Online-Only Supplemental Material

Personalized Postprandial Glucose Response-Targeting Diet versus Mediterranean Diet for Glycemic Control in Prediabetes

The Personalized Nutrition Project for Prediabetes (PNP3)
Randomized Clinical Trial

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Table S1. Related to Methods section. Complete Eligibility Criteria

Inclusion Criteria	Exclusion Criteria*
Prediabetes defined by meeting 2 glycemic criteria: HbA1c 5.7 – 6.5% (39-48 mmol/mol), inclusive Fasting Glucose 100 – 125 mg/dl (5.6-6.9 mmol/L), inclusive	Recent treatment (last 3 months) with antibiotics/antifungal
Age ≥ 18 and ≤ 65	Use of anti-diabetic and/or weight-loss medication
Capable of working with a smartphone application	Chronic use of medications that affect glucose metabolism (e.g. steroids, thiazide diuretics). Other drugs that may have some effect on glucose metabolism but are very commonly used and therefore were not excluded from the study: oral contraceptive, antidepressants (e.g. SSRI's), methylphenidate (Ritalin), cardiovascular medications: beta-blockers, statins, and vasodilators)
	People under another diet regime and/or a dietitian consultation/another study
	Pregnancy, fertility treatments
	Chronic diseases/medical conditions that affect energy/glucose metabolism or that require specific and intensive dietary management (HIV, Cushing syndrome, CKD, acromegaly, hyperthyroidism or imbalanced hypothyroidism, liver cirrhosis, inflammatory bowel diseases, cardiomyopathy, cardiac arrhythmia, COPD, Triglycerides>500 mg/dl). Other medical conditions (comorbidities) which are highly prevalent (e.g. dyslipidemia, hypertension, etc.) or that require minor dietary adaptations (e.g. nephrolithiasis), were not excluded. In these cases, the dietitian provided participants with additional dietary advice on how to adapt the assigned diet and comply with specific dietary requirements for these conditions
	Anemia (Hb<11.7 for women, Hb<13.1 for men)
	Hemoglobinopathies (including sickle cell anemia and thalassemia)
	Cancer and recent anticancer treatment in the last 5 years
	Psychiatric disorders (including antipsychotic medication such as- chlorpromazine, perphenazine, clozapine, iloperidone, olanzapine, paliperidone, quetiapine, risperidone)
	Coagulation disorders
	Bariatric surgery
	Alcohol or substance abuse

*Exclusion criteria were selected to: (1) ensure participants' safety; (2) avoid conditions that would affect the outcomes (i.e. minimize competing risk); (3) make recruitment targets realistic; (4) amplify generalizability of study results; (5) maximize participants' adherence with study procedures. Any other special medical history that arose during the screening procedures were reviewed by the trial physician who judged every case individually for inclusion or exclusion.

Table S2. Related to Results section. Changes in dietary intake during the intervention*

	MED diet	MED diet	PPT diet	PPT diet	P-value†	Mean Between-Group Difference (95% CI)
	n=	Mean (SD)	n=	Mean (SD)		
Total Energy intake, kcal/day						
baseline	111	1873.7 (427.4)	113	1881.0 (431.0)	0.9	-7.2 (105.8 to -120.3)
3m	112	1706.4 (329.4)	113	1693.3 (281.5)	0.749	13.1 (93.6 to -67.5)
6m	95	1666.0 (324.9)	99	1637.6 (283.2)	0.516	28.4 (114.9 to -58.0)
Carbohydrate, g/day						
baseline	111	189.8 (46.3)	113	182.7 (47.6)	0.263	7.0 (19.4 to -5.3)
3m	112	178.2 (35.1)	113	94.4 (22.8)	0.0	83.8 (91.6 to 76.0)
6m	95	176.4 (36.9)	99	83.3 (22.8)	0.0	93.2 (101.9 to 84.4)
Carbohydrate, % of energy						
baseline	111	40.7 (5.3)	113	39.0 (6.2)	0.025	1.7 (3.3 to 0.2)
3m	112	41.9 (4.2)	113	22.4 (4.5)	0.0	19.5 (20.6 to 18.4)
6m	95	42.4 (4.0)	99	20.4 (4.3)	0.0	22.0 (23.2 to 20.9)
Protein, g/day						
baseline	111	84.1 (20.6)	113	85.9 (20.4)	0.505	-1.8 (3.6 to -7.2)
3m	112	85.8 (20.7)	113	91.8 (19.5)	0.026	-6.0 (-0.7 to -11.3)
6m	95	83.3 (20.5)	99	88.4 (19.2)	0.074	-5.1 (0.5 to -10.8)
Protein, % of energy						
baseline	111	18.1 (2.6)	113	18.4 (2.9)	0.295	-0.4 (0.3 to -1.1)
3m	112	20.1 (2.6)	113	21.7 (3.2)	0.0	-1.6 (-0.9 to -2.4)
6m	95	20.0 (3.0)	99	21.7 (3.5)	0.001	-1.7 (-0.7 to -2.6)
Total fat, g/day						
baseline	111	81.9 (22.2)	113	84.9 (25.2)	0.335	-3.1 (3.2 to -9.3)
3m	112	66.6 (15.6)	113	101.1 (20.3)	0.0	-34.4 (-29.7 to -39.2)
6m	95	64.5 (14.7)	99	101.6 (20.3)	0.0	-37.1 (-32.1 to -42.1)
Total fat, % of energy						
baseline	111	39.1 (3.9)	113	40.4 (5.4)	0.037	-1.3 (-0.1 to -2.6)
3m	112	35.1 (4.0)	113	53.6 (4.8)	0.0	-18.5 (-17.4 to -19.7)
6m	95	34.8 (3.7)	99	55.8 (4.8)	0.0	-21.0 (-19.8 to -22.2)
Saturated Fat, g/day						
baseline	111	23.3 (7.3)	113	25.2 (8.9)	0.08	-1.9 (0.2 to -4.0)
3m	112	17.6 (4.5)	113	28.3 (7.5)	0.0	-10.7 (-9.1 to -12.3)
6m	95	17.3 (4.4)	99	28.8 (7.5)	0.0	-11.5 (-9.7 to -13.2)
Saturated Fat, % of energy						
baseline	111	11.1 (2.1)	113	12.0 (2.5)	0.007	-0.8 (-0.2 to -1.4)
3m	112	9.3 (1.5)	113	15.0 (2.7)	0.0	-5.7 (-5.2 to -6.3)

