Cover Page for Study Protocol and Informed Voluntary Consent Form

Principal Investigator Name:	Funda GARGACI TUNCER
NCT Number:	Not yet
Official Title of Study:	Effects of Carbohydrate Counting Training Versus Standard Nutritional and Medical Therapy on Glycemic Control and Sarcopenia in Type 2 Diabetes: A Randomized Controlled Trial
Document Date:	March 24, 2022

Study Protocol

The Aim of the Study

The aim of this study to evaluate the effects of advanced carbohydrate counting training on anthropometric/body composition, biochemical, and clinical parameters of patients with type 2 diabetes mellitus who receive intensive insulin therapy. In addition, this study also evaluated the sarcopenia status of the patients and the effect of carbohydrate counting training on sarcopenia.

Study Design and Participants:

This study was planned as a randomized, controlled, 24-week study and was conducted with the voluntary participation of individuals with type 2 diabetes who were treated with intensive insulin. The study sample consisted of patients who received outpatient treatment at the Uskudar Diabetes Polyclinic of the Fatih Sultan Mehmet Training and Research Hospital Additional Service Building of the Ministry of Health of the Republic of Turkey between August 2022 and December 2024.

Inclusion criteria included, a history of type 2 diabetes mellitus or using insulin for more than one year, being over 18 years old, receiving basal and bolus insulin treatment, not having received carbohydrate counting training before, not receiving treatment with sulfanylurea drugs from oral anti-diabetics. Exclusion criteria included being pregnant or breastfeeding or planning a pregnancy during the study period, discontinuing insulin treatment during the study period, having active proliferative retinopathy, severe nephropathy, severe or newly diagnosed cardiac disease within 6 months or stage III–IV heart failure, having dementia or psychosis and cognitive impairment, receiving glucocorticoid treatment, being fed enterally or parenterally and receiving nutrition treatment due to another/concomitant disease, having end-stage renal or hepatic/ renal disease (other than

proteinuria); having liver disease/microangiopathy diseases, having clinical cancer, chronic respiratory disease or amputations, or having any disease or condition that would prevent completion of the study.

The study was conducted in accordance with the Declaration of Helsinki with the document number E-10840098-772.02-2030 and permission dated 24.03.2022 from the Istanbul Medipol University Non-Interventional Clinical Research Ethics Committee. Informed Voluntary Consent Form was obtained from all volunteers participating in the study.

Research Protocol

The training protocol, duration and content of each training session were decided by doctors and diabetes dietitians. Individual trainings on carbohydrate counting were organized for patients in the training group in 3 sessions on different dates in parallel with carbohydrate counting training levels. The first level training session (90 minutes), provided basic information on nutrition and health and comprised of topics as, macronutrients in food groups and their effect on plasma glucose, glycemic index, healthy food preferences and meal planning, carbohydrate contents in various food items, concept of carbohydrate change (portions of foods containing 15 grams of carbohydrate) were taught. In the second session (45 minutes), the patients calculated the carbohydrate contents of the meals they consumed based on the knowledge from the previous session. In addition, the meal plans, snacking and main meal frequencies, physical activities, effect of insulin doses on plasma glucose, noted down by each patient in their data sheets of each patient were reviewed. In the third training (45 minutes), calculation of the total carbohydrate content of the consumed meal were repeated, in order to reinforce the patient's training in this field. Furthermore, the patients were informed about the Insulin Correction Factor and Carbohydrate/Insulin ratios, and the insulin doses to be taken per meal were calculated and exemplified based on their meal plan data sheets.

Evaluation

A questionnaire form containing sociodemographic information was completed by face-to-face interview method. In addition, biochemical (HbA1c, fasting plasma glucose (FPG), C peptide, total cholesterol, HDL, LDL, triglyceride, estimated glomerular filtration rate) and anthropometric and body composition (weight-height, BMI, fat mass, fat ratio, muscle mass, lean tissue mass, waist circumference), hand grip strength, blood pressure and physical activity measurements of the patients were taken at the beginning, 12th week and 24th week of the study.

Biochemical analyzes of the participants were performed in the Biochemistry Laboratory of Fatih Sultan Mehmet Training and Research Hospital. The individuals participating in the study were asked to give blood in the morning after 10-12 hours of fasting after dinner. Plasma glucose levels were analyzed by hexokinase method in colorimetric-spectrophotometric Aeroset (Roche Hitachi cobas 6000) autoanalyzer and total cholesterol, HDL and triglyceride levels were analyzed by enzymatic colorimetric method. LDL levels were calculated using the Fridewald equation [1]. In addition, HbA1c levels were measured by high-performance liquid chromatography (HPLC) method. Serum C peptide levels were determined by Enzyme-Linked Immunosorbent Assay (ELISA) method (Roche Hitachi cobas 6000) using a commercial kit in an autoanalyzer.

