Supplementary Material

Gkouskou KK, Grammatikopoulou MG, Lazou E, Sanoudou D, Goulis DG, Eliopoulos AG. Genetically-guided medical nutrition therapy in type 2 diabetes mellitus and prediabetes: a series of n-of-1 superiority trials. Frontiers in Nutrition 2022; 9: 772243.

Detailed methodology

Inclusion and exclusion criteria

Patients were eligible to participate provided that 1) they were adults; 2) with a diagnosis of type 2 diabetes mellitus (T2DM) or prediabetes based on the American Diabetes Association (ADA) standards of medical care (1); 3) willing to adhere to a lifestyle treatment including a personalized diet; 4) without any known malignancies, endocrine or metabolism-related diseases (e.g. renal disease) or genetic disorders affecting metabolism and nutritional status; 5) providing consent to the study participation; and 6) be able to communicate and understand the Greek language. Exclusion criteria involved 1) minors; 2) with comorbidities affecting metabolism; 3) unwilling to consent or adhere to lifestyle therapy; 4) unable to communicate in the Greek language.

Intervention and comparator

The energy requirements of all participants were estimated using the Mifflin-St. Jeor prediction equations (2,3) and the level of physical activity of each patient. The appropriate energy deficit level was calculated based on the estimated energy requirements (EER) and the goal for a 5% body weight (BW) loss, as suggested in the clinical practice guidelines for the treatment of prediabetes and T2DM (4). Two interventions were administered in a cross-over, non-randomized design. The procedure of each trial is detailed in Figure 1. Each patient was initiated with 8 weeks of conventional medical nutrition therapy (MNT) or prediabetes based on the American Diabetes Association (ADA) standards of medical care (1); 3) willing to adhere to a lifestyle treatment including a personalized diet; 4) without any known malignancies, endocrine or metabolism-related diseases (e.g. renal disease) or genetic disorders affecting metabolism and nutritional status; 5) providing consent to the study participation; and 6) be able to communicate and understand the Greek language. Exclusion criteria involved 1) minors; 2) with comorbidities affecting metabolism; 3) unwilling to consent or adhere to lifestyle therapy; 4) unable to communicate in the Greek language.

As for all cases response to the treatment was inadequate according to the predefined NCP outcomes within 8 weeks of the initial intervention, the genetic profile of each patient was used to guide a more personalized lifestyle intervention (diet and exercise), based on the available scientific evidence. No changes were made to the pharmacological treatment of participants. Baseline and end of treatment period measurements were recorded for each participant.

Adherence to the dietary treatment
Adherence to the treatment was assessed qualitatively, through telephone interviews by experienced dietitians (M.G.G, E.L). Previous 24h diet recalls were collected through these interviews regarding the diet of the participants. This is a retrospective short-term method for assessing dietary and food intake often performed through telephone interviews. Adherence to the oral nutrient supplementation (ONS) treatment (whenever applicable) was self-reported and exercise adherence was evaluated in the telephone interviews using specific questions assessing adherence and possible barriers.

**Phenotypic profiling related to diabetes and weight status**

Detailed phenotypic profiling of patients was performed by a multidisciplinary team of registered dietitians (E.L. and M.G.G) and endocrinologists (D.G.G), including a complete assessment of their dietary intake, physical activity levels and diabetes risk factors.

**Anthropometric characteristics**

BW and height of participants were measured during morning hours, by an experienced dietitian, with the use of a digital scale (SECA 813, SECA Group, Hamburg, Germany) and a wall-mounted stadiometer (SECA 216, SECA Group, Hamburg, Germany). At least duplicate measurements were taken. Body mass index (BMI) was calculated for each patient and weight status was defined according to the World Health Organization BMI thresholds (10). Waist circumference was measured at the iliac crest, with patients on a horizontal plane, and central obesity was diagnosed in those with a perimeter exceeding 94 cm (11).

**Assay of blood markers**

Morning fasting blood samples were collected from all patients for blood glucose and a lipidemic profile assay, including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol. Moreover, glycosylated haemoglobin (HbA1c) of all patients was assessed from a capillary fingerstick using a DCA 2000 analyzer (Bayer Diagnostics, Tarrytown, NY, USA). With respect to the availability of micronutrients, ferritin serum levels were assessed according to the International Committee for Standardization in Haematology (12) and 25-hydroxy vitamin D (25(OH)D) concentrations were evaluated via liquid chromatography-tandem mass spectrometry (LC-MS).

**Blood pressure**

Blood pressure (BP) was measured with patients in a seated, relaxed position, in the arm exhibiting the highest BP value, using an oscillometric device (Microlife Exact BP, Microlif AG, Widnau, Switzerland). Duplicate measurements were taken.

**Outcomes of interest**

For all participants, primary outcomes involved change (Δ) in BW (kg) and fasting plasma glucose (FPG) concentrations (mg/dL). Secondary outcomes were systolic and diastolic involved blood pressure (BP) (mm Hg), depressive symptoms (whenever applicable) and possible adverse events.

**Depressive symptoms**
For patients exhibiting depression traits, the Beck Depression Inventory (BDI) was used to assess possible improvement (13). The scale consists of 21 questions each with four possible answers in a Likert scale. Each answer provides a score of 0–3, giving a pooled total score that can range from 0 (complete lack of depression) to 63. The scale has been officially translated and validated in the Greek language and used in a plethora of research.