6m	95	9.3 (1.5)	99	15.7 (2.7)	0.0	-6.4 (-5.8 to -7.0)
Fiber, g/day						
baseline	111	22.5 (8.1)	113	20.9 (6.6)	0.109	1.6 (3.5 to -0.4)
3m	112	26.9 (7.9)	113	16.3 (4.6)	0.0	10.5 (12.2 to 8.8)
6m	95	26.4 (8.7)	99	15.6 (5.6)	0.0	10.8 (12.9 to 8.7)
Fiber, g/1000 kcal						
baseline	111	12.2 (3.9)	113	11.4 (3.8)	0.163	0.7 (1.7 to -0.3)
3m	112	15.8 (3.9)	113	9.7 (2.4)	0.0	6.1 (7.0 to 5.3)
6m	95	15.8 (4.0)	99	9.6 (3.3)	0.0	6.2 (7.2 to 5.2)

*Dietary data was calculated from self-reported intake that participants logged on a designated smartphone application throughout the trial. Baseline intake was calculated from logging data during the run-in period (only participants with at least 2 days of >600kcal were included); 3 months (3m) intake was calculated from logging data during the first 3 months of intervention (only participants with at least 5 days of >600kcal were included); 6 months (6m) intake was calculated from logging data during the last 3 months of intervention (only participants with at least 5 days of >600kcal were included). Also, only days with carbohydrates content between 20 grams to 400 grams were included to avoid incomplete logs or logs with errors. The magnitude of decrease in energy intake was similar in the diet groups. During the intervention, the PPT diet group consumed lower amounts of dietary carbohydrates and fiber, and higher amounts of fat and protein, as compared to the MED diet group.

†P-values for between-groups differences were calculated using t-test.

Figure S1. Related to Methods section. Dietary Recommendations Construction Process

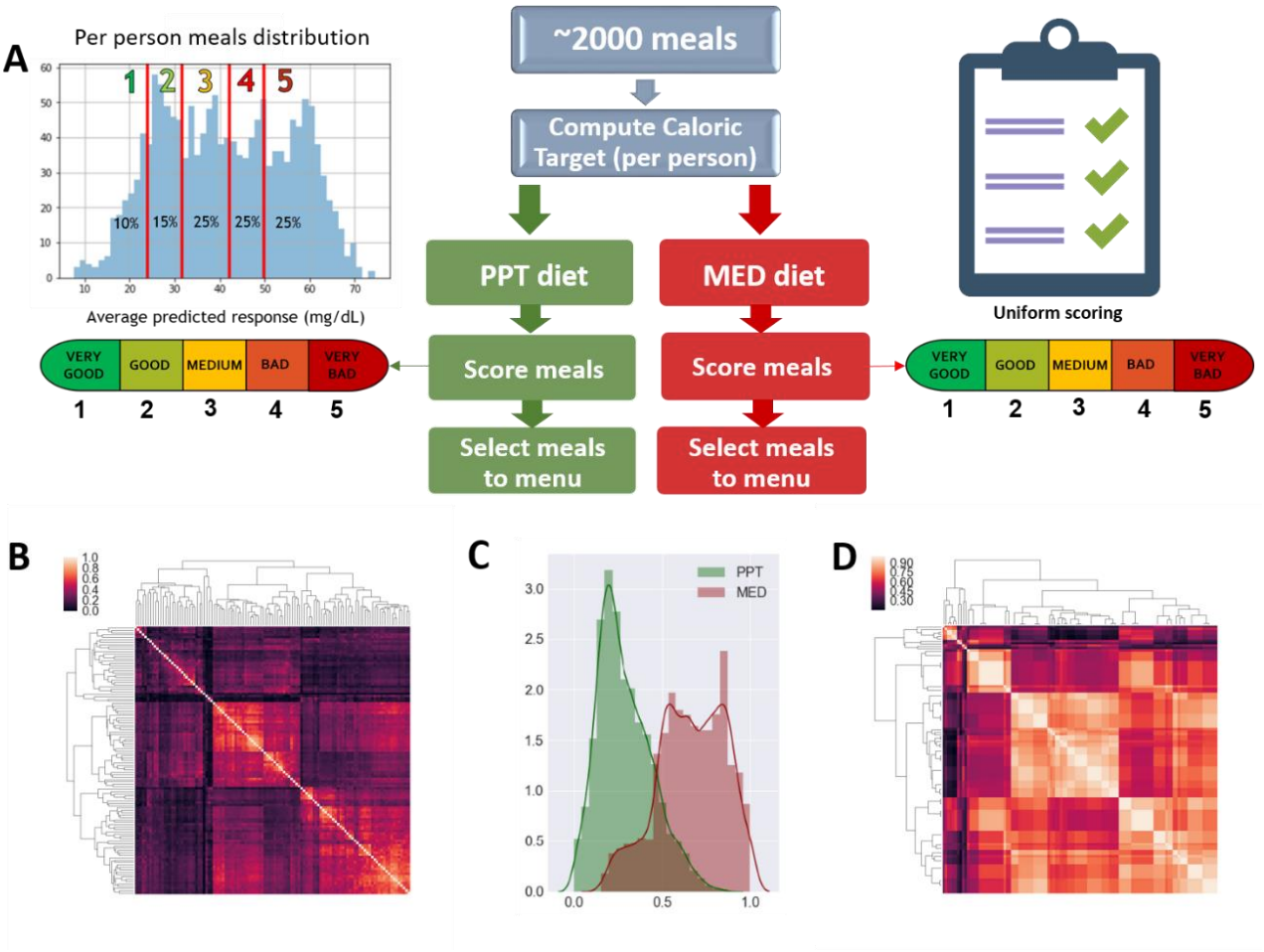

Figure S1. Dietary Recommendations Construction Process. (A) Illustration of dietary recommendations construction process in MED diet (right) and PPT diet (left). (B) Pairwise similarity heat map for PPT diet indicating that the percent of recommended meals each pair of participants share in common in their menu was low, hence more personalized, in this diet group. (C) Distribution of pairwise similarities in menus in the two arms. (D) Pairwise similarity heat map for MED diet indicating that the percent of recommended meals each pair of participants share in common in their menu was high in this diet group.

