 77	1840 ± 97	1701 ± 52	1664 ± 104	18 ± 99	.852	1642 ± 62	1600 ± 119	−3 ± 103	.976
Carbohydrates (E%)	42.1 ± 1.2*	45.9 ± 1.7	12.5 ± 1.0	48.5 ± 0.7	−31.9 ± 2.1	<.001	13.4 ± 1.2	48.4 ± 1.0	−30.5 ± 2.2	<.001
Protein (E%)	19.4 ± 0.8	18.9 ± 0.8	22.9 ± 0.6	21.4 ± 0.8	1.1 ± 1.3	.391	23.4 ± 0.7	22.5 ± 1.0	0.4 ± 1.3	.779
Fat (E%)	38.2 ± 1.2*	34.5 ± 1.9	64.5 ± 1.0	29.5 ± 1.0	31.0 ± 2.2	<.001	63.2 ± 1.2	28.3 ± 1.0	30.6 ± 2.2	<.001
Saturated fat (g/day)	24.8 ± 1.7	21.7 ± 3.0	44.2 ± 1.8	16.1 ± 1.5	24.0 ± 3.2	<.001	40.7 ± 2.2	15.6 ± 1.3	21.5 ± 3.2	<.001
Monounsaturated fat (g/day)	20.8 ± 1.6	18.5 ± 2.7	36.1 ± 1.7	13.6 ± 1.3	19.9 ± 3.1	<.001	33.0 ± 1.8	12.5 ± 1.1	17.7 ± 3.1	<.001
Polyunsaturated fat (g/day)	8.6 ± 0.7	8.2 ± 0.8	16.5 ± 0.9	7.6 ± 0.6	8.2 ± 1.5	<.001	15.8 ± 1.2	7.0 ± 0.7	8.1 ± 1.5	<.001
Dietary fibre (g/day)	21.8 ± 1.1	24.3 ± 1.4	16.0 ± 0.9	31.2 ± 2.2	−13.2 ± 1.8	<.001	15.9 ± 1.1	30.1 ± 2.1	−11.9 ± 1.9	<.001
Sugars (g/day)	46.2 ± 3.1	54.6 ± 5.9	22.4 ± 1.2	55.4 ± 4.1	−26.0 ± 4.6	<.001	22.2 ± 1.5	52.1 ± 4.9	−22.1 ± 4.8	<.001

Note: The *P* values show the significance levels of the effect of intervention in the LCD group corrected for any change in the control group and baseline differences. This is given as the mean difference in change (mean ± SE). Other data are given as means ±SEM.

Abbreviations: E%, percentage of total energy intake; LCD, low-carbohydrate diet.

*$P \leq .05$ versus control at baseline.

Our results provide evidence that a non-calorie–restricted LCD, which is feasible under free-living conditions, has clinically relevant effects on glycaemic control and body composition and is safe with respect to cardiovascular risk factors, hypoglycaemia, and adverse events in patients with T2D. Further long-term studies are, however, needed to show that these effects are sustained beyond 6 months.

This randomized study shows a highly clinically relevant effect of a LCD on HbA1c in patients with T2D with a between-group decrease of 8.9 mmol/mol after 3 months, which was largely sustained after 6 months. Our finding is in line with a recent meta-analysis,[11] which showed that the greater the carbohydrate restriction, then the greater the reduction in HbA1c in RCTs. In fact, the magnitude of the reduction in HbA1c in our study is comparable with two other studies restricting carbohydrate intake in patients with T2D to the same (i.e. 14 E%) low level,[16,22] and which in fact showed the largest between-group decreases in HbA1c in this meta-analysis.[11] However, in the study conducted by Saslow et al.,[22] both the LCD and the control groups reduced their energy intake by 700-800 kcal/day, and in the study conducted by Tay et al.,[16] the LCD was combined with both calorie restriction (500-1000 kcal/day) and an exercise programme, which could have enhanced the decrease in HbA1c, as shown for weight loss in a recent meta-analysis.[25] On the other hand, reduction in antidiabetic medicine in these studies could have attenuated the glucose-lowering effect of the LCD. In another study, a 6-month reduced-calorie LCD (20 E% carbohydrates) without instructions for changes in physical activity reduced HbA1c by only 4.8 mmol/mol in the LCD group, and there was no between-group difference compared with a low-fat diet.[20] In that study, the use of insulin was reduced among the one-third of patients with T2D treated with insulin, whereas no significant reduction in oral glucose-lowering medication was observed.[20] Thus, in addition to the degree of carbohydrate restriction, factors such as calorie restriction, changes in physical activity and antidiabetic medication, as well as the proportion of insulin users, may explain the differences between studies. In our study, the marked effect of LCD on HbA1c compared with the control diet was obtained despite maintaining participants' daily intake of energy and their levels of physical activity. Moreover, in contrast to the aforementioned studies,[11,20,22] our study participants were instructed to maintain their non-insulin glucose-lowering medication, although it was reduced in 20% of participants because of decreasing HbA1c rather than hypoglycaemia.

Dietary fibre may exert beneficial effects on glycaemic control, dyslipidaemia, and all-cause mortality in patients with T2D.[7,30] By contrast, a higher intake of saturated fat may increase the risk of CVD and mortality,[15] although this association has been difficult to prove in meta-analyses.[31,32] Therefore, a diet low in SFA and high in MUFA was recommended as this may reduced systolic and diastolic blood pressure.[33] However, the intake of SFA in the LCD group increased to 2.7-fold higher levels than in the control group. Despite this increase in dietary SFA, corresponding to 29% of daily energy intake, and a lower intake of dietary fibre, the classical cardiovascular risk factors measured in this study did not worsen in response to a 6-month LCD. We could not confirm the small improvements in triglycerides and HDL previously reported after carbohydrate restriction in some studies,[10,14,16,18] whereas the lack of changes in LDL cholesterol and blood pressure is consistent with findings in other studies and recent meta-analyses.[13,14,18,20,22,23] In some of these studies, blood lipids could have been influenced by calorie restriction and exercise,[14,34] which was not the case in our study. Although we observed a small transient increase in HDL after 3 months in the LCD group, this increase is probably of little clinical importance. Overall, the LCD approach used in this study appeared safe with regard to traditional cardiovascular risk factors, although long-term studies are still necessary, as well as an evaluation of other risk factors, such as the circulating fatty acid composition and markers of inflammation.

Recent RCTs and meta-analyses of carbohydrate restriction in patients with T2D conclude that carbohydrate-restricted diets have the same effect on body weight, BMI, and waist circumference as low-fat control diets.[12,14,16,20,22] However, many of these studies included hypocaloric diets and increased physical activity in both groups,[16,20,22] whereas in our study, the greater reductions in weight, BMI, and waist circumference in response to the LCD compared with the control diet were obtained despite no reduction in calorie intake or increase in the level of physical activity. Our data lend support to well-controlled studies showing that isocaloric substitution of fat for carbohydrates results in slightly higher energy expenditure.[10,35,36] The mechanism could include changes in hormones such as catecholamines and thyroid hormones, although changes in hormones regulating appetite and satiety could also play a role.[10] Studies of changes in fat mass and lean mass in response to a LCD in patients with T2D are rare. In a study conducted by Tay et al.,[16] the authors found similar reductions in total fat mass and lean mass in participants undergoing either a LCD or low-fat diet combined with caloric restriction and an exercise programme for 24 weeks. In our study, we observed not only a decrease in total fat mass in response to a non-calorie–restricted LCD, but also significant reductions in waist circumference and abdominal fat mass compared with the control diet, and hence an improvement in the body fat distribution, which is known to confer protection against CVD and mortality.[37] However, total lean mass also decreased in response to the LCD. This was expected because a LCD reduces glycogen levels in muscle (~400 g) and liver (~100 g), leading to a loss of body water (3 g of water per gram of glycogen), which could explain a lean mass loss of 1-2 kg.[10,38] Of importance, the between-group decreases in weight and BMI observed after 3 months on a LCD were even larger after 6 months, indicating a high degree of compliance to the dietary instructions in our study.

In addition to being safe with respect to cardiovascular risk factors, the non-calorie–restricted LCD was also safe with respect to hypoglycaemia and adverse effects, except for an increased frequency of gastrointestinal complaints. The lack of differences in hypoglycaemic events was observed despite participants randomized to a LCD being instructed to maintain their non-insulin glucose-lowering medication. By randomization, most participants treated with SGLT2 inhibitors followed the LCD diet, but this apparently did not affect the risk of hypoglycaemia or other outcomes, most probably because the LCD was non-calorie–restricted. Following the initiation of our study,

there were reported cases of euglycaemic ketoacidosis in younger patients with T2D experiencing a major weight loss in response to concurrent treatment with a SGLT2 inhibitor and a hypocaloric VLCKD.[39,40] Although it remains to be established if this rare adverse effect of SGLT2 inhibitors is also possible in response to a non-calorie–restricted LCD, discontinuation of SGLT2 inhibitors should be considered in T2D patients with known contributing factors such as severe acute illness, surgery, dehydration, excessive alcohol intake, extreme physical activity, low caloric and fluid intake, or reduction or discontinuation of insulin.[41]

The strengths of our study include the randomized design, the well-matched study groups, and the ability to examine the isolated effect of a non-calorie–restricted LCD in patients with T2D and with no intention to change their level of physical activity or non-insulin antidiabetic medication. Adjustment for any differences in medication, sex, age, diabetes duration, and smoking, or subgroup analysis excluding users of sulphonylureas and insulin or non-users of glucose-lowering medication, did not affect the effect of LCD on the primary outcome (i.e. HbA1c). The limitations include the open-label approach, the self-reported glucose measurements and symptoms of hypoglycaemia, the lack of continuous assessment of physical activity, and the lack of strict control with regard to changes in non-insulin antidiabetic medication, energy intake and diet macronutrient composition, the latter possibly explaining the higher intake of saturated fat and reduced intake of dietary fibre. Moreover, the sample size was calculated to detect a change in HbA1c, but this may not be sufficient to identify an effect of LCD on other outcomes. In particular, the use of HOMA-IR as an estimate of insulin sensitivity may only be suitable for larger population studies.

In summary, our study shows that a 6-month, non-calorie–restricted LCD in patients with T2D instructed to maintain their level of physical activity and non-insulin glucose-lowering medication significantly reduced HbA1c, body weight, BMI, and abdominal adiposity compared with a control diet, whereas no changes in other cardiovascular risk factors were seen. We observed no increased risk of hypoglycaemia or adverse events in the LCD group, except for more gastrointestinal complaints. Our results provide evidence that a non-calorie–restricted LCD, which is feasible under free-living conditions, has clinically relevant effects on glycaemic control and body composition, and appears to be safe with regard to cardiovascular risk factors, hypoglycaemia, and other adverse events in patients with T2D.

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CONFLICT OF INTEREST

The authors declare that there is no duality of interest associated with this manuscript.