Genotyping and genetic scores for obesity, habitual coffee consumption, T2DM and elevated fasting plasma glucose

Buccal swabs were stored at 4°C and processed for DNA extraction within 24 hours using the Purelink Genomic DNA extraction mini kit (Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Single nucleotide polymorphism (SNP) status was assessed on an Open Array Quant Studio 12X flex thermocycler (Applied Biosystems, Waltham, USA). The success rate of genotyping was 99%.

Genetic risk score (GRS) determining habitual coffee consumption (that affected recommendations for fat intake in the proposed diet) was calculated on the basis of 8 SNPs (GCKR rs1260326, ABCG2 rs1481012, AHR rs4410790 and rs6968554, Max-like protein X interacting protein-like rs7800944, POR rs17685, BDNF rs6265, CYPIA1 rs2470893, CYPIA2 rs2472297, and EFCAB5 rs9902453), with the use of a previously reported weighting method (14).

The GRS for T2DM (driving recommendations for the prescription of optimal protein intake) was calculated on the basis of the 31 SNPs ADAMTS9 rs4607103, ADCY5 rs11708067, BCL11A rs243021, CDC123-CAMK1D rs12779790, CDKL1 rs10946398, CDKN2A/B rs10811661, CNTD2 rs1552224, CHCHD9 rs13292136, DGKB-TMEM195 rs2191349, GCK rs4607517, GCKR rs780094, HMG2A2 rs1531343, HNF1A rs7957197, IGF2BP2 rs4402960, IRS1 rs2943641, JAZF1 rs864745, KCNQ1 rs231362, KLF14 rs972283, MTNR1B rs10830963, NOTCH2 rs10923931, PPARG rs1801282, PRC1 rs8042680, PROX1 rs340874, RBMS1-ITGB6 rs7593730, SLC30A8 rs13266634, THADA rs7578597, TP53INP1 rs896854, TSPAN8-LGR5 rs7961581, WFS1 rs10010131, ZBED3 rs4457053 and the ZFAND6 rs11634397, with the use of a previously reported weighting method (15).

The GRS for elevated FPG (influencing recommendations for optimal fat intake in precision MNT pattern) was calculated on the basis of the 14 SNPs PROX1 rs340874, G6PC2 rs560887, GCKR rs780094, SLC2A2 rs11920090, ADCY5 rs11708067, DGKB-TMEM195 rs2191349, GCK rs4607517, GLIS3 rs7034200, ADRA2A rs10885122, FADS1 rs174550, CRY2 rs11605924, MADD rs7944584, MTNR1B rs10830963, and C2CD4B rs11071657, as suggested by Wang (16).

The GRS for DM (that was modified by the adoption of a Western dietary pattern) was calculated on the basis of 10 SNPs HHEX rs1111875, CDKAL1 (rs7756992), IGF2BP2 (rs4402960), SLC30A8 rs13266634, WFS1 (rs10010131), CDKN2A/B (rs564398 and rs10811661), TCF7L2 (rs12255372), PPARG (rs1801282), and KCNJ11 (rs5219), as previously suggested (16).

The GRS for obesity was calculated as the weighted sum of risk alleles across 32 SNPs indicating the highest association with BMI and/or WHR (and affected recommendations for protein intake in proposed diet) (17–19).

Summary of the genetic association results that were used for the present study are available on the GIANT consortium website (20) for BMI (‘SNP_gwas_mc_merge_nogc.tbl.uniq.gz’) (21).
Furthermore, independent SNPs that could be used to modify each patient’s dietary, lifestyle (including chrono-nutrition) and physical activity habits, were additionally genotyped. SNPs were studied in relation to the optimal macronutrient intake, fibre intake and diabetes status, including the MTNR1B rs1387153, APOA5 rs662799, CRY1 rs2287161, PCSK7 rs236918, GIPR rs2287019, IRS1 rs2943641 PLIN-1 rs894160, PPM1K rs1440581, DHCR7 rs12785878, CLOCK rs1801260 and rs4580704, LEPR rs3790433 GCKR rs780094, TCF7L2 rs7903146 and rs12255372 (22). Studied SNPs that are relevant to micronutrient supplementation and DM included the MTNR1B rs10830963, MTHFR rs1801133 (23) and the SLC30A8 rs13266634 (24). The IRS1 rs2943641 was additionally assessed, as its effect on insulin is modified by circulating 25(OH)D concentrations (25). SNPs relevant to chrononutrition, lifestyle and physical activity choices included the MTNR1B rs10830963 (26,27), SLC30A8 rs13266634 (28), as well as the PPARG rs1801282 (29).

Data monitoring

Three experts (A.G.E., D.G.G. and D.S.) comprised the data monitoring committee (DMC) according to the SPENT guidelines. The DMC had full access to the anonymously coded dataset of the study.

Statistical analyses

As each patient received a different, personalized treatment, this heterogeneity does not allow for a quantitative synthesis of data. Thus, for each patient, outcomes at the end of each intervention cycle were compared to the start of the intervention period (treatment-by-period interaction) (30). No missing data were apparent in the dataset.

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