An Isoenergetic Multifactorial Diet Reduces Pancreatic Fat and Increases Postprandial Insulin Response in Patients With Type 2 Diabetes: A Randomized Controlled Trial

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OBJECTIVE

To compare the effect of an isocaloric multifactorial diet with a diet rich in monounsaturated fatty acids (MUFA) and similar macronutrient composition on pancreatic fat (PF) and postprandial insulin response in type 2 diabetes (T2D).

RESEARCH DESIGN AND METHODS

According to a randomized controlled parallel-group design, 39 individuals with T2D, 35–75 years old, in satisfactory blood glucose control, were assigned to an 8 week isocaloric intervention with a multifactorial diet rich in MUFA, polyunsaturated fatty acids, fiber, polyphenols, and vitamins ($n = 18$) or a MUFA-rich diet ($n = 21$). Before/after the intervention, PF content was measured by the proton-density fat fraction using a three-dimensional mDIXON MRI sequence, and plasma insulin and glucose concentrations were measured over a 4 h test meal with a similar composition as the assigned diet.

RESULTS

After 8 weeks, PF significantly decreased after the multifactorial diet (from 15.7 ± 6.5% to 14.1 ± 6.3%; $P = 0.024$), while it did not change after the MUFA diet (from 17.1 ± 10.1% to 18.6 ± 10.6%; $P = 0.139$) with a significant difference between diets ($P = 0.014$). Postprandial glucose response was similar in the two groups. Early postprandial insulin response (incremental postprandial areas under the curve [$iAUC_{0–120}$]) significantly increased with the multifactorial diet (from 36,340 ± 34,954 to 44,138 ± 31,878 pmol/L/min; $P = 0.037$), while it did not change significantly in the MUFA diet (from 31,754 ± 18,446 to 26,976 ± 12,265 pmol/L/min; $P = 0.178$), with a significant difference between diets ($P = 0.023$). Changes in PF inversely correlated with changes in early postprandial insulin response ($r = −0.383$; $P = 0.023$).

CONCLUSIONS

In patients with T2D, an isocaloric multifactorial diet, including several beneficial dietary components, markedly reduced PF. This reduction was associated with an improved postprandial insulin response.

Ectopic fat is defined as the deposition of triglycerides within cells of nonadipose sites (1), such as the liver, skeletal muscle, heart, kidney, and pancreas, and is related to the impaired fat storage capacity of the adipose tissue (2). Ectopic fat

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deposition in different tissues and organs is one of the key pathophysiological mechanisms of the most common cardiometabolic diseases worldwide, representing an impacting social health concern (3). While great attention has been paid to liver and heart fat deposition for a long time, the interest for the fat accumulation in the pancreas [i.e., pancreatic steatosis or nonalcoholic fatty pancreas disease (4)] has developed only in the last decade. Accumulation of fat in the pancreas is common in the general population (4), and in the long term, it may worsen both exocrine functions, leading to acute and chronic pancreatitis, pancreatic fibrosis, pancreatic cancer (5,6), and endocrine functions, possibly impairing insulin secretion, thus affecting glucose metabolism (6–8). In this respect, some evidence shows that pancreatic fat (PF) is positively associated with β-cell dysfunction and insulin secretion worsening in healthy individuals (9,10) and in patients with prediabetes (11) or type 2 diabetes (T2D) (8,12).

Consequently, any potential approach to reduce PF might beneficially impact insulin secretion, a key factor involved in diabetes pathophysiology (13), preventing the onset of T2D or improving blood glucose control in patients with T2D. Weight loss achieved by different approaches represents the main therapeutical goal to treat obesity and reduce visceral fat (14). However, so far, only very large weight reductions, as achievable with very-low-calorie diets or bariatric surgery, have been able to induce a clinically significant lowering of PF (6,15–20).

Despite the consistent evidence that the larger the body weight loss, the greater the PF reduction, too-radical strategies might be unfeasible on a large scale (21), and the long-term maintenance of weight reduction following very-low restricted-calorie diets represents a major challenge (22). Changes in diet composition, independently of changes in energy intake, are effective in reducing ectopic fat in different body districts (23,24), offering a more feasible and safe alternative treatment to energy restriction.

However, very little is known about the effects of diet composition per se, independently of body weight reduction, on PF. To elucidate this point, in overweight/obese patients with T2D, we evaluated the effects on PF content of an isocaloric multifactorial diet characterized by high contents of polyunsaturated fatty acids (PUFA), especially PUFA n-3, monounsaturated fatty acids (MUFA), fiber, polyphenols, vitamins D, E, and C, compared with an isocaloric diet rich in MUFA and similar for the other macronutrients. A further aim of this study was to evaluate whether the possible reduction in PF content was associated with an improvement in postprandial insulin response.

RESEARCH DESIGN AND METHODS

Participants

This study reports the results of the two intervention diets, primarily evaluated for their effects on liver fat (25), in the study participants for whom PF measurement was available. More details on the study design, characteristics of participants, and dietary interventions have been published elsewhere (25). Briefly, patients with T2D of both sexes, age range 35–75 years, were recruited for this dietary intervention. Other inclusion criteria were: abdominal obesity (waist circumference ≥102 cm for men and ≥88 cm for women), stable blood glucose control (glycated hemoglobin [HbA_{1c}] levels ≤7.5% [58.5 mmol/mol]) with diet alone or diet plus oral glucose-lowering drugs (metformin, repaglinide, dipeptidyl peptidase-4 inhibitors, or sulfonylureas), fasting plasma concentrations of triglyceride ≤3.95 mmol/L, and LDL cholesterol ≤3.36 mmol/L with or without a stable treatment with lipid-lowering drugs.