AUTHOR CONTRIBUTIONS

EMG-K, CDH, AK, HB-N, MHO, and KH contributed to the conception and design of the study. EMG-K, CDH, and MBH identified the eligible participants, and EMG-K, CDH, MBH, and JMJ conducted the intervention study. JCB and APH contributed to accelerometer and DXA data. EMG-K, HB-N, MHO, JCB, and KH analysed and interpreted data, and EMG-K and KH wrote the manuscript. All the authors have revised the manuscript critically for important intellectual content and given final approval of the version to be published. KH and AK are guarantors of this work, and as such had full access to all the data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/dom.14633.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Cho NH, Shaw JE, Karuranga S, et al. IDF Diabetes Atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res Clin Pract*. 2018;138:271-281.
2. Stratton IM, Adler AI, Neil HA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ*. 2000;321(7258):405-412.
3. Garber AJ, Abrahamson MJ, Barzilay JI, et al. Consensus statement by the American Association of Clinical Endocrinologists and American College of Endocrinology on the comprehensive type 2 diabetes management algorithm - 2019 executive summary. *Endocr Pract*. 2019;25(1):69-100.
4. The Danish Ministry of Food Agriculture and Fisheries The official dietary guidelines: Food-based dietary guidelines. https://altomkost.dk/raad-og-anbefalinger/de-officielle-kostraad/. Accessed December 12, 2021.
5. Sheard NF, Clark NG, Brand-Miller JC, et al. Dietary carbohydrate (amount and type) in the prevention and management of diabetes: a statement by the american diabetes association. *Diabetes Care*. 2004;27(9):2266-2271.
6. American Diabetes Association. 5. Lifestyle management: standards of medical care in diabetes-2019. *Diabetes Care*. 2019;42(suppl 1):S46-S60.
7. Evert AB, Boucher JL, Cypress M, et al. Nutrition therapy recommendations for the management of adults with diabetes. *Diabetes Care*. 2014;37(suppl 1):S120-S143.
8. Feinman RD, Pogozelski WK, Astrup A, et al. Dietary carbohydrate restriction as the first approach in diabetes management: critical review and evidence base. *Nutrition*. 2015;31(1):1-13.
9. Turton J, Brinkworth GD, Field R, Parker H, Rooney K. An evidence-based approach to developing low-carbohydrate diets for type

2 diabetes management: a systematic review of interventions and methods. *Diabetes Obes Metab*. 2019;21(11):2513-2525.
10. Kirkpatrick CF, Bolick JP, Kris-Etherton PM, et al. Review of current evidence and clinical recommendations on the effects of low-carbohydrate and very-low-carbohydrate (including ketogenic) diets for the management of body weight and other cardiometabolic risk factors: a scientific statement from the National Lipid Association Nutrition and Lifestyle Task Force. *J Clin Lipidol*. 2019;13(5):689-711.
11. Snorgaard O, Poulsen GM, Andersen HK, Astrup A. Systematic review and meta-analysis of dietary carbohydrate restriction in patients with type 2 diabetes. *BMJ Open Diabetes Res Care*. 2017;5(1):e000354.
12. Sainsbury E, Kizirian NV, Partridge SR, Gill T, Colagiuri S, Gibson AA. Effect of dietary carbohydrate restriction on glycemic control in adults with diabetes: a systematic review and meta-analysis. *Diabetes Res Clin Pract*. 2018;139:239-252.
13. Meng Y, Bai H, Wang S, Li Z, Wang Q, Chen L. Efficacy of low carbohydrate diet for type 2 diabetes mellitus management: a systematic review and meta-analysis of randomized controlled trials. *Diabetes Res Clin Pract*. 2017;131:124-131.
14. van Zuuren EJ, Fedorowicz Z, Kuijpers T, Pijl H. Effects of low-carbohydrate compared with low-fat-diet interventions on metabolic control in people with type 2 diabetes: a systematic review including GRADE assessments. *Am J Clin Nutr*. 2018;108(2):300-331.
15. Guasch-Ferre M, Babio N, Martinez-Gonzalez MA, et al. Dietary fat intake and risk of cardiovascular disease and all-cause mortality in a population at high risk of cardiovascular disease. *Am J Clin Nutr*. 2015;102(6):1563-1573.
16. Tay J, Luscombe-Marsh ND, Thompson CH, et al. A very low-carbohydrate, low-saturated fat diet for type 2 diabetes management: a randomized trial. *Diabetes Care*. 2014;37(11):2909-2918.
17. Westman EC, Tondt J, Maguire E, Yancy WS Jr. Implementing a low-carbohydrate, ketogenic diet to manage type 2 diabetes mellitus. *Expert Rev Endocrinol Metab*. 2018;13(5):263-272.
18. Davis NJ, Tomuta N, Schechter C, et al. Comparative study of the effects of a 1-year dietary intervention of a low-carbohydrate diet versus a low-fat diet on weight and glycemic control in type 2 diabetes. *Diabetes Care*. 2009;32(7):1147-1152.
19. Iqbal N, Vetter ML, Moore RH, et al. Effects of a low-intensity intervention that prescribed a low-carbohydrate vs. a low-fat diet in obese, diabetic participants. *Obesity*. 2010;18(9):1733-1738.
20. Guldbrand H, Dizdar B, Bunjaku B, et al. In type 2 diabetes, randomisation to advice to follow a low-carbohydrate diet transiently improves glycaemic control compared with advice to follow a low-fat diet producing a similar weight loss. *Diabetologia*. 2012;55(8):2118-2127.
21. Sato J, Kanazawa A, Hatae C, et al. One year follow-up after a randomized controlled trial of a 130 g/day low-carbohydrate diet in patients with type 2 diabetes mellitus and poor glycemic control. *PLoS One*. 2017;12(12):e0188892.
22. Saslow LR, Kim S, Daubenmier JJ, et al. A randomized pilot trial of a moderate carbohydrate diet compared to a very low carbohydrate diet in overweight or obese individuals with type 2 diabetes mellitus or prediabetes. *PLoS One*. 2014;9(4):e91027.
23. Yamada Y, Uchida J, Izumi H, et al. A non-calorie-restricted low-carbohydrate diet is effective as an alternative therapy for patients with type 2 diabetes. *Intern Med*. 2014;53(1):13-19.
24. Watson N, Dyer K, Buckley J, et al. Effects of low-fat diets differing in protein and carbohydrate content on cardiometabolic risk factors during weight loss and weight maintenance in obese adults with type 2 diabetes. *Nutrients*. 2016;8(5):289.
25. Gummesson A, Nyman E, Knutsson M, Karpefors M. Effect of weight reduction on glycated haemoglobin in weight loss trials in patients with type 2 diabetes. *Diabetes Obes Metab*. 2017;19(9):1295-1305.
26. American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2019. *Diabetes Care*. 2019;42(suppl 1):S13-S28.
27. Gerrior S, Juan W, Basiotis P. An easy approach to calculating estimated energy requirements. *Prev Chronic Dis*. 2006;3(4):A129.
28. International Hypoglycaemia Study Group. Glucose concentrations of less than 3.0 mmol/L (54 mg/dL) should be reported in clinical trials: a joint position statement of the American Diabetes Association and the European Association for the study of diabetes. *Diabetes Care*. 2017;40(1):155-157.
29. Skotte J, Korshoj M, Kristiansen J, Hanisch C, Holtermann A. Detection of physical activity types using triaxial accelerometers. *J Phys Act Health*. 2014;11(1):76-84.
30. Chandalia M, Garg A, Lutjohann D, von Bergmann K, Grundy SM, Brinkley LJ. Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. *N Engl J Med*. 2000;342(19):1392-1398.
31. Jakobsen MU, O'Reilly EJ, Heitmann BL, et al. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. *Am J Clin Nutr*. 2009;89(5):1425-1432.
32. Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. *Am J Clin Nutr*. 2010;91(3):535-546.
33. Abbasnezhad A, Falahi E, Gonzalez MJ, Kavehi P, Fouladvand F, Choghakhori R. Effect of different dietary approaches in comparison with high/low-carbohydrate diets on systolic and diastolic blood pressure in type 2 diabetic patients: a systematic review and meta-analysis. *Prev Nutr Food Sci*. 2020;25(3):233-245.
34. McArdle PD, Greenfield SM, Rilstone SK, Narendran P, Haque MS, Gill PS. Carbohydrate restriction for glycaemic control in type 2 diabetes: a systematic review and meta-analysis. *Diabet Med*. 2019;36(3):335-348.
35. Hall KD, Chen KY, Guo J, et al. Energy expenditure and body composition changes after an isocaloric ketogenic diet in overweight and obese men. *Am J Clin Nutr*. 2016;104(2):324-333.
36. Ebbeling CB, Feldman HA, Klein GL, et al. Effects of a low carbohydrate diet on energy expenditure during weight loss maintenance: randomized trial. *BMJ*. 2018;363:k4583.
37. Cuthbertson DJ, Steele T, Wilding JP, et al. What have human experimental overfeeding studies taught us about adipose tissue expansion and susceptibility to obesity and metabolic complications? *Int J Obes*. 2017;41(6):853-865.
38. Adam-Perrot A, Clifton P, Brouns F. Low-carbohydrate diets: nutritional and physiological aspects. *Obes Rev*. 2006;7(1):49-58.
39. Tougaard NH, Faber J, Eldrup E. Very low carbohydrate diet and SGLT-2-inhibitor: double jeopardy in relation to ketoacidosis. *BMJ Case Rep*. 2019;12(4):e227516.
40. Earle M, Ault B, Bonney C. Euglycemic diabetic ketoacidosis in concurrent very low-carbohydrate diet and sodium-glucose transporter-2 inhibitor use: a case report. *Clin Pract Cases Emerg Med*. 2020;4(2):185-188.
41. Fleming N, Hamblin PS, Story D, Ekinci EI. Evolving evidence of diabetic ketoacidosis in patients taking sodium-glucose cotransporter 2 inhibitors. *J Clin Endocrinol Metab*. 2020;105(8):dgaa200.

SUPPORTING INFORMATION

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

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Supporting Information

METHODS

Diet intervention

This was a free-living study and no food was provided to the participants. At the baseline visit, a licensed dietitian thoroughly (1 hour) instructed participants in both groups individually to follow the principles of their allocated diet until it was fully understood. This included documents with information about the dietary guidelines and pie charts illustrating the recommended intake of macronutrients for each group. Participants assigned to the LCD were instructed to compose their diet with a maximum of 20 E% of carbohydrates, mainly complex and water soluble, 50-60 E% fat and 25-30 E% protein. A high intake of monounsaturated fatty acids (MUFA) and an intake of SFA as low as possible was encouraged. Participants assigned to the control diet were instructed to follow the official Danish dietary guidelines, consisting of 50-60 E% carbohydrates mainly from fruit, vegetables and whole-grain sources, 20-30 E% fat, where < 10 E% should be SFA, and protein 20-25 E%.[1] Written material and a five-day start-up diet plan based on the calculated baseline caloric intake was provided. The participants energy requirement was calculated using the Harris Benedict equation for basal metabolic rate taking sex, height, weight and physical activity level into account.[2,3] This formula have an accuracy of 63% in overweight individuals[2,4] and is still widely used in clinical practice. Thus, for women we used the equation [655 + 9.563 x weight (kg) + 1.850 x height (cm) – 4.676 x age (years)] and for men the equation [66.5 + 13.75 x weight (kg) + 5.003 x height (cm) – 6.755 x age (years)]. These calculations of the basal metabolic rate were adjusted to the self-reported physical activity level of the participants (using a factor 1.2 for sedentary activity, 1.4 for light activity, 1.5 for moderate activity, 1.7 for high activity, 1.9 for extremely high activity). These estimates were used as a guideline for non-calorie-restricted intake during the entire intervention period. Processed carbohydrates and sugar were discouraged in both groups. Participants were instructed to eat until comfortable satiety and not to restrict calories, in addition to try to maintain weight-stability.