Menu construction process

The menu construction process is illustrated in **Figure S1**. Menus were based on a meal bank that we generated for this study, with over 2,000 meals representative of the Israeli typical diet, with a variety of different food combinations. Meals size were determined for each participant for every meal type (breakfast, lunch, dinner, and snack) based on his calculated daily caloric target. All meals were scored for every participant using a score scale of 1-5 (best to worst), based on the scoring principles of the diet group they were assigned to, as described hereafter. Meals with scores 1 and 2 were selected to menus, with attention to personal dietary preferences as reported by participants on a food preferences questionnaire.

Caloric target

In order to provide participants with diets that support their energetic needs (without aiming at weight loss by caloric restriction) we computed a daily caloric target for each participant as average between:

- EER (Estimated Energy Requirements)-calculated with Mifflin equation[1], a predictive equation for Resting Energy Expenditure (REE), multiplied by Physical Activity (PA) factor based on the level of PA that the person does on a regular basis.
- BMR (Basic Metabolic Rate) value obtained from Tanita device (BC-418MA), divided by 0.7 (as BMR accounts for ~65-70% of TEE)
- Average daily caloric intake obtained from participant's log in the app during the profiling stage.

Meal scoring in MED diet

Meal scoring for the MED diet was based on the principles of a Mediterranean-style standard-of-care diet as advised in clinical practice. We used the help of 4 outside independent certified dietitians who scored the meals in our meal bank as recommended/non recommended based on their food content. We then used these binary scores to apply scores 1 to 5 to all meals, depending on how many dietitians marked the meal as recommended or non-recommended (**Figure S1A**).

Meal scoring in PPT diet

We developed a scoring system that is size-independent to avoid the effect of calorie content of a meal on prediction and compare between meals in an unbiased manner. For that end, for each person meals were theoretically scaled to fixed calorie contents (20%, 25% and 30% of daily caloric target for main meals; 10% and 15% of daily caloric target for snacks). We then computed the predicted glycemic response for each meal in all caloric targets and used the distribution of meals by their average predicted response (separate distributions for main meals and snacks). The distribution of meals/snacks was divided into 5 percentiles of 10%, 15%, 25%, 25%, 25% and scores of 1 to 5 (best to worst) were assigned to meals respectively (**Figure S1A**). This scoring system was also used to score any other desired food or meal logged on the app by participants in the PPT diet group, using an interactive 'traffic light' feature that we developed for that purpose, which provided to participants real-time feedback on the meals they log (**Figure S2E**).

Algorithm for PPGR prediction

Algorithm for personalized prediction of postprandial glucose response (PPGR) originated from a previous work in our lab[2] and was adjusted for the usage in a clinical setting. Among the features used to predict PPGR to meals were anthropometrics, blood tests (FPG, HbA1c% and Hemoglobin), lifestyle features derived from questionnaires, and microbiome features: abundances of species estimated by MetaPhlAn2 and functional modules estimated with mapping to KEGG database. Additionally, macro- and micronutrient composition of the meal was used and features extracted from person's CGM (percentiles of blood glucose). Since no events around the meal were used for prediction, trained predictor could predict response for any profiled participant to any given meal. We used stochastic gradient boosting regression, such that 80% of the samples and 40% of the features were randomly sampled for each estimator. The depth of the tree at each estimator was not limited, but leaves were restricted to have at least 60 instances (meals). We used 5000 estimators with a learning rate of 0.005. Xgboost library v 0.6 was used.

Monitoring adherence by feedback reports provided to participants

In order to achieve high-quality data and good adherence to dietary recommendations by the study participants, we developed semi-automatic feedback reports that were sent to participants every two weeks in order to motivate them and convey to them their compliance to the diet principles in terms of meals composition and calorie consumption in the past two weeks, based on their logging in the app. These feedback reports included composite grades on a scale of 0-100 (from worse to best) for diet composition and calorie intake separately, for the entire two-week period. The scores were calculated as follows:

- PPT diet grade - indicates how well the participant stick to predictor-based meal scores. Each meal score was assigned with a grade as follows: meal score 1=grade 100; meal score 2=80; meal score 3=50; meal score 4=25; meal score 5=0. The grades are averaged calorie-wise (with food energy trimmed to be within (100,500) kcal interval)- $\sum \text{kcal}_{(i)} \cdot \text{grade}_{(i)}$.
  For example, if a person ate three meals: 600 kcal of meal score 2, 1000 kcal of meal score 5 and 80 kcal of meal score 1, he will receive a feedback grade of: (500*80+500*0+100*100) / (500+500+100) = 45.
  If too few (100 by default) calories are logged (overall), we did not compute a score.
- MED diet grade – indicates how well the participant stick to control diet principles. The grade was composed of four elements:
  - Carbs (% of calories): if above 45%, the score is 100; below 25% -- 0; in between -- linear interpolation
  - Fat (% of calories): if below 35%, the score is 100; above 50% -- 0; in between -- linear interpolation
  - Saturated fat (% of calories): if below 10%, the score is 100; above 20% -- 0; in between -- linear interpolation
  - Fibers (grams per 1000 kcal) : if above 15, the score is 100; at 0 -- 0; in between -- linear interpolation

  Then a composite grade was calculated, using the weighted mean score: the basic scores are averaged with weights 2, 2, 1, 1 correspondingly, to enhance the influence of fat and carbs basic scores.
  * If too few (100 by default) calories are logged (overall), we did not compute the score
- Calorie score (both arms) - indicates how well the participant stick to caloric target
  - When caloric intake deviates within 15% of caloric target (CT) → score 100
  - When caloric intake deviation exceeds 60% of CT → score 0
  - When caloric intake deviation is between 15% to 60% → linear penalty on score depending on the deviation.

In addition to grades, feedback reports also included a list of 'top 10' meals and 'worst 10' meals (by meal score) to highlight the best and worst meals consumed on that time period (as logged by the participant), and emphasize which meals should be avoided and which should be adopted to their normal eating routine. The best and worst meals lists were generated systematically and reviewed by the escorting dietitian of each participant, such that manual editing of these lists could be done according to nutritional considerations and dietitian judgement. For MED diet reports this was specifically important as we did not have a full systematic capability to judge/score any given meal logged by the users since the MED diet principles are referring to whole diet composition and foods and not to meal composition specifically.