Individuals were excluded if they were diagnosed with any disease severely affecting health status, unstable body weight (>3 kg changes during the last 6 months), supplementation with vitamins/nutraceuticals/antioxidants, current smoking, unstable food habits, or regular moderate to strenuous physical activity.

The study protocol, performed in accordance with the Declaration of Helsinki for clinical trials, was approved by the Ethics Committee of Federico II University (Naples, Italy) in March 2017. All study participants gave their informed consent for participation. The first patient started the dietary intervention in April 2017. At the time of registration at ClinicalTrials.gov (NCT03380416), 13 patients (~25% of all participants) had completed the trial.

Study Design

The study was conducted according to a randomized, controlled, parallel-group design and consisted of a 3-week run-in period during which participants were stabilized on a dietary pattern reproducing the habitual diet of Italian patients with T2D (26), followed by an 8-week dietary intervention. As already reported (25), after the run-in, participants were randomly assigned to a MUFA diet or a multifactorial diet. The two diets were isoenergetic and with a similar composition in the global content of protein, carbohydrate, and fat; however, they differed for a higher content of fiber, polyphenols, PUFA (especially PUFA n-3), and antioxidants in the multifactorial diet. Alcohol was not allowed during the study. Nutritional differences between the two diets were due to different food and beverage patterns, as illustrated in Supplementary Fig. 1 (25). Compliance with the dietary treatments was evaluated by a 7 day food record filled in by the participants at 4 and 8 weeks.

Experimental Procedures

All measurements were performed at baseline and after 8 weeks of the dietary intervention. Body weight, height, and waist circumference were measured according to standardized procedures. BMI was calculated as weight (in kilograms) divided by the square of height (in meters). At baseline and end of intervention, after 12 h of overnight fast, the participants consumed the same 800-kcal meal with a similar composition as the diet they had been assigned to by the randomization. The meal corresponding to the MUFA diet consisted of rice, tomato sauce, egg, beef cured meat (bresaola) and filet steak, peas, extra virgin olive oil, banana, and water. The meal corresponding to the multifactorial diet consisted of pasta, beans, salmon, arugula, extra virgin olive oil, orange, and decaffeinated green tea. The energy content and nutrient composition of the two meals are reported in Supplementary Table 1. Before and 30, 60, 90, 120, 150, 180, 210, and 240 min after the meal, blood samples were collected for the measurement of insulin and glucose plasma concentrations. Plasma β-hydroxybutyrate and serum triglyceride fatty acid composition were evaluated at fasting.

Blood samples were collected by an antecubital vein, immediately placed on

ice, centrifuged at 4°C, separated, and stored at −80°C until analyses. Radiologists and laboratory staff were masked to the treatment assignment.

PF Content

Each subject underwent an upper-abdominal MRI examination performed on a 3T magnetic resonance (MR) scanner (dStream; Philips Healthcare, Eindhoven, the Netherlands) equipped with the dStream Torso coil, placed on the chest of the patients, and the dStream Posterior coil, allowing for abdominal imaging. A commercially available version of mDIXON sequence package was used to acquire fat and water images of the upper abdomen. The mDIXON technique combines a two-point DIXON method with the implementation of flexible echo times. The following imaging parameters were analyzed: three-dimensional T1-weighted fast field echo sequence, two echoes (echo time 1, 1.2 ms; and echo time 2, 2.3 ms); repetition time, 3.2 ms; flip angle 10°; SENSE with acceleration factor 1.5 in phase-encoding direction, matrix 264 × 218; field of view 393 × 323 × 200 mm^3; and voxel size 0.98 × 0.98 × 2.00 mm^3.

Data for each two-echo mDIXON sequence were reconstructed on the MR system using the available standard single-peak spectral model of fat. Each sequence yielded four images per slice: water only, fat only, in-phase, and out-phase. A voxel-wise proton density fat fraction (PDFF) map was calculated by means of in-house software from the water and fat images automatically computed by the scanner using the following equation: PDFF (%) = $100 \times S_f/(S_f + S_w)$, where S_f and S_w are the pixel signal intensities on the fat and water MR images, respectively (27).

For quantitative assessment of PF content, circular regions of interest covering an area of ∼100 mm^2 were drawn into the pancreatic head (caput), body (corpus), and tail (cauda) in different slices of PDFF maps by using the free tool ITK-snap (http://www.itksnap.org/pmwiki/pmwiki.php). Finally, PDFF values over the selected regions of interest ($PDFF_{head}$, $PDFF_{body}$, and $PDFF_{tail}$) were computed.

Metabolic Parameters

Plasma glucose was assayed by enzymatic colorimetric method (Roche Diagnostics, Milan, Italy, and ABX Diagnostics, Montpellier, France) on an ABX Pentra 400 (HORIBA Medical, Montpellier, France). Plasma insulin concentrations were measured by ELISA (DIAsource ImmunoAssay S.A., Louvain-la-Neuve, Belgium) on a Triturus Analyzer (Diagnostic Grifols S.A., Barcelona, Spain). Plasma β-hydroxybutyrate concentrations were evaluated by an enzymatic end point method (DiaSys Diagnostic Systems, Holzheim, Germany) on an automated photometric analyzer (ABX Pentra 400; HORIBA Medical). Palmitic and linoleic acid proportions in the serum triglyceride