After 1 week, all participants were contacted by telephone by the dietitian address dietetic challenges and assess compliance and after two weeks by the trial investigators to follow up on the diet. Hereafter, the dietitian contacted participants in both groups once a month and on-demand throughout the study period to ensure adherence to 1) the macronutrient composition of their diet, 2) choice of food sources, and 3) calorie intake, as well as to discuss 4) any loss or gain of weight, 5) current motivation, 6) everyday challenges with adherence to the diet. Regardless of group allocation, both the participants and the dietitian reviewed the need for extra counselling on-demand.

In addition to the these individual telephone contacts, every week, the dietitian mailed out newsletters with suggestions on season specific recipes, food-items on offer, tips and tricks and practical news from the trial. Moreover, the dietitian arranged group-specific discussion meetings of 1.5 hours every other month.

The participants were introduced and advised to keep track of energy intake and diet composition through a Danish internet-based food diary (MadLog Aps, Kolding, Denmark), and as a minimum report their food intake three days a month. Participants had access to a group-specific recipe-collection in MadLog, which was continuously updated. A pie chart illustrating the distribution of the recommended macronutrient intake (E%) was visible in Madlog each time they entered their own food diary. Incomplete days were discarded in data collection. The dietitian had access to the nutritional logs. Data was collected at the end of study.

Glucose lowering medication and safety monitoring

All participants were instructed to continue their non-insulin glucose-lowering medication, unless they experienced hypoglycemia and were adviced by the study staff to reduce or discontinue medication for glycemic safety. Thus, glucose-lowering drugs with a low risk of hypoglycemia (metformin, dipeptidyl peptidase-4 (DPP4) inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists and sodium-glucose cotransporter-2 (SGLT-2) inhibitors) remained unadjusted in both groups in the majority of the participants. In insulin-treated participants (n=2) allocated to LCD, the daily dose was reduced by 20% at baseline, and they were instructed to report blood glucose levels at least once weekly until establishment of glycemic control without hypoglycemia. Participants in the LCD group treated with sulfonylureas (n=4) were instructed to

report blood glucose levels in the same manner and to reduce the dose if they experienced hypoglycemia. Participants with normal or subnormal BMI treated with a SGLT-2 inhibitor received a blood ketone measuring device (see below) and instructions on use and actions. Nevertheless, in the LCD group, the dose of metformin (n=3) was reduced either by the patients themselves or by their general practitioners, and SGLT-2 inhibitors (n=2) or sulfonylureas (n=3) were discontinued by the general practitioners due to decreasing HbA1c despite absence of hypoglycemia. GLP-1 treatment was reduced by study personnel in one patient (n=1) due to gastrointestinal complaints. In the placebo group, the dose of a DDP4 inhibitor (n=1), insulin (n=2) or a GLP-1 receptor agonist (n=1) was increased by the general practitioners due to increasing HbA1c, the latter with a concomitant reduction in insulin dose.

All participants were instructed to measure plasma glucose levels regularly on their own glucose measuring device before meals and nighttime and report measurements for at least 3 separate days at each visit (Supplemental Table S1). They were also instructed to measure plasma glucose levels if they experienced symptoms of hypoglycemia, and report any hypoglycemic episodes as soon as possible. Symptoms of hypoglycemia and a plasma glucose ≤ 3.9 mmol/l was defined as documented symptomatic hypoglycemia, a plasma glucose < 3.0 mmol/l as clinically important biochemical hypoglycemia and severe hypoglycemia as needing the help of another person for recovery.[5] A glucose-measuring device (Bayer Contour® Next One (Ascensia Diabetes Care Holdings, Switzerlands) was provided if needed with instructions on use.

For safety monitoring, the participants were instructed to report any consultations at their general practitioners, hospital visits and potential adverse events related to the diet.

Anthropometrics, biochemical analysis and DXA

At all three visits, anthropometric data was collected in a standardized manner; height was measured without shoes on a wall-mounted stadiometer (SECA model 216, Munich Germany) and weight in light in-door clothing. Waist circumference was measured mid-abdominal (between the iliac crest and the lowest rib) after expiration with soft measuring tape and hip circumference was measured perpendicular to the floor over the femoral tuberculi. Blood pressure was measured in a sitting position every three minutes over 30 minutes with MobiloGraph ® (IEM GmbH).

Overnight fasting blood samples were drawn for analyses of plasma glucose, serum insulin, HbA1c, blood beta-hydroxybutyrate, total cholesterol, HDL and LDL cholesterol and triglycerides. HOMA-IR was calculated as fasting serum insulin (mU//l) x plasma glucose (mmol/l)/22.5. Plasma glucose was analyzed on Radiometer ABL800 FLEX Blood Gas Analyzer (Radiometer Medical ApS, Bronshoj, Denmark). Blood beta-hydroxybutyrate levels (mmol/l) were measured using an Abbott FreeStyle Precision Neo Blood Glucose and Ketone Monitoring System (Abbott Laboratories A/S, Abbott Diabetes Care). Plasma total cholesterol, HDL-cholesterol and -triglycerides were analyzed on heparinized plasma with absorbtion photometry on Cobas 8000 (Roche Diagnostics International Ltd., California). Plasma LDL cholesterol was calculated with Friedwalds formula. Serum insulin and C-peptide levels were analyzed on Cobas e411 (Roche Diagnostics International Ltd., California).

At baseline and after 6 months (last visit), total and regional fat mass and lean mass were assessed with Dual-Energy X-ray Absorptiometry-scan (DXA) (Lunar Prodigy; General Electric Corporation, Madison, Wisconsin).

Physical activity

Participants were instructed to maintain their usual physical activity level throughout the study period. Two 3-axis logging accelerometers (AX3, Axivity Ltd, Newcastle upon Tyne, UK) were attached to the skin on right thigh and lower back for 24-hour recordings on seven consecutive days at baseline and after six months. The participants were requested to uphold their usual physical activity when carrying accelerometers.

All analysis of accelerometer data were restricted to the period starting at 6:00 AM and exactly for 16, 17 or 18 hours. Wear time was scored considering both activity- and temperature monitoring. The algorithm used to identify activity types from acceleration were as described by Skotte et al.[6] Activity types included everyday activities such as walking, running, cycling, sitting, walking stairs and standing. Activity intensity was divided into four categories: 1) sedentary (lying, standing, sitting), 2) light, 3) moderate (time spent in the moderate intensity domain (>light and <vigorous) and 4) vigorous. Due to low volumes among participants, moderate and vigorous activity were combined in the analysis.

References:

1. The Danish Ministry of Food, Agriculture and Fisheries. The official dietary guidelines Food-based dietary guidelines https://altomkost.dk/raad-og-anbefalinger/de-officielle-kostraad/.
2. Gerrior S, Juan W, Basiotis P. An easy approach to calculating estimated energy requirements. *Prev Chronic Dis.* 2006;3(4):A129.
3. Harris JA, Benedict FG. A Biometric Study of Human Basal Metabolism. *Proc Natl Acad Sci U S A.* 1918;4(12):370-373.
4. Madden AM, Mulrooney HM, Shah S. Estimation of energy expenditure using prediction equations in overweight and obese adults: a systematic review. *J Hum Nutr Diet.* 2016;29(4):458-476.
5. International Hypoglycaemia Study Group. Glucose Concentrations of Less Than 3.0 mmol/L (54 mg/dL) Should Be Reported in Clinical Trials: A Joint Position Statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care.* 2017;40(1):155-157.
6. Skotte J, Korshoj M, Kristiansen J, Hanisch C, Holtermann A. Detection of physical activity types using triaxial accelerometers. *J Phys Act Health.* 2014;11(1):76-84.

Supplemental Table S1. Self-reported plasma glucose measurements

	Baseline		**3 months**				**6 months**			
Plasma glucose (mmol/l)	**LCD (n = 40-44)**	**Control (n = 18-19)**	**LCD (n = 38-44)**	**Control (n = 18-21)**	**Mean-difference in change**	***p*-value**	**LCD (n = 38-41)**	**Control (n = 18-19)**	**Mean-difference in change**	***p*-value**
Breakfast	8.4 ± 0.3	8.6 ± 0.4	6.8 ± 0.2	8.7 ± 0.4	-1.6 ± 0.3	< 0.001	6.8 ± 0.2	8.6 ± 0.5	-1.5 ± 0.3	< 0.001
Lunch	7.5 ± 0.3	8.4 ± 0.6	6.3 ± 0.2	8.4 ± 0.5	-1.1 ± 0.4	0.010	6.3 ± 0.2	8.0 ± 0.5	-0.7 ± 0.4	0.144
Dinner	8.0 ± 0.3	8.6 ± 0.5	6.3 ± 0.2	8.2 ± 0.4	-1.4 ± 0.5	0.006	6.1 ± 0.2	8.5 ± 0.6	-1.9 ± 0.5	< 0.001
Night	8.4 ± 0.3	8.8 ± 0.5	6.6 ± 0.2	9.0 ± 0.4	-2.0 ± 0.4	< 0.001	6.5 ± 0.2	9.3 ± 0.4	-2.4 ± 0.4	< 0.001

Self-reported plasma glucose measurements before breakfast, lunch and dinner and before night. The *p*-values show the significance levels of the effect of intervention in the LCD group corrected for any change in the control group and baseline differences. This is given as the mean-difference in change (means ± SE). Other data are given as means ± SEM

Supplemental Table S2: Physical activity by accelerometer

	Baseline		**6 months**			
Movement measurements	**LCD (n = 48)**	**Control (n= 22)**	**LCD (n = 42)**	**Control (n = 18)**	**Mean-difference in change**	***p*-value**
Activity intensity						
Sedentary activity (hrs/day)	12.5 ± 0.2	13.3 ± 0.3	12.6 ± 0.2	13.0 ± 0.3	0.3 ± 0.3	0.277
Sedentary time (%)	80.3 ± 0.8	82.7 ± 1.2	80.1 ± 0.8	81.4 ± 1.4	0.6 ± 1.4	0.696
Light activity (min/day)	169 ± 7.9	155 ± 11	174 ± 6.9	164 ± 10	-0.7 ± 13.3	0.960
Moderate/vigorous activity (min/day)	14.7 ± 2.3	12.7 ± 2.9	13.2 ± 1.9	12.6 ± 3.1	0.5 ± 2.2	0.808
Activity type						
Sitting (hrs/day)	10.7 ± 0.3	11.0 ± 0.5	10.3 ± 0.3	11.5 ± 0.7	-0.9 ± 0.8	0.287
Walk (min/day)	79 ± 4.1	61 ± 6.2	78 ± 4.1	66 ± 9.4	-4.6 ± 7.4	0.538
Steps (no/day)	4617 ± 274	3627 ± 526	4773 ± 297	4094 ± 713	-148 ± 516	0.775