Figure S2. Related to Methods and Results sections. Monitoring Diet Adherence

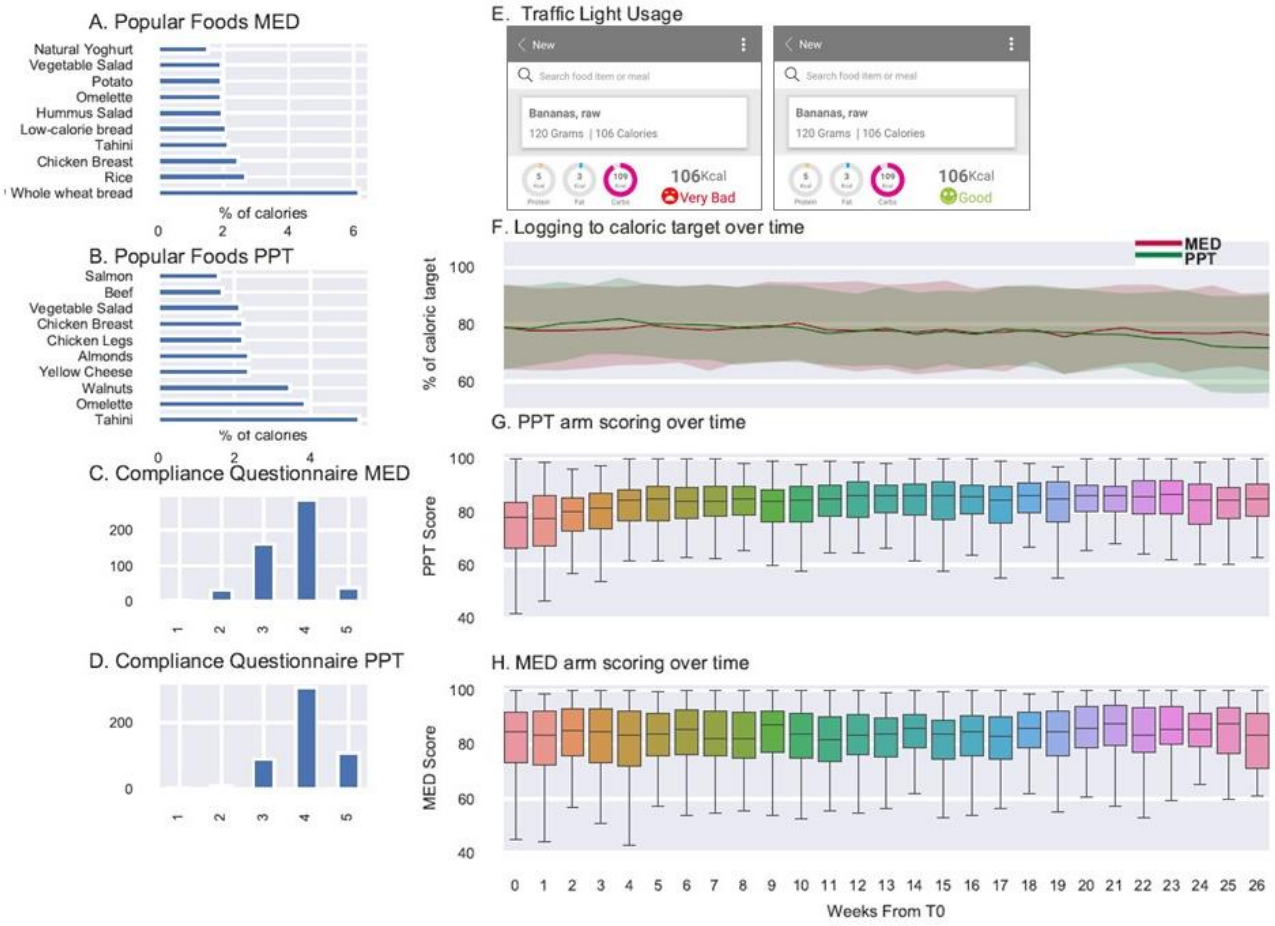

Figure S2. Monitoring Diet Adherence. (A,B) Top 10 popular foods logged by MED and PPT diet participants, respectively. (C,D) Distributions of responses in the MED and PPT diet groups, respectively, to the question in follow up questionnaire inquiring about level of adherence to the dietary recommendations, on a scale of 1 to 5 (low to high). (E) Example of a 'traffic light' output on the app for two different participants for the same food (banana). (F) Mean amount of calories logged by MED participants (red) and PPT participants (green) as percent from caloric target, throughout the intervention period (26 weeks). (G,H) Box plots of mean diet score in PPT diet group and MED diet group, respectively, throughout the intervention period (26 weeks). Vertical bars indicate standard errors.

Figure S3. Related to Table S2. Effect of dietary carbohydrates on glycemic control