Anthropometric measurements of the participants were taken by a dietician. The height of the participants was measured with a wall-type stadiometer with barefoot heels touching each other, arms hanging to the side, feet 45^{o} apart and head in the Frankfort plan. Body weight and body composition were measured with TANITA BC-418MA bioelectrical impedance analyzer. BMI values of the participants were calculated based on weight and height measurement data. In waist circumference measurement, the midpoint between the

bottom of the rib bone and the iliac crest was determined and the circumference of this area was measured with a tape measure.

The participants' hand grip strength was measured three times with a digital hand dynamometer (CAMRY-EH101, USA) and the mean value in kg was calculated. In addition, the threshold values determined for hand grip strength in the European Working Group on Sarcopenia in Older People guideline are 15 kg for women and 20 kg for men [2]. In this context, individuals below this threshold value were evaluated as low muscle strength. Skeletal muscle mass data obtained from the BIA measurement results and skeletal muscle according to the height obtained from this data were calculated by dividing the skeletal muscle mass by the square of the height. In this context, in the European Working Group on Sarcopenia in Older People guideline, the threshold values for evaluation within the scope of sarcopenia were determined as 15 kg for women and 20 kg for men for skeletal muscle, while 5.5 kg/m2 for women and 7 kg/m2 for men for skeletal muscle by height [2]. In this context, individuals below this threshold value were evaluated as having low muscle mass.

In the timed walk-up test, the patient is asked to get up from the chair, walk at a normal walking speed on a flat surface of 3 meters, turn around, walk back and sit back in the chair [3]. The time between the patient getting up from the chair, walking and sitting down again is recorded and the patient's dynamic balance during walking is also evaluated. In addition, the threshold value of the test duration in the evaluation of sarcopenia was determined as 10.85 s in the study by Christopher et al. and individuals above this threshold value were evaluated as low physical performance [4].

Blood pressure of the participants was measured with an automatic sphygmomanometer (SCIAN LD-533, Germany). Three repeated measurements were made on the right arm in the sitting position with the arm at the level of the heart and after a rest period of at least 10 minutes and the mean was calculated.

In addition, a 24-hour retrospective food consumption record was applied to evaluate the energy and nutrient intakes. The calculation of the energy, macro and micro-nutrient intakes of the participants was carried out using the Turkish Nutrition Database Ebispro for Windows program (version 7.2) [5].

Reference

1. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18(6):499–502.
2. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing. 2019;48(1):16–31.
3. Bischoff HA. Identifying a cut-off point for normal mobility: a comparison of the timed 'up and go' test in community-dwelling and institutionalised elderly women. Age Ageing. 200;32(3):315–20.
4. Ebispro for Windows & Turkish Version 7.2. Stuttgart: Germany. Data bases: Bundeslebenmittelschlüssel, II.3 and other sources.; 2020.

Informed Voluntary Consent Form

PLEASE TAKE TIME TO READ THIS DOCUMENT CAREFULLY

We invite you to participate in the study titled "Effects of Carbohydrate Counting Training Versus Standard Nutritional and Medical Therapy on Glycemic Control and Sarcopenia in Type 2 Diabetes: A Randomized Controlled Trial" conducted by the Üsküdar Diabetes Polyclinic of the Fatih Sultan Mehmet Hospital Additional Services Building. Before you decide whether to participate in this study, you need to know why and how the study will be conducted. Therefore, it is very important that you read and understand this form. If there is anything you do not understand or that is not clear to you, or if you would like more information, please ask us.

Participation in this study is completely voluntary. You have the right not to participate in the study or to withdraw from the study at any time after participating. Your response to the study will be interpreted as your consent to participate in the study. Do not be under pressure or suggestion from anyone while answering the questions on the forms given to you. The information obtained from these forms will be used solely for research purposes.

1. Information Regarding the Research:

a. Purpose of the Research: It is aimed to evaluate the effect of carbohydrate counting training on disease prognosis and sarcopenia development in T2DM individuals receiving intensive insulin treatment

b. Content of the Research: Blood findings, anthropometric (body weight, fat and muscle mass) measurements and muscle strength and function of patients with type 2 diabetes will be evaluated.

c. Reason for the Research: Scientific research

d. Estimated Duration of the Research: 1 year

e. Number of Participants/Volunteers Expected to Participate in the Research: 50 people

f. Place of the Research: Fatih Sultan Mehmet Education and Research Hospital Additional Service Building Üsküdar Diabetes Polyclinic

2. Consent to Participate in the Study:

I have read the information provided above and that should be given to the participant before the research and I fully understand the scope and purpose of the study in which I am asked to participate and the responsibilities that fall upon me as a volunteer. The researcher named below provided a written and verbal explanation about the study, I had the opportunity to ask questions and discuss them, and I received satisfactory answers. The possible risks and benefits of the study were also explained to me verbally. I understand that I can leave this study at any time and without having to give any reason, and that I will not face any negative consequences if I do so.

Under these conditions, I agree to participate in the study of my own free will, without any pressure or coercion.

Participant's (in his/her own handwriting)

Name-Surname:

Signature:

Note: This form is prepared in two copies. One of these copies is given to the volunteer in return for a signature, and the other is kept by the researcher.