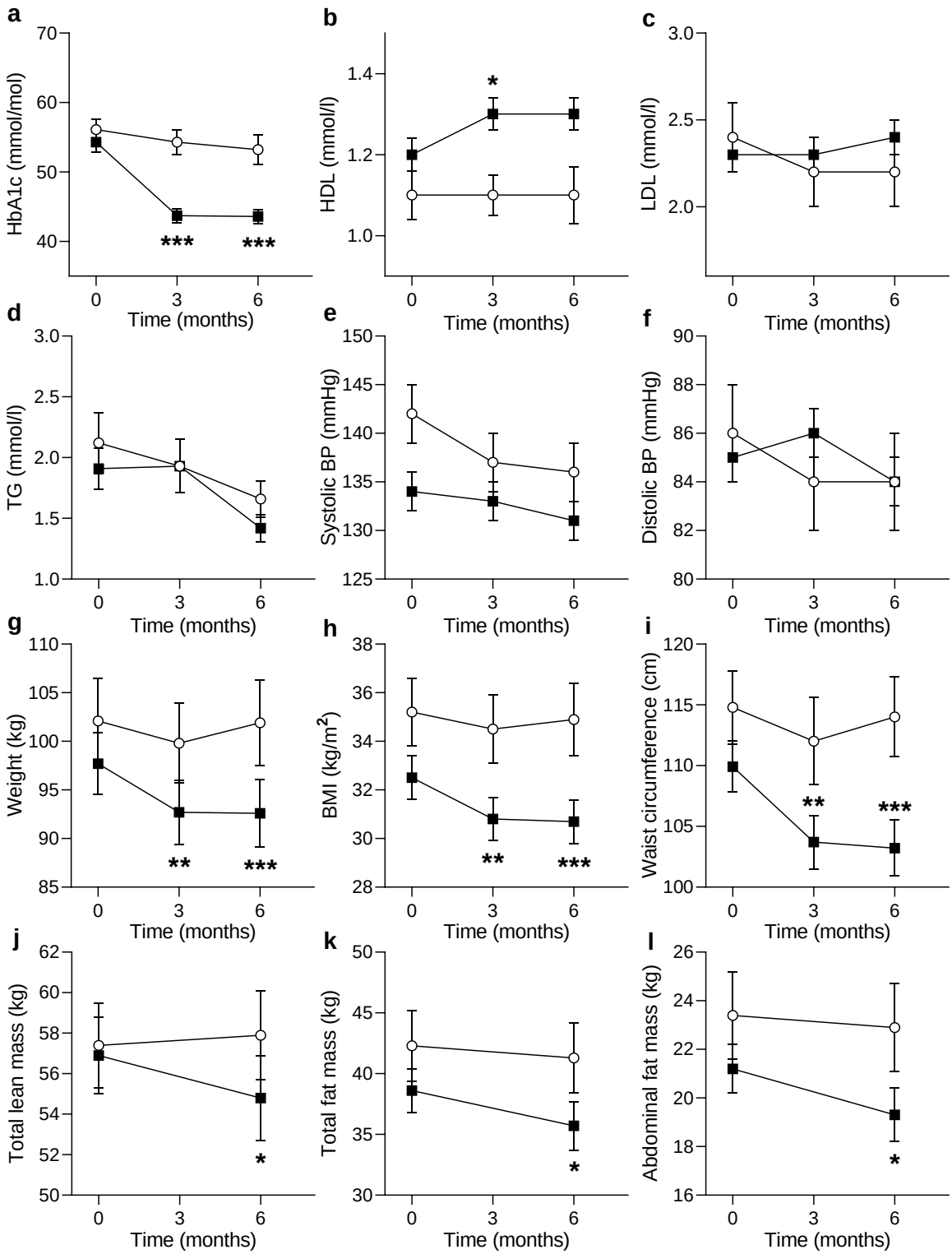
The *p*-values show the significance levels of the effect of intervention in the LCD group corrected for any change in the control group and baseline differences. This is given as the mean-difference in change (means ± SE). Other data are given as means ± SEM

Supplemental Table S3: Hypoglycemic episodes and adverse events

	LCD	**Control**
Hypoglycemic episodes		
Documented symptomatic hypoglycemia	1	1
Clinically important biochemical hypoglycemia	1	0
Severe hypoglycemia	0	0
Adverse events		
Gastrointestinal complaints (constipation, n= 5; diarrhea, n = 2; abdominal discomfort n = 3)	10*	0
Stroke	0	1
Exacerbation of asthma	1	0
Pneumonia	1	0
Tonsillitis	1	0
Urinary tract infections	2	0
Leg erysipelas	0	1
Restless Legs Syndrome	2	0
Leg cramps	2	0
Foot ulcer	1	0
Orthostatic hypotension	2	0
Allergic skin reaction	0	2
Recurrence of kidney stone	1	0
Recurrence of pancreatitis	1	0
Worsening of depression	1	0

*$p < 0.05$ vs control.

Supplemental Fig. S1



Legend Supplemental Fig. S1

HbA1c (**a**), blood lipids (**b-d**), blood pressure (BP) (**e-f**), weight, BMI, waist circumference and body composition (**g-k**) in patients with type 2 diabetes at baseline and after 3 and 6 months on either a LCD (black squares) or a control diet (white circles). Data are means ± SEM. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ for between-group differences in change. BP, blood pressure; LCD, low-carbohydrate diet; TG, triacylglycerols.

A six-month low-carbohydrate diet high in fat does not adversely affect endothelial function or low-grade inflammation in patients with type 2 diabetes. An open-label randomized controlled trial

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Abstract

Background: While a low-carbohydrate diet (LCD) reduces HbA1c in patients with type 2 diabetes (T2D), the associated high intake of fat may adversely affect cardiovascular risk factors such as impaired endothelial function and low-grade inflammation. Here, we examined the effect of a non-calorie-restricted LCD high in fat for six months on measures of endothelial function and low-grade inflammation in T2D.

Methods: In an open-label randomized controlled trial, 71 patients with T2D were randomized 2:1 to either a LCD (<20 E% carbohydrates, 50-60 E% fat) or a control diet (50-60 E% carbohydrates, 20-30 E% fat) for six months. Flow-mediated vasodilation (FMD) and nitroglycerine-dependent vasodilation (NID) were assessed by ultrasound in the brachial artery at baseline and after six months, along with plasma interleukin-6 (IL-6) and serum high-sensitivity C-reactive protein (hsCRP).

Results: The FMD and NID were unaltered in both groups after six months, and there were no between-group differences in change of either FMD ($p=0.34$) or NID ($p=0.53$) in response to the interventions. The circulating hsCRP and IL-6 levels decreased only in response to LCD (both $p<0.05$). However, comparing changes over time with the control diet, the LCD did not reduce either IL-6 ($p=0.25$) or hsCRP ($p=0.07$) levels. The lack of changes in FMD and NID in response to LCD persisted after adjustment for cardiovascular risk factors.

Conclusion: A LCD high in fat for six months does not adversely affect endothelial function or low-grade inflammation, which together with unaltered blood pressure and lipid levels suggests that this nutritional approach is safe with respect to cardiovascular risk factors.

Introduction

One of the major causes of mortality in type 2 diabetes (T2D) is cardiovascular disease (CVD) [1], which contributes to a reduced life span [2]. A number of factors contribute to an increased risk of CVD in T2D such as hypertension, dyslipidemia, poor glycemic control, abdominal obesity and insulin resistance [3]. These factors are associated with chronic low-grade inflammation with increased circulating levels of pro-inflammatory markers such as high-sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL6) [4]. The endothelium is highly susceptible to low-grade inflammation, and endothelial dysfunction (ED) is one of the earliest signs of progressing arteriosclerosis [5]. In ED, the normal regulation of the vessel tone, inflammatory processes and anticoagulation to maintain vascular homeostasis are disrupted [6,7], with impaired ability to react to physiological stimuli [8]. Flow-mediated vasodilation (FMD) in the brachial artery, measured as the percentage change in artery diameter after reactive hyperemia, is significantly lower in hypertensive patients with T2D compared with hypertensive persons without T2D [9]. Reduced FMD is recognized as an independent risk factor for future development of CVD such as cardiovascular death, myocardial infarction, need of revascularization of coronary arteries, and stroke [5,10,11].

T2D management includes lifestyle changes involving both diet and physical activity and is important to improve glycemic control and decrease risk of CVD [3]. Low-carbohydrate diets have beneficial effects on HbA1c compared to control diets over six months [12], but the increase in dietary fat, in particular saturated fat, have raised concerns about an increased risk of developing CVD [13]. Smaller cross-sectional studies have shown that a higher habitual intake of dietary fat [14], and saturated fat [15] is associated with impaired endothelial function. However, the reported effects of carbohydrate-restricted diets high in fat on endothelium-dependent vasodilation in non-diabetic individuals are inconsistent. Thus, in healthy obese individuals, low carbohydrate diets did not affect FMD over six weeks [16], 12 weeks [17] nor 12 months [18], regardless of whether it included additional approaches such as a reduced caloric intake or increased exercise or not. In contrast, a meta-analysis found decreased FMD in response to carbohydrate-restricted diets (≤ 45 E% carbohydrate) [19], while another report pointed to improved vasoreactivity after a very low-carbohydrate diet (VLCD) for 6 weeks compared to a low-fat diet [20]. In individuals with T2D, the effect of carbohydrate-restricted diets high in fat on measures of endothelial function is not very well examined. Wycherley *et al* reported no effect on brachial FMD in patients with T2D randomized to a VLCD (14 E% carbohydrates) compared with a high-carbohydrate diet low

in fat after 12 months [21], and with no effect on endothelial function in either groups after 24 months [22]. However, in both groups, the diets were energy-restricted (~30%) and combined with a supervised exercise program leading to significant but similar reductions in weight and HbA1c, which may have obscured the true effect of the diets on FMD [21-23]. Interestingly, Barbosa-Yañez *et al* [24] found that the brachial FMD increased more than 50% after three weeks of a hypocaloric low fat diet compared with no change in response to a hypocaloric VLCD (5-10 E% carbohydrates) in patients with T2D suggesting a potential harmful effect of carbohydrate-restriction on endothelial function.

Along with endothelial dysfunction, circulating markers of low-grade inflammation such as hsCRP and IL-6 are often elevated in obesity and T2D and associated with a higher risk of cardiovascular events [25-27]. So far, however, studies comparing the effect of carbohydrate restriction with low fat diets on circulating hsCRP and IL-6 levels in patients T2D have been scarce and inconclusive [28,29]. In particular, the effect of restricting carbohydrate intake to 10-25 E% (defined as a low-carbohydrate diet, LCD) on these pro-inflammatory markers in patients with T2D remains to be established.

We have previously reported that six months of a LCD high in fat reduced HbA1c, weight and abdominal adiposity compared to a control diet in patients with T2D, whereas blood pressure or lipid levels were unaffected [30]. In the present study, we aimed to test the hypothesis that a LCD high in fat for six months adversely affects CVD risk factors such as endothelial function assessed as FMD and nitroglycerine-dependent dilation (NID) and markers of chronic low-grade inflammation compared to a control diet in patients with T2D instructed to maintain their daily intake of energy and levels of physical activity.

Methods

Study design and participants

As previously reported [30] the study was an out-patient, open-labelled, randomized controlled trial (RCT) of the effects of a LCD compared to a control diet for six months in patients with T2D. The study was conducted from November 2016 to May 2019 at Odense University Hospital. Participants with established T2D were recruited through mainly public advertisement and social media, in addition to invitations to patients, who had attended previous studies. Results from this study including changes in glycaemic control, measures of body composition, blood lipids and blood pressure were recently reported [30]. In the present study, we report the prespecified secondary outcome endothelial function as well as markers of low-grade inflammation.