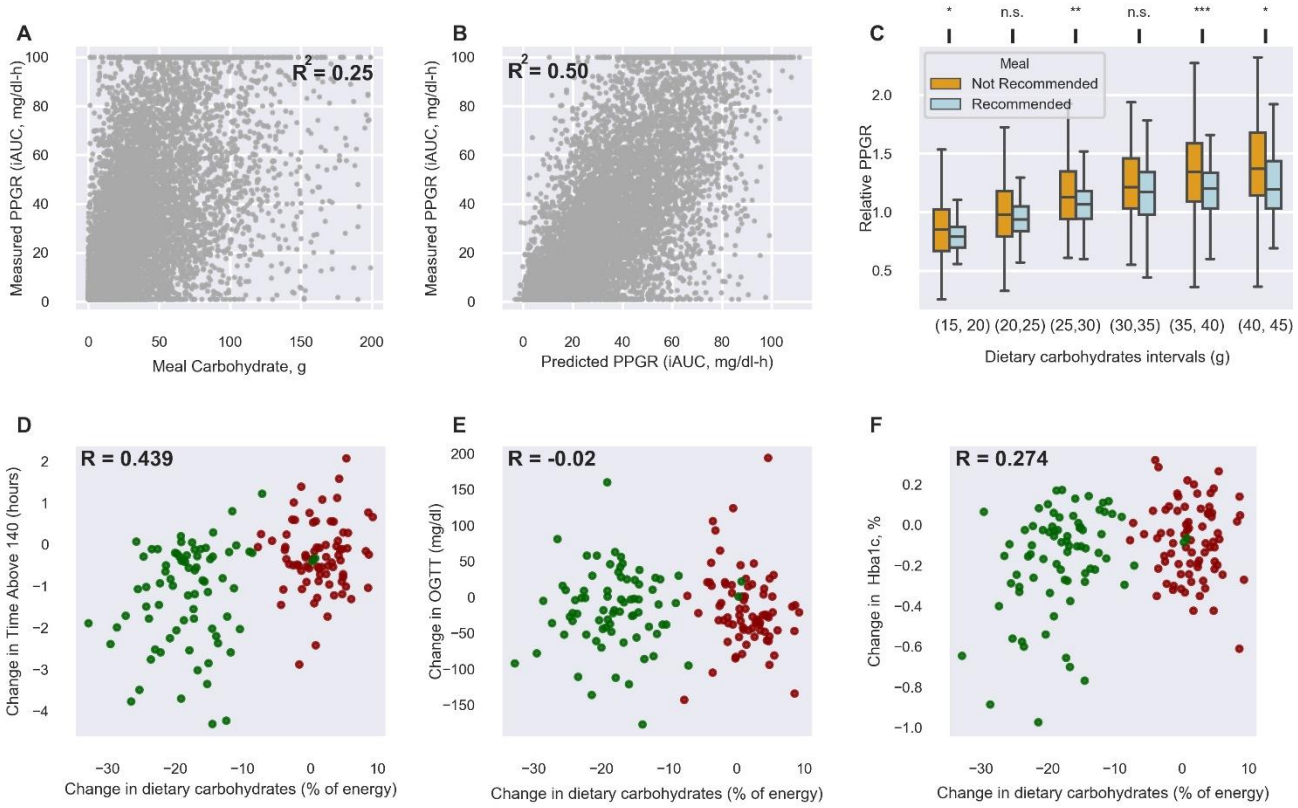

Figure S3. Effect of dietary carbohydrates on glycemic control. (A-B) PPGR predictions. Dots represent predicted (x axis) and CGM-measured PPGR (y axis) for meals reported by participants in the PPT diet group during the run-in period, in a model based only on the meal's carbohydrate content (A) or our algorithm (B). The coefficient of determination, (R^2, computed from Pearson correlation) is indicated. (C) Box plot of relative CGM-measured PPGRs per person by meal's carbohydrate content, for recommended meals (blue, scores 1-2) and not recommended meals (orange, scores 3-5) based on our algorithm and scoring system. Asterisks indicate significant between-groups difference. * p-value<0.05; ** p-value<0.01; *** p-value<0.001; n.s, not significant. (D) Pearson correlation of 'time above 140' change and dietary carbohydrate change. Dots represent participants from MED diet group (red) and PPT diet group (green). (E) As in D but for OGTT outcome. (F) As in D but for HbA1c outcome.

Figure S4. Related to Figure 2A. Sensitivity Analysis for Primary Outcomes

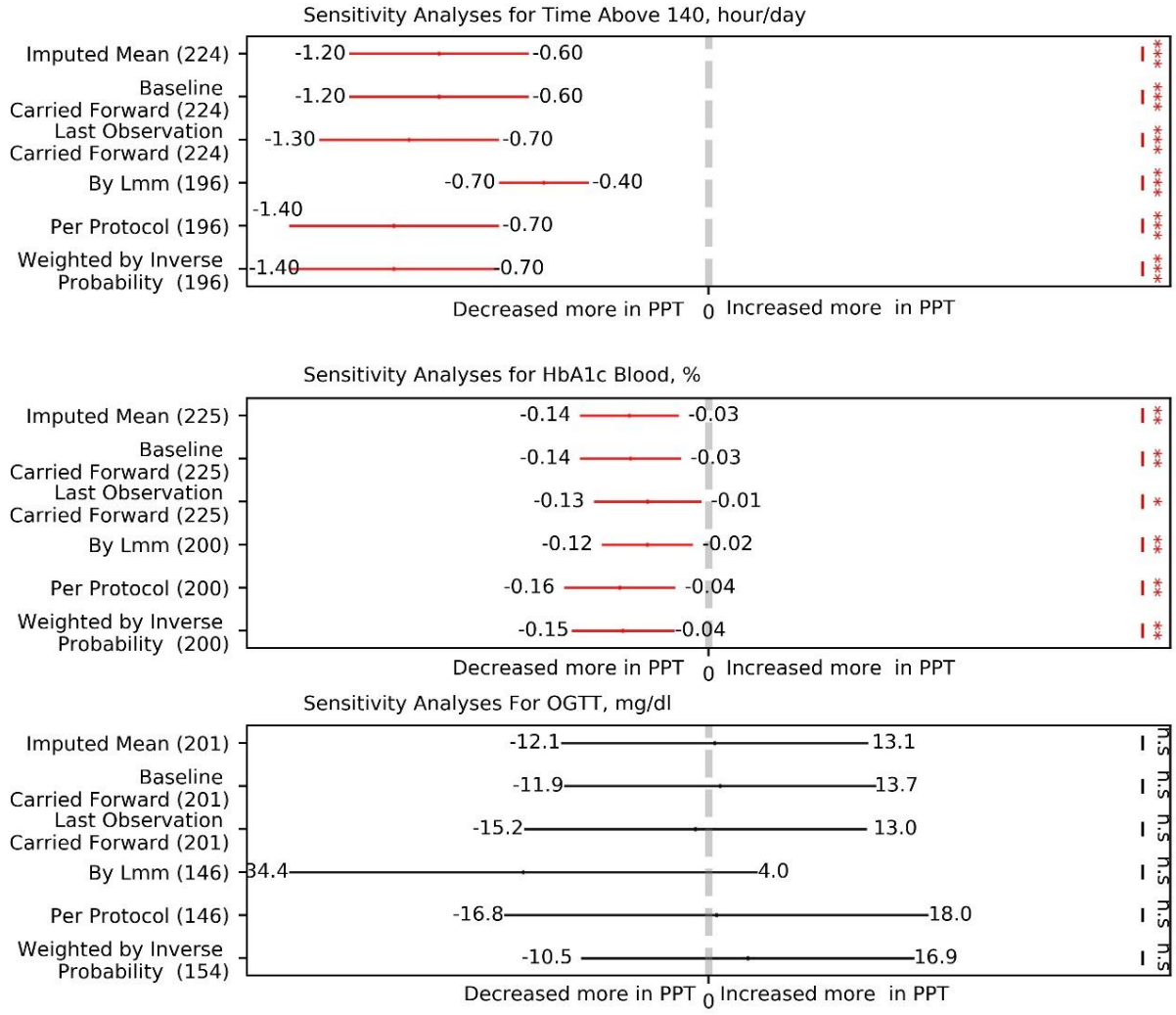

Figure S4. Sensitivity Analysis for Primary Outcomes. Sensitivity analysis for 'time above 140', HbA1c% and OGTT, in top, middle and bottom panels, respectively. The 95% confidence interval for the change difference between treatments in primary outcomes, using different imputation methods. Values in parentheses indicate the number of participants which their data was used in each imputation method. Asterisks indicate significant between-groups difference. * p-value<0.05; ** p-value<0.01; *** p-value<0.001. Lmm, linear mixed models.