Briefly described, the inclusion criteria included an established diagnosis of T2D [31], age older than 18 years, an HbA1c of more than 48 mmol/mol, a diabetes duration of six months to five years, but up to 10 years if treated with ≤ 2 non-insulin antidiabetic drugs, and stable glucose lowering therapy >3 months prior to inclusion. To prevent changes in cholesterol lowering treatment during the study, the inclusion criteria included a LDL cholesterol < 2.5 mmol/l and a total cholesterol < 4.5 mmol/l. However, if the patients could not tolerate statin and/or refused treatment, a higher LDL-cholesterol was accepted. Exclusion criteria were significant co-morbidities or significant diabetic comorbidities that could risk safety during diet change or affect diet compliance. Other exclusion criteria were continuous use of steatosis-inducing drugs or glucocorticoids, following a carbohydrate-restricted diet prior to inclusion, excessive weight loss before enrollment (defined as > 10 kilos over three months) or pregnancy/planned pregnancy.

Out of 345 persons eligible for screening [30], 73 were enrolled and randomized 2:1, stratified on sex and number of antidiabetic drugs (0-1 and ≥ 2), to either the LCD intervention or a control diet. Two sets of family members were randomized as one unit each, and two participants withdrew consent before commencing to the study, leaving 49 participants for inclusion in the LCD group and 22 in the control group at baseline. After 6 months of the study, five in the LCD group and two in the control group had dropped out [30].

Informed consent was obtained from all individuals before participation. The study was approved by the Regional Committees on Health Research Ethics for Southern Denmark and was performed in accordance with the Declaration of Helsinki Declaration II. The RCT was registered at ClinicalTrials.gov (NCT03068078).

Diet intervention and physical activity

The LCD group was instructed to follow a diet consisting of a maximum of 20 E% carbohydrates, 50-60 E% fats 25-30 E% protein with a recommendation of a high intake monounsaturated fatty acids (MUFAs) and as low intake of saturated fatty acids as possible. The control diet group were instructed to follow a diet according to the current official Danish dietary guidelines with a recommended intake of 50-60 E% carbohydrates, 20-30 E% fat, where <10 E% should be from saturated fat, and 20-25 E% protein [32].

All participants were individually introduced to the diet by a licensed clinical dietitian, had opportunity for on-demand visits and could attend group-specific discussion meetings supervised by the dietitian. The dietitian contacted every participant per telephone one week after starting the new diet and hereafter every month. A five-days startup menu plan based on pre-study calorie intake was provided as well as weekly newsletters to all participants. Participants had access to a recipe-database that was continuously updated and were instructed to register food intake in MadLog (MadLog ApS, Kolding, Denmark). Based on the estimated energy requirement at baseline, participants were guided to maintain their calorie-intake during the entire intervention. Furthermore, the participants were instructed maintain their usual physical activity level throughout the study period. As reported [30], this was examined by accelerometer-based assessment of physical activity for seven days at baseline and after six months. This showed no change in physical activity levels in the two study groups. For further details regarding the diet and accelerometry please refer to our previous report [30].

Assessment of flow-mediated vasodilation (FMD and nitroglycerine induced dilation (NID)

FMD and NID in the brachial artery were assessed by a single investigator at baseline and after six months of diet change using a Phillips iE33 ultrasound machine with a L15-7io linear array transducer and automated settings for FMD/NID. The participant's right arm was examined in the morning after an overnight fast, minimum 8 hours. The participants were instructed to discontinue antihypertensive medication, vitamins and sildenafil three days before the examination and to refrain from strenuous exercise, tea and juice for 48 hours and coffee, alcohol and nicotine for 12 hours prior to the examination. Any ongoing cholesterol-lowering treatment continued, but was not taken on the day of examination. One participant was examined at noon both times.

Following a 15 minutes rest in supine position, blood pressure was measured in the left arm to ensure cuff inflation minimum 20 mmHg above systolic blood pressure (SBP) (minimum 200 mmHg). A rapid inflation/deflation cuff was applied with upper crease in the cubital fossa on the right forearm (Hokanson E20, Bellevue USA), where after a suitable segment of the brachial artery proximal of the cubital fossa was identified. Anatomical markers and cuff pressure were noted for follow-up examinations. The resting brachial artery diameter (FDM RD) was recorded at least three times for 60 seconds, lifting the transducer between each recording. The cuff was then inflated for five minutes and recordings were resumed five seconds before cuff deflation and continued for five minutes to determine FMD. The resting brachial artery (NID RD) was assessed after another 15-minutes rest with another three recordings after which one spray of 400 µg of sublingual glyceryl trinitrate was administered. After that the recordings continued for 9.4 minutes (6 x 94 seconds) to determine NID.

Sequences were exported as AVI-files or DICOM for off-line analyses. The same trained person who executed the FMD measurements also analyzed offline the individual sequences blinded for patient ID and clinical data using a semi-automated, commercial software (Brachial Analyzer, Medical Imaging Application, version 6.9.1, Coralville, Iowa, USA) [33]. Auto-gated, end-diastolic diameters were used. To examine intra-observer reliability for FMD and NID, a random sample of twelve volunteers were scanned on two consecutive days, assessing FMD. The resulting intra-observer reliability coefficient was 0.968, which is similar to that reported in similar studies [34].

The following variables were estimated: The resting diameters (FMD RD, NID RD) were calculated by using the mean of the 60-second resting end-diastolic diameter measurements. The maximal flow-mediated vasodilation (FMD max) was calculated as the mean of minimum five consecutive auto-gated diameters after reaching peak dilation, which was obtained through visual inspection of the end-diastolic diameters. The maximal nitroglycerine-induced dilation (NID max) was estimated as the mean of minimum 60 seconds measurements after the individual peak dilation was reached. FMD and NID were calculated and reported as the percentage change (%) compared to the resting diameter. The ratio between FMD and NID (FMD/NID) was calculated as an estimate of endothelial-dependent vasodilatory function adjusted for endothelium-independent vasodilatory capacity. The cut-off values for FMD and NID used to distinguish those without an increased risk of CVD from those with an increased risk, were FMD < 7.1 % and NID<15.6 %, respectively [35].

Out of the 71 FMD- and NID sequences obtained at baseline, the FMD and NID in one person from the control group could not be analyzed due to low image quality and the NID from one person in the LCD group was missing due to damaged files (LCD), leaving a total of 70 FMD and 69 NID sequences for analysis at baseline. Given the seven drop-outs (see above), 64 FMD- and NID sequences were available for analysis at the 6 month follow-up. None of the participants FMD- or NID data were excluded due to outliers.

Other outcome measures

All participants attended three visits during the study (baseline, three months and six months) with collection of fasting blood samples for measuring lipids, insulin, HbA1c, fasting plasma glucose and blood-ketones as reported [30]. Anthropometric measurements were assessed at all three visits. Participants were scanned with a dual-energy x-ray absorptiometry (DXA) scan (Hologic, Marlborough, MA) at baseline and after six months.

Serum hsCRP was measured in duplicates by an in-house ELISA using commercially available monoclonal antibodies and reagents (Biotechne, R&D Systems, MN, USA) according to the manufacturers instructions. The limit of detection was 0.05 µg/L and the intra- and inter-assay CVs were below 15%. Circulating IL-6 was measured in singlets on fasting EDTA plasma by the human high-sensitive IL-6 ELISA assay essentially as described (R&D Systems, Abingdon, UK). Mean CV% between runs was 6.9% (EDTA plasma pool, level 8.1 pg/ml). CV% of assay controls were 20% (level 0.5 pg/ml), 12.6%, (level 3.2 pg/ml) and 18.5% (level 5.9 pg/ml).

Statistical analysis

Statistical analysis was done with STATA for Windows (STATA 16.0, StataCorp LLC, Texas, USA). The vascular function was one of the pre-specified secondary outcomes in this study. Even though the power calculations were based on the primary outcome, HbA1c, a non-inferiority analysis showed that to detect a 20% change in FMD% with a SD of 20% and a power of 80% with 2:1 randomization, 34 participants (23 + 11) would have been necessary.

All residuals were tested for normal distribution, and if the residuals did not meet criteria of normal distribution, the dependent variables were log-transformed prior to the statistical analyses. The Students *t*-test for unequal variances was used for comparison of continuous variables and the chi-squared test for comparison of categorical variables between the two groups at baseline. For analyses of changes over time within and

between groups, a mixed model with randomization- and time interaction was applied. The mean difference in change (MDIC) between groups from baseline to 6 month is reported as the effect of LCD versus control diet. The relationship between FMD and NID and several measured CVD risk factors including circulating IL-6 and hsCRP levels at baseline was examined using univariate linear regression or Spearman's rank correlation coefficient if residuals were not normally distributed. All data are reported as mean ± SEM. Statistical significance was assumed at $p < 0.05$.

Results

Baseline characteristics and changes in clinical parameters.

Among study participants with valid FMD measurements at baseline (n=70), the two groups were comparable with respect to age, duration of diabetes, HbA1c, gender distribution, smoking status, lipid levels and BMI (Table 1 and Supplemental Table S1). The systolic blood pressure was higher in the control group at baseline. As reported [30], there were no significant differences in the types or number of glucose- or blood pressure lowering drugs between the groups. Moreover, the LCD caused a reduction of HbA1c, weight, BMI, total fat mass and waist circumference after 6 month compared with the control diet, whereas no changes in blood lipids or blood pressure were seen (Supplemental Table S1). After 6 months, 35 of 44 (80%) in the LCD groups and 8 of 20 (40%) in the control group had HbA1c ≤ 48 mmol/mol (p=0.002). The LCD group reduced their self-reported carbohydrate intake to 13.4 E% while fat intake was increased to 63.2 E% compared to an intake of 48.4 E% carbohydrate and 28.3 E% of fat in the control group after 6 months [30]. At the end of the study, the self-reported intake of saturated fatty acids was 2.6 fold higher in the LCD group compared with the control group (Supplemental Table S1).

Measures of endothelial function

While there were no differences between groups in resting brachial artery diameters (FMD RD or NID RD) or maximal dilation (FMD max or NID max) at baseline, the LCD group had higher values of both FMD (5.2±0.3% vs. 4.2±0.4%, p=0.04) and NID (16.7±0.6% vs. 13.6±1.1%, p=0.02) compared with the control group (Table 2). At baseline, 41 of 49 (84%) in the LCD group and 20 of 21 (95%) in the control group had FMD < 7.1% (p=0.42), and 20 of 48 (42%) in the LCD group and 14 of 21 (67%) in the control group had NID < 15.6 % (p=0.06). These proportions did not change significantly after 6 months (data not shown). In the LCD group, the resting diameters (FMD RD and NID RD) and maximal dilations (FMD max and NID max) decreased, whereas FMD and NID were unaltered after 6 months on the diet. In the control group, no changes were observed (Table 2). Moreover, the FMD/NID ratios were similar in the groups at baseline, and did not change in either of the groups after 6 month. Importantly, there were no effects of LCD on the resting diameters or maximal dilations (all p>0.46), or on FMD (MDIC: -0.44±0.47%, p= 0.34), NID (MDIC:

+0.59±0.93%, p=0.53) or FMD/NID (MDIC: -0.02±0.04, p=0.52) compared with the control diet (Fig.1 and Table 2).

Measures of low-grade inflammation

There were no differences between groups in circulating levels of hsCRP or IL-6 at baseline (Fig. 2). In the LCD group, both the hsCRP (p=0.004) and IL-6 levels (p=0.013) decreased after 6 months on the diet, whereas no significant changes were seen in the control group (Table 4). However, when comparing changes over time, the LCD did not significantly reduce either IL-6 (MDIC: p=0.247) or hsCRP (MDIC: p=0.065) compared with the control diet.

Using Spearman's rank correlation coefficient in the total cohort (n=70) at baseline, we found that plasma Il-6 levels correlated positively with BMI (p<0.001), and abdominal (p=0.004) and total body fat percentage (p=0.011), but not with the other CVD risk factors listed in Table 3. The serum levels of hsCRP correlated positively with female sex (p=0.036), triglycerides (p=0.024), BMI (p<0.001), and abdominal (p<0.001) and total body fat percentage (p<0.001).

Linear regression analysis of covariates

To adjust the analysis for between-group differences in CVD risk factors at baseline, we evaluated the relationship between FMD and NID and all the CVD risk factors listed in Table 3. This univariate linear regression analysis showed that FMD correlated significantly with age, systolic BP and resting diameter (FDM RD) at baseline, whereas NID correlated significantly with age and resting diameter (NID RD).

When adjusting our analysis for these significant covariates, the difference in FMD (corrected for age, FMD RD and systolic BP) between groups at baseline disappeared (p=0.197), whereas NID (corrected for age and NID RD) remained higher in the LCD group at baseline (p=0.007). After adjusting for the same covariates, there was, however, still no effect of LCD for 6 months on FMD (adjusted MDIC: -0.44±0.45%, p=0.335) or NID (adjusted MDIC: +0.56±0.92 %, p=0.543).

Description of changes in cholesterol and blood pressure lowering medication

There were no changes in cholesterol lowering treatment during the intervention period, although two participants on LCD reported to only take statins sporadically during the study. In the LCD-group, one patient

treated with both an angiotensin-converting enzyme (ACE) inhibitor and an angiotensin II receptor blocker (ARB) had the ACE inhibitor discontinued. Another patient in the LCD group discontinued thiazide treatment and was reduced in beta-blocker treatment. In two other patients in the LCD group, the dose of ARB treatment (25 mg and 50 mg, respectively) was reduced due to orthostatism. In one patient in the control group the dosage of ACE inhibitor combined with hydrochlorthiazide was increased.

Discussion

In this second study from an open-label RCT [30], we report the effect of a 6-months non-calorie-restricted LCD on measures of endothelial function in the brachial artery and markers of low-grade inflammation compared to a control diet in patients with T2D. In contrast to our hypothesis, the LCD did not cause changes in either endothelium-dependent or endothelium-independent arterial dilatation assessed as brachial artery FMD and NID, respectively, compared to the control diet in patients with T2D. Although circulating IL-6 and hsCRP levels decreased only the LCD group, there were no significant between-group differences in the change of IL-6 or hsCRP levels over time. These findings together with the previously published lack of changes in blood lipids and blood pressure in this study support that a non-calorie-restricted LCD without changes in physical activity is safe with respect to cardiovascular risk despite a high intake of fat. However, longer studies are needed to confirm these findings beyond 6 months.

Population-based studies have provided evidence that correction of a suboptimal diet may be a powerful approach to reduce the risk of CVD [36]. Our findings extend results from the reports by Wycherley *et al* [21] and Tay *et al* [22], who found no between-group differences in change of brachial FMD when comparing a VLCD low in saturated fat to a high-carbohydrate diet low in fat, both combined with calorie-restriction and a supervised exercise program in patients with T2D. In the study by Wycherley *et al* [21], the authors speculated that this might be due to a significant weight loss observed in both groups during the study, in addition to a decreased intake of saturated fat while maintaining intake of dietary fibers. The prescribed exercise program in both groups may also have affected the outcome in these studies [21,22], as exercise training has been demonstrated to improve endothelial function [37]. In the present study, the participants in both groups were instructed to maintain their level of physical activity and intake of calories according to their baseline energy requirement, and the LCD group increased their intake of saturated fat and lost weight compared to the control group. These findings suggest that the lack of changes in FMD reported in the previous reports [21,22] may not be explained by a calorie restriction-induced weight loss or exercise training, and that neither an increase nor a decrease in saturated fat in these diets affect endothelial function. In support, a previous report found that a diet-induced weight loss of ~10 kg in abdominally obese individuals did not improve brachial FMD compared with a control group without weight-loss [38]. In addition, a study of individuals with obesity without diabetes found that weight-loss achieved by either a LCD or a low-fat diet did not affect FMD [18].

There might, however, be transient effects of an increased intake of fat on endothelial function that are attenuated or eventually lost over time. Thus, the brachial FMD increased markedly in response to a low-fat diet for 3 weeks, but not with a VLCD in patients with T2D, even though both groups lost abdominal weight [24]. The authors also found a positive correlation between FMD and protein and fat intake in the low-fat diet group after 3 weeks, but not in the VLCD group. This positive effect of a low-fat diet on FMD was observed despite a significant reduction of HbA1c only in the VLCD group suggesting that a short-term improvement in glycemic control per se does not improve FMD [24]. Intriguingly, an inverted U-shape association between FMD and HbA1c has been reported, with a lower FMD in T2D patients with an HbA1c < 48 mmol/mol than those with an HbA1c of 48-63 mmol/mol and similar to those with an HbA1c higher than that [39]. In contrast, a higher degree of coronary atherosclerosis and lower FMD were observed in T2D patients with a poor glycemic control compared to those with an appropriate glycemic control [40]. In line, a greater improvement in FMD (69%) was reported in T2D patients with poor glycemic control on intensified antidiabetic treatment for 12 months, who achieved an HbA1c≤48 mmol/mol [41]. In our study, the majority (80%) of participants randomized to the LCD achieved an HbA1c≤48 mmol/mol compared with less than half of the participants on the control diet (40%), however, the larger reduction in HbA1c in the LCD group was not accompanied by an improvement in FMD. Furthermore, consistent with other studies [42], we did not observe an association between HbA1c and FMD at baseline. Thus, while we cannot exclude the possibility, that the improvement in HbA1c may have counteracted a potential negative effect of the increased intake of saturated fat on FMD, the lack of associations between FMD and HbA1c at baseline and the lack of changes in other CVD risk factors such as blood lipids and blood pressure suggest that this is not the case.

Markers of systemic low-grade inflammation such as IL-6 and hsCRP are often elevated in obesity and T2D and associated with an increased risk of CVD [25-27]. Moreover, a meta-analysis has shown that weight-loss causes a reduction in circulating levels of IL-6 and hsCRP [43], In line, we found that both hsCRP and IL6 levels were positively associated with BMI and abdominal fat (%) at baseline. After 6 months, we found a small but significant reduction in both IL-6 and hsCRP levels in the LCD group, whereas no changes were observed in the control group. However, we could not demonstrate a significant difference in change of IL-6 or hsCRP levels over time between the groups. This lack of difference between the diets was seen despite a greater reduction in body weight and HbA1c in the LCD group. Previous studies reporting the effect of carbohydrate-restriction versus low fat diet for 6 months on pro-inflammatory markers in patients with T2D have shown

somewhat variable results [28,29]. Thus, in one study, plasma IL-6 increased only in response to the low fat diet, whereas plasma hsCRP remained unchanged in both groups [28]. Conversely, in another study, serum hsCRP decreased only in response to the low fat diet, whereas serum IL-6 was unchanged in both groups [29]. These changes were observed despite similar weight losses in both diet groups in these studies [28,29]. However, in line with our findings, none of the studies reported a mean difference in change of IL-6 or hsCRP levels between the groups. Moreover, a randomized cross-over study of a carbohydrate-restricted diet high in protein versus a conventional diet low in fat for 6 weeks in patients with T2D found no changes in either hsCRP or IL-6 levels arguing against a missed short-term effect in our study [44]. Taken together, these results provide evidence that carbohydrate-restriction to different extent (E14% to E34%) does not negatively affect markers of systemic low-grade inflammation. Larger and longer clinical trials are, however, needed to examine if a larger reduction of weight and HbA1c in response to a LCD (E% 10-25%) could reduce systemic low-grade inflammation despite an increased intake of fat.

The strengths of the present study include the randomized design, the well-matched study groups, and the sample size, which according to a non-inferiority analysis was sufficiently large to rule out a change in FMD higher than 20%. In addition, the participants were instructed to maintain their level of physical activity and medication, which allowed us to study the isolated effect of a non-calorie-restricted LCD, which is feasible for patients with T2D under free-living conditions. The limitations include the open-label approach, the lack of strict control with regard to changes in physical activity, medication, and diet macronutrient composition, the latter leading to a higher intake of saturated fat than recommended. Moreover, the inability to demonstrate a between-group difference in change of IL-6 and hsCRP levels despite a reduction of both markers in the LCD group suggests that a larger sample size would have been needed to make a conclusion whether a LCD reduces low-grade-inflammation.

In summary, the present study provides evidence that a LCD high in fat for 6 months in patients with T2D instructed to maintain their daily energy intake and level of physical activity does not adversely affect either the endothelium-dependent (FMD) or –independent (NID) vasodilation in the brachial artery or markers of systemic low-grade inflammation compared with a control diet low in fat. These findings together with the previously reported lack of changes in blood lipids and blood pressure [29] suggest that this nutritional approach is safe with respect to several well-established cardiovascular risk factors although studies of longer duration are needed.

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Conflict of interest

The authors declare that there is no duality of interest associated with this manuscript.

Author contributions

EMG-K, CDH, AK, HB-N, MHO, and KH contributed to the conception and design of the study. EMG-K, CDH, MBH, and JMJ conducted the intervention study. EMG-K, TBO and AH contributed to the ultrasound and biomarker analyses. EMG-K, CDH, AK, HB-N, MHO, TBO, AH and KH analysed and interpreted data, and EMG-K and KH wrote the manuscript. All the authors have revised the manuscript critically for important intellectual content and given final approval of the version to be published. KH and AK are guarantors of this work, and as such had full access to all the data in the study and take full responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. (IDF) IDF. Diabetes and cardiovascular disease. In. https://idf.org/our-activities/care-prevention/cardiovascular-disease/cvd-report.html#sub-content-tab-nav2016:www.idf.org.
2. Sattar N, Rawshani A, Franzén S, et al. Age at Diagnosis of Type 2 Diabetes Mellitus and Associations With Cardiovascular and Mortality Risks. *Circulation.* 2019;139(19):2228-2237.
3. 10. Cardiovascular Disease and Risk Management: Standards of Medical Care in Diabetes-2021. *Diabetes Care.* 2021;44(Suppl 1):S125-s150.
4. Calle MC, Fernandez ML. Inflammation and type 2 diabetes. *Diabetes Metab.* 2012;38(3):183-191.
5. Halcox JP, Schenke WH, Zalos G, et al. Prognostic value of coronary vascular endothelial dysfunction. *Circulation.* 2002;106(6):653-658.
6. Lerman A, Burnett JC, Jr. Intact and altered endothelium in regulation of vasomotion. *Circulation.* 1992;86(6 Suppl):Iii12-19.
7. Alley H, Owens CD, Gasper WJ, Grenon SM. Ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery in clinical research. *J Vis Exp.* 2014(92):e52070.
8. Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. *Arterioscler Thromb Vasc Biol.* 2003;23(2):168-175.
9. Bruno RM, Penno G, Daniele G, et al. Type 2 diabetes mellitus worsens arterial stiffness in hypertensive patients through endothelial dysfunction. *Diabetologia.* 2012;55(6):1847-1855.
10. Matsuzawa Y, Kwon TG, Lennon RJ, Lerman LO, Lerman A. Prognostic Value of Flow-Mediated Vasodilation in Brachial Artery and Fingertip Artery for Cardiovascular Events: A Systematic Review and Meta-Analysis. *J Am Heart Assoc.* 2015;4(11).
11. Inaba Y, Chen JA, Bergmann SR. Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. *Int J Cardiovasc Imaging.* 2010;26(6):631-640.
12. Kirkpatrick CF, Bolick JP, Kris-Etherton PM, et al. Review of current evidence and clinical recommendations on the effects of low-carbohydrate and very-low-carbohydrate (including ketogenic) diets for the management of body weight and other cardiometabolic risk factors: A scientific statement from the National Lipid Association Nutrition and Lifestyle Task Force. *J Clin Lipidol.* 2019;13(5):689-711 e681.