Figure S5. Related to Figure 2A. Subgroup Analysis for Primary Outcomes

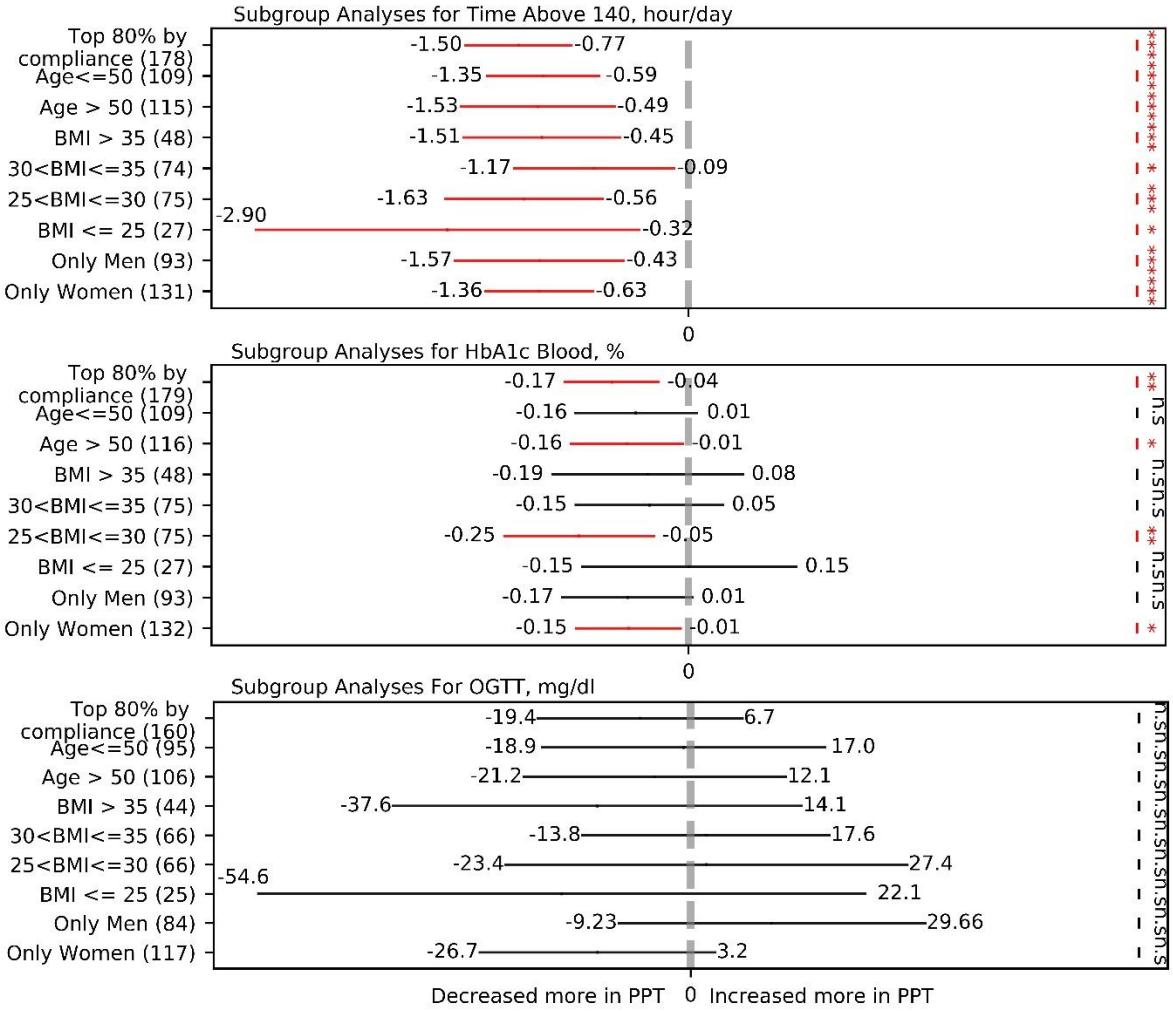

Figure S5. Subgroup Analyses for Primary Outcomes. Subgroup analyses for 'time above 140', HbA1c% and OGTT, in top, middle and bottom panels, respectively. The 95% confidence interval for the change difference between treatments in primary outcomes, among specific subgroups, was done using t-test. Values in parentheses indicate the number of participants which their data was used in each subgroup. Asterisks indicate significant between-groups difference. * p-value<0.05; ** p-value<0.01; *** p-value<0.001; n.s, not significant.

Figure S6. Related to Results section. Changes in outcomes during intervention and post-intervention follow up