13. Hooper L, Summerbell CD, Higgins JP, et al. Dietary fat intake and prevention of cardiovascular disease: systematic review. *BMJ (Clinical research ed).* 2001;322(7289):757-763.

14. Dow CA, Stauffer BL, Greiner JJ, DeSouza CA. Influence of habitual high dietary fat intake on endothelium-dependent vasodilation. *Appl Physiol Nutr Metab.* 2015;40(7):711-715.

15. Lambert EA, Phillips S, Belski R, et al. Endothelial Function in Healthy Young Individuals Is Associated with Dietary Consumption of Saturated Fat. *Front Physiol.* 2017;8:876.

16. Hwang CL, Ranieri C, Szczurek MR, et al. The Effect of Low-Carbohydrate Diet on Macrovascular and Microvascular Endothelial Function is Not Affected by the Provision of Caloric Restriction in Women with Obesity: A Randomized Study. *Nutrients.* 2020;12(6).

17. Ruth MR, Port AM, Shah M, et al. Consuming a hypocaloric high fat low carbohydrate diet for 12 weeks lowers C-reactive protein, and raises serum adiponectin and high density lipoprotein-cholesterol in obese subjects. *Metabolism.* 2013;62(12):1779-1787.

18. Mohler ER, 3rd, Sibley AA, Stein R, et al. Endothelial function and weight loss: comparison of low-carbohydrate and low-fat diets. *Obesity (Silver Spring).* 2013;21(3):504-509.

19. Schwingshackl L, Hoffmann G. Low-carbohydrate diets impair flow-mediated dilatation: evidence from a systematic review and meta-analysis. *Br J Nutr.* 2013;110(5):969-970.

20. Ballard KD, Quann EE, Kupchak BR, et al. Dietary carbohydrate restriction improves insulin sensitivity, blood pressure, microvascular function, and cellular adhesion markers in individuals taking statins. *Nutr Res.* 2013;33(11):905-912.

21. Wycherley TP, Thompson CH, Buckley JD, et al. Long-term effects of weight loss with a very-low carbohydrate, low saturated fat diet on flow mediated dilatation in patients with type 2 diabetes: A randomised controlled trial. *Atherosclerosis.* 2016;252:28-31.

22. Tay J, Thompson CH, Luscombe-Marsh ND, et al. Effects of an energy-restricted low-carbohydrate, high unsaturated fat/low saturated fat diet versus a high-carbohydrate, low-fat diet in type 2 diabetes: A 2-year randomized clinical trial. *Diabetes Obes Metab.* 2018;20(4):858-871.

23. Tay J, Luscombe-Marsh ND, Thompson CH, et al. Comparison of low- and high-carbohydrate diets for type 2 diabetes management: a randomized trial. *Am J Clin Nutr.* 2015;102(4):780-790.

24. Barbosa-Yañez RL, Dambeck U, Li L, Machann J, Kabisch S, Pfeiffer AFH. Acute Endothelial Benefits of Fat Restriction over Carbohydrate Restriction in Type 2 Diabetes Mellitus: Beyond Carbs and Fats. *Nutrients.* 2018;10(12).

25. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA.* 2001;286(3):327-334.

26. Kolb H, Mandrup-Poulsen T. The global diabetes epidemic as a consequence of lifestyle-induced low-grade inflammation. *Diabetologia.* 2010;53(1):10-20.

27. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med.* 2000;342(12):836-843.

28. Jonasson L, Guldbrand H, Lundberg AK, Nystrom FH. Advice to follow a low-carbohydrate diet has a favourable impact on low-grade inflammation in type 2 diabetes compared with advice to follow a low-fat diet. *Ann Med.* 2014;46(3):182-187.

29. Davis NJ, Crandall JP, Gajavelli S, et al. Differential effects of low-carbohydrate and low-fat diets on inflammation and endothelial function in diabetes. *J Diabetes Complications.* 2011;25(6):371-376.

30. Gram-Kampmann EM, Hansen CD, Hugger MB, et al. Effects of a 6-month, low-carbohydrate diet on glycaemic control, body composition, and cardiovascular risk factors in patients with type 2 diabetes: An open-label randomized controlled trial. *Diabetes Obes Metab.* 2022.

31. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2021. *Diabetes Care.* 2021;44(Suppl 1):S15-s33.

32. Fisheries MoFAa. The official dietary guidelines Food-based dietary guidelines https://altomkost.dk/raad-og-anbefalinger/de-officielle-kostraad/.

33. Sonka M, Liang W, Lauer RM. Automated analysis of brachial ultrasound image sequences: early detection of cardiovascular disease via surrogates of endothelial function. *IEEE Trans Med Imaging.* 2002;21(10):1271-1279.

34. Wycherley TP, Brinkworth GD, Noakes M, Buckley JD, Clifton PM. Effect of caloric restriction with and without exercise training on oxidative stress and endothelial function in obese subjects with type 2 diabetes. *Diabetes Obes Metab.* 2008;10(11):1062-1073.

35. Maruhashi T, Kajikawa M, Kishimoto S, et al. Diagnostic Criteria of Flow-Mediated Vasodilation for Normal Endothelial Function and Nitroglycerin-Induced Vasodilation for Normal Vascular Smooth Muscle Function of the Brachial Artery. *J Am Heart Assoc.* 2020;9(2):e013915.

36. Health effects of dietary risks in 195 countries, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet (London, England).* 2019;393(10184):1958-1972.

37. Lee JH, Lee R, Hwang MH, Hamilton MT, Park Y. The effects of exercise on vascular endothelial function in type 2 diabetes: a systematic review and meta-analysis. *Diabetol Metab Syndr.* 2018;10:15.

38. Joris PJ, Plat J, Kusters YH, et al. Diet-induced weight loss improves not only cardiometabolic risk markers but also markers of vascular function: a randomized controlled trial in abdominally obese men. *Am J Clin Nutr.* 2017;105(1):23-31.

39. Yamaji T, Harada T, Hashimoto Y, et al. Inconvenient relationship of haemoglobin A1c level with endothelial function in type 2 diabetes in a cross-sectional study. *BMJ Open.* 2021;11(6):e045415.

40. Chen S, Shen Y, Liu YH, et al. Impact of glycemic control on the association of endothelial dysfunction and coronary artery disease in patients with type 2 diabetes mellitus. *Cardiovascular diabetology.* 2021;20(1):64.

41. Lambadiari V, Pavlidis G, Kousathana F, et al. Effects of Different Antidiabetic Medications on Endothelial Glycocalyx, Myocardial Function, and Vascular Function in Type 2 Diabetic Patients: One Year Follow-Up Study. *J Clin Med.* 2019;8(7).

42. Naka KK, Papathanassiou K, Bechlioulis A, et al. Determinants of vascular function in patients with type 2 diabetes. *Cardiovascular diabetology.* 2012;11:127.

43. Byers T, Sedjo RL. Does intentional weight loss reduce cancer risk? *Diabetes Obes Metab.* 2011;13(12):1063-1072.

44. Skytte MJ, Samkani A, Astrup A, et al. Effects of a highly controlled carbohydrate-reduced high-protein diet on markers of oxidatively generated nucleic acid modifications and inflammation in weight stable participants with type 2 diabetes; a randomized controlled trial. *Scand J Clin Lab Invest.* 2020;80(5):401-407.

Table 1: Baseline characteristics

	LCD (n=49)	**Control (n = 21)**
Age (years)	55.2 ± 0.9	57.1 ± 2.8
Duration of diabetes, years	5.2 ± 0.5	5.0 ± 0.5
Female sex	26 (53)	12 (57)
Smoking status		
Never smoked	24 (49)	10 (48)
Active smoker	2 (4)	1 (5)
Exsmoker	23 (47)	10 (48)
Smoking pack years, years	21.4 ± 3.1	14.0 ± 5.4
Body Mass Index	32.5 ± 0.9	35.5 ± 1.5
Systolic blood pressure	134 ± 2*	141 ± 3
Diastolic blood pressure	85 ± 1	85 ± 2
MAP	107 ± 2	111 ± 2
Pulse	77 ± 2	83 ± 4
Hypertension treatment	32 (65)	15 (71)
No. of blood pressure lowering agents		
0	17 (35)	6 (29)
1	14 (29)	7 (33)
2	10 (20)	5 (24)
3	5 (10)	1 (5)
4	2 (4)	2 (10)
5	1 (2)	0 (0)
No. of glucose-lowering agents		
0	5 (10)	3 (14)
1	23 (47)	11 (52)
2	16 (33)	4 (19)
> 3	5 (10)	3 (14)
Cholesterol-lowering treatment	27 (55)	11 (52)

Data are means ± SEM or number (%). *p = 0.046

Table 2: Endothelial function

	Baseline		6 months			
	LCD (n=49)	**Control (n=21)**	**LCD (n=44)**	**Control (n=20)**	**MDIC**	**p-value**
FMD RD (mm)	4.28 ± 0.11	4.37 ± 0.14	4.17 ± 0.11*	4.35 ± 0.15	+0.00 ± 0.04	0.93
FMD max (mm)	4.50 ± 0.11	4.55 ± 0.15	4.38 ± 0.11**	4.54 ± 0.15	-0.008 ± 0.04	0.85
FMD (%)	5.19 ± 0.28$^{\#}$	4.17 ± 0.39	5.00 ± 0.32	4.52 ± 0.50	-0.44 ± 0.47	0.34
NID RD (mm)	4.29 ± 0.11	4.39 ± 0.14	4.14 ± 0.11**	4.37 ± 0.15	-0.04 ± 0.05	0.46
NID max (mm)	5.00 ± 0.12	4.98 ± 0.15	4.86 ± 0.12*	4.97 ± 0.16	-0.02 ± 0.06	0.75
NID (%)	16.66 ± 0.56$^{\#}$	13.64 ± 1.07	17.47 ± 0.52$^{(*)}$	14.03 ± 1.10	+0.59 ± 0.93	0.53
FMD%/NID%	0.32 ± 0.02	0.33 ± 0.03	0.29 ± 0.02	0.33± 0.03	-0.02 ± 0.04	0.52

Data are means ± SEM. Abbreviations: FMD, flow-mediated vasodilation; LCD, low carbohydrate diet; MDIC, mean difference in change; NID, nitroglycerin-induced vasodilation; RD, resting diameter**.** $^{\#}p<0.05$ vs control at baseline; $^{(*)}p<0.10$, *p<0.05 or **p<0.01 vs baseline

Table 3: Association of endothelial function with cardiovascular risk factors

	FMD (%)	**NID (%)**
	Coefficients ± SE	Coefficients ± SE
Sex (female)	0.42 ± 0.47	-0.35 ± 1.07
Diabetes duration, y	-0.04 ± 0.08	-0.09 ± 0.18
Age	-0.08 ± 0.03**	-0.13 ± 0.06*
Smoking status		
Non-smoker	(base)	(base)
Active smoker	-0.16 ± 1.20	2.79 ± 2.61
Previous smoker	-0.03 ± 0.49	2.15 ± 1.07*
Smoking pack years	-0.01 ± 0.02	-0.05 ± 0.04
BMI	-0.01 ± 0.04	-0.07 ± 0.08
Systolic blood pressure	-0.05 ± 0.02**	-0.06 ± 0.04
Diastolic blood pressure	-0.04 ± 0.03	-0.04 ± 0.06
LDL-cholesterol	-0.11 ± 0.31	0.48 ± 0.70
Triglycerides	0.08 ± 0.20	0.39 ± 0.44
HbA1c	-0.01 ± 0.03	-0.00 ± 0.06
Resting diameter	-0.91 ± 0.31**	-1.89 ± 0.72*
Body fat, %	0.01 ± 0.03	-0.03 ± 0.07
Abdominal fat, %	0.01 ± 0.03	-0.04 ± 0.07
hsCRP	0.01 ± 0.07	-0.06 ± 0.15
IL-6	0.11 ± 0.09	-0.09 ± 0.20

Abbreviations: BP, blood pressure; hsCRP, high sensitivity CRP. *p<0.05 and **p<0.01

Figure legends

Fig.1

Individual changes in (**A**) flow-mediated vasodilation (FMD) and (**B**) nitroglycerine-induced dilation (NID) from baseline to 6 months in patients with type 2 diabetes randomized to either a LCD (red circles/lines) or a control diet (blue circles/lines).

Fig.2

Changes in (**A**) plasma IL-6 and (**B**) serum hsCRP from baseline to 6 months in patients with type 2 diabetes randomized to either a LCD (red boxplots) or a control diet (blue bloxplots). *$p < 0.05$, within group change.

Fig. 1

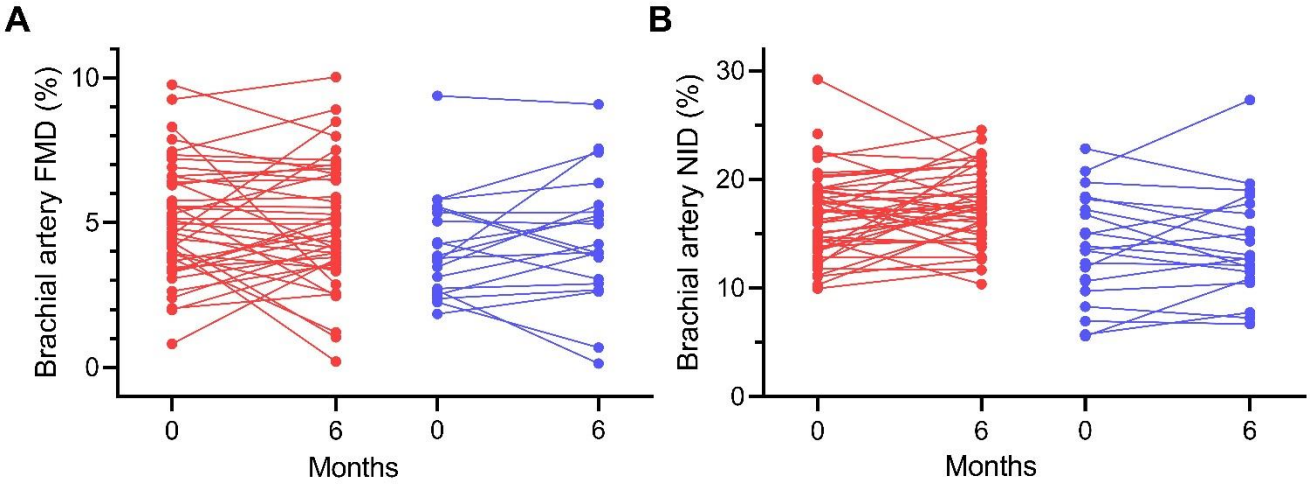
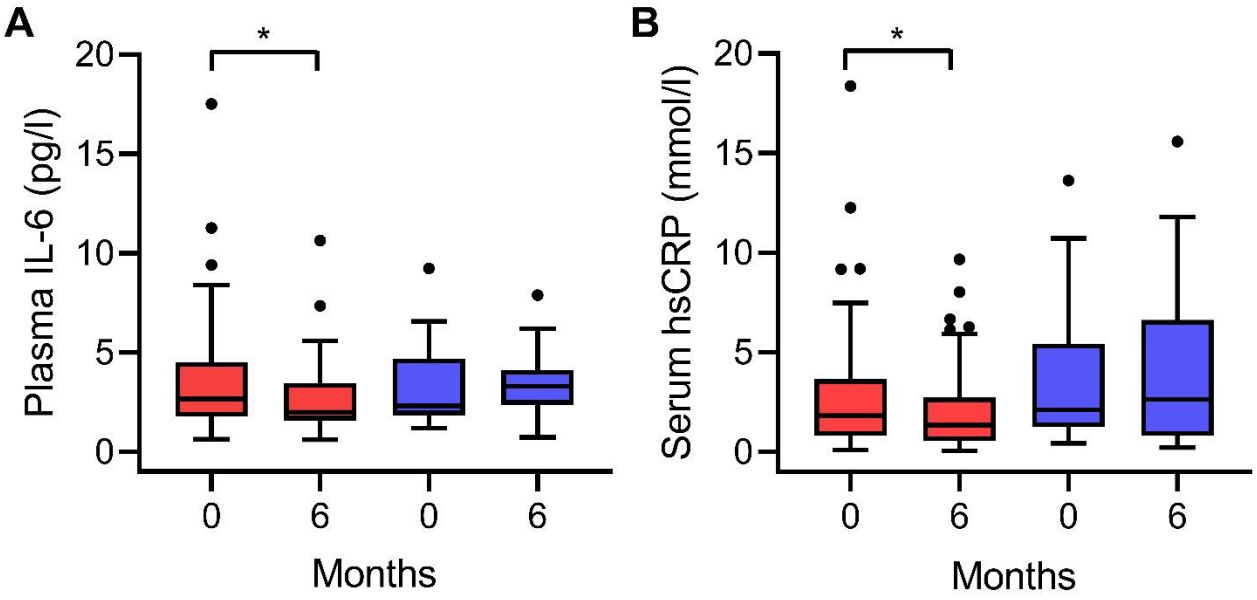

A
Brachial artery FMD (%)
10
5
0
0
6
0
6
Months
B
Brachial artery NID (%)
30
20
10
0
0
6
0
6
Months

Fig. 2


A
Plasma IL-6 (pg/l)
20
15
10
5
0
*
0
6
0
6
Months
B
Serum hsCRP (mmol/l)
20
15
10
5
0
*
0
6
0
6
Months

Supplemental Table S1. Glycemic control, cardiovascular risk factors, body composition and dietary data

	Baseline		**6 months**			
	LCD (n = 49)	**Control (n = 21)**	**LCD (n = 44)**	**Control (n = 20)**	**MDIC**	***p*-value**
HbA1c (mmol/mol)	54.3 ± 1.4	56.5 ± 1.5	43.5 ± 1.0	53.2 ± 2.1	-7.4 ± 2.1	< 0.0001
Systolic BP (mmHg)	134 ± 2*	141 ± 3	131 ± 2	136 ± 3	0.2 ± 2.9	0.936
Diastolic BP (mmHg)	85 ± 1	85 ± 2	84 ± 1	84 ± 2	1.2 ± 1.9	0.538
Serum LDL (mmol/l)	2.3 ± 0.1	2.4 ± 0.2	2.4 ± 0.1	2.2 ± 0.2	0.3 ± 0.2	0.078
Serum HDL (mmol/l)	1.2 ± 0.04	1.1 ± 0.06	1.3 ± 0.04	1.1 ± 0.07	0.1 ± 0.0	0.128
Serum TG (mmol/l)	1.91 ± 0.17	2.14 ± 0.26	1.42 ± 0.11	1.66 ± 0.15	-0.02 ± 0.22	0.920
BMI ($kg \cdot m^2$)	32.5 ± 0.9	35.5 ± 1.5	30.7 ± 0.9	34.9 ± 1.5	-1.3 ± 0.5	0.004
Weight (kg)	97.8 ± 3.2	103.1 ± 4.4	92.6 ± 3.5	101.9 ± 4.4	-3.8 ± 1.3	0.004
Waist circumference (cm)	110 ± 2	116 ± 3	103 ± 2	114 ± 3	-5 ± 1	< 0.001
Hip circumference (cm)	108 ± 2	114 ± 3	105 ± 2	112 ± 3	-2 ± 1	0.105
Serum creatinine (µmol/l)	71.3 ± 2.2	73.2 ± 4.6	68.2 ± 2.2	72.7 ± 4.9	-1.3 ± 2.2	0.549
DXA scans	**(n = 49)**	**(n = 21)**	**(n = 44)**	**(n = 20)**		
Total lean mass (kg)	56.9 ± 1.9	57.9 ± 2.1	54.8 ± 2.1	57.9 ± 2.2	-1.3 ± 0.6	0.018
Total fat mass (kg)	38.6 ± 1.8	42.7 ± 3.0	35.7 ± 2.0	41.3 ± 2.9	-2.2 ± 1.0	0.029
Total lean mass (%)	58.3 ± 1.1	56.8 ± 1.6	59.2 ± 1.2	57.4 ± 1.7	1.0 ± 0.6	0.071
Total body fat (%)	38.9 ± 1.1	40.7 ± 1.6	37.9 ± 1.3	40.0 ± 1.7	-1.2 ± 0.6	0.043
Dietary data	**(n = 36)**	**(n = 18)**	**(n = 39)**	**(n = 19)**		
Kcal per day	1805 ± 77	1817 ± 100	1642 ± 62	1660 ± 119	-24 ± 121	0.841
Carbohydrates (E%)	42.1 ± 1.2*	47.0 ± 1.3	13.4 ± 1.2	48.4 ± 1.0	-30.0 ± 2.7	< 0.001
Protein (E%)	19.4 ± 0.8	19.1 ± 0.8	23.4 ± 0.7	22.5 ± 1.0	0.5 ± 1.7	0.773
Fat (E%)	38.2 ± 1.2*	33.3 ± 1.6	63.2 ± 1.2	28.3 ± 1.0	29.9 ± 2.7	< 0.001
Saturated fat (g/day)	24.8 ± 1.7	20.5 ± 2.9	40.7 ± 2.2	15.6 ± 1.3	20.5 ± 3.7	< 0.001

The *p*-values show the significance levels of the effect of intervention in the LCD group corrected for any change in the control group and baseline differences. This is given as the mean-difference in change (MDIC) as means ± SE. Other data are given as means ± SEM. *$p < 0.05$ vs control at baseline. Abbreviations: BP, blood pressure; DXA, dual-energy x-ray absorptiometry; E%, percent of total energy intake; TG, triglycerides.