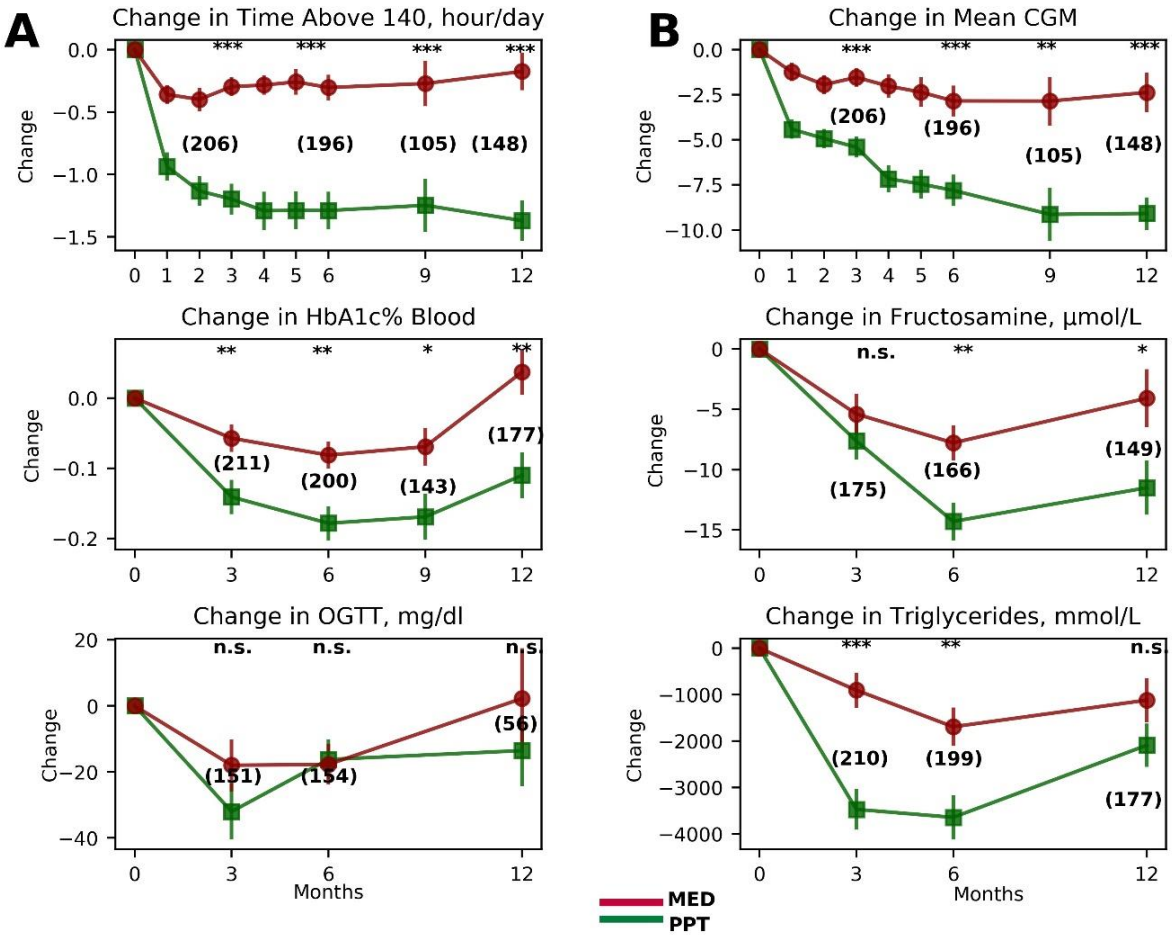

Figure S6. Changes in outcomes during intervention and post-intervention follow up. (A) Changes in primary outcomes over time. 'Per-protocol' analysis of the mean change in primary outcomes from intervention start, in the MED diet (red) and PPT diet (green). To statistically evaluate the changes in outcomes over time, the repeated measures ANOVA test was used and the difference between groups at each time point was assessed by t-test. The p-values for the interaction between diet group and time are <0.001 for 'time above 140' and HbA1c, and 0.8 for OGTT. (B) Selected secondary outcomes. 'Per-protocol' analysis of the mean change in three selected secondary outcomes from intervention start, in the MED diet (red) and PPT diet (green). The p-values for the interaction between diet group and time are <0.001 for mean CGM glucose and triglycerides, and 0.007 for fructosamine. Values in parentheses indicate the number of participants that contributed data at every time point. Asterisks indicate significant difference between the groups, at each time point; * p-value<0.05; ** p-value<0.01; *** p-value<0.001; n.s, not significant.

Figure S7. Related to Figure 2. Paired Confidence Intervals for Change in Outcomes

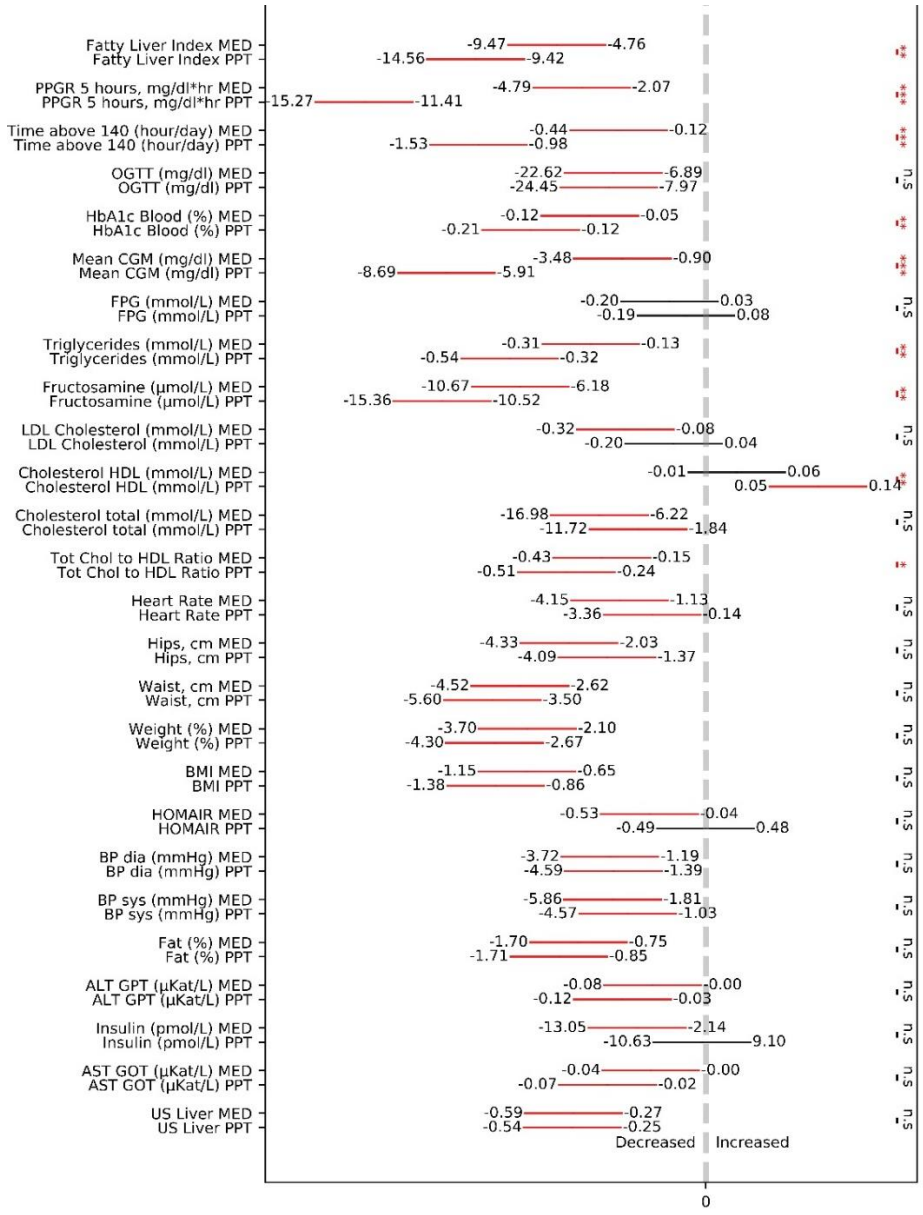

Figure S7. Paired Confidence Intervals for Change in Outcomes. The 95% confidence interval (CI) for change in primary and secondary outcomes on both diet groups, was assessed using t-test. Red lines indicate significant ($p<0.05$) change compared to baseline in each treatment. Black lines indicate non-significant change. Asterisks indicate significant between-group difference. * p-value<0.05; ** p-value<0.01; *** p-value<0.001; n.s, not significant. BMI, Body Mass Index. HOMA-IR, homeostasis model assessment of insulin resistance. To convert CGM glucose values from conventional units (mg/dl) to SI units (mmol/L) multiply by 0.05551. To convert FPG values from SI units (mmol/L) to conventional units (mg/dl) divide by 0.05551. To convert HDL, LDL and total cholesterol values from SI units (mmol/L) to conventional units (mg/dl) divide by 0.02586. To convert triglycerides values from SI units (mmol/L) to conventional units (mg/dl) divide by 0.01129. To convert insulin values from SI units (pmol/L) to conventional units (µU/ml) divide by 6.945. To convert liver enzymes ALT and AST from SI units (µKat/L) to conventional units (U/L) divide by 0.017.

Figure S8. Related to Figure 3. Changes in additional secondary outcomes during the intervention

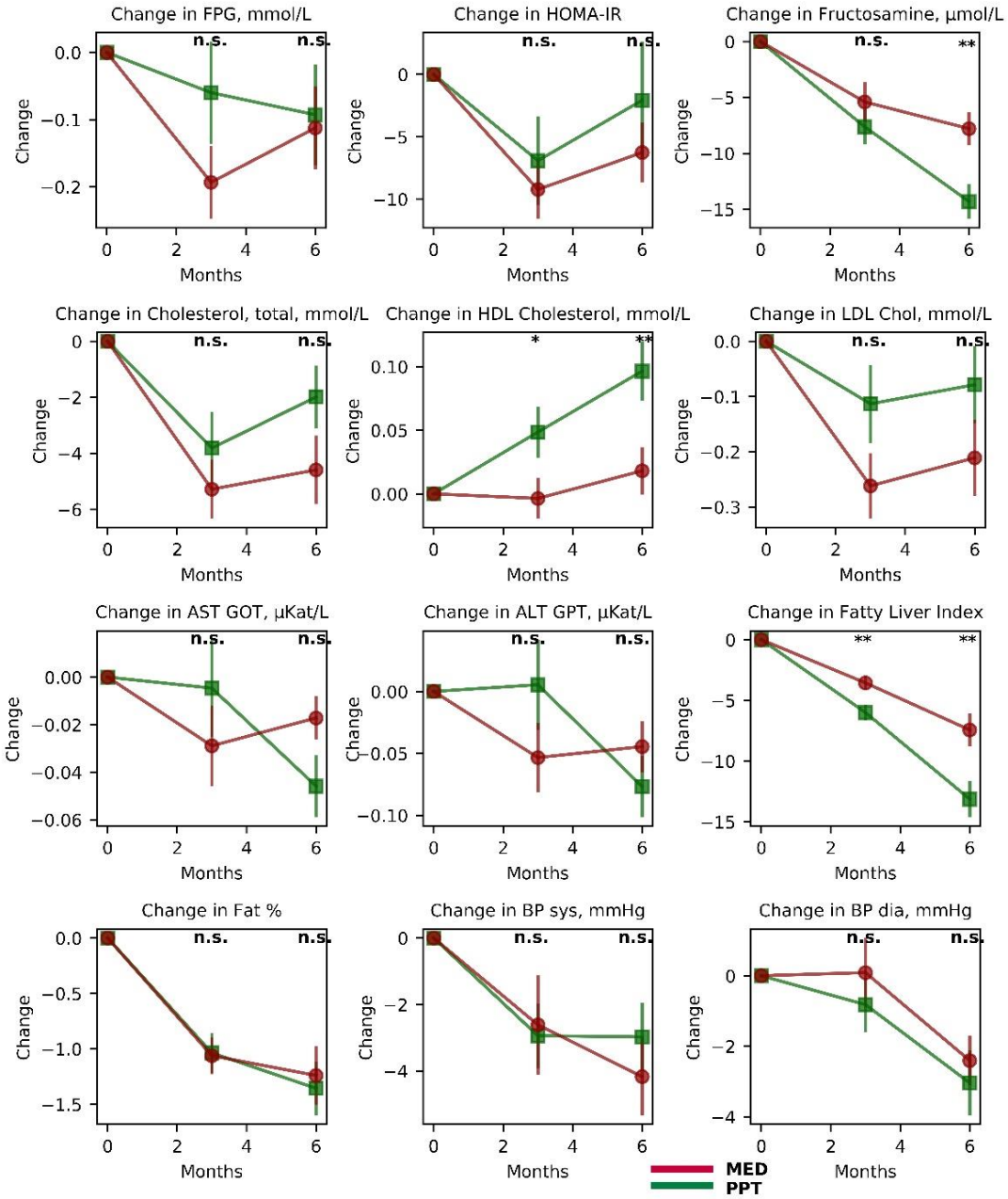

Figure S8. Changes in additional secondary outcomes during the intervention. Changes in secondary outcomes over time, in the MED diet (red) and PPT diet (green). Analysis was done based on intention-to-treat principle. To statistically evaluate the changes in outcomes over time, the repeated measures ANOVA test was used and the difference between groups at each time point was assessed by t-test for all outcomes except HOMA-IR, for which a Mann-Whitney non-parametric test was used. Asterisks indicate significant difference between the groups at each time point. * p-value<0.05; ** p-value<0.01; *** p-value<0.001; n.s, not significant. To convert FPG values from SI units (mmol/L) to conventional units (mg/dl) divide by 0.05551. To convert HDL, LDL and total cholesterol values from SI units (mmol/L) to conventional units (mg/dl) divide by 0.02586. To convert liver enzymes ALT and AST from SI units (µKat/L) to conventional units (U/L) divide by 0.017.

References:

1. Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. *Am J Clin Nutr*. 1990;51(2):241-247. doi:10.1093/ajcn/51.2.241

2. Zeevi D, Korem T, Zmora N, et al. Personalized nutrition by prediction of glycemic responses. *Cell*. 2015;163(5):1079-1094. doi:10.1016/j.cell.2015.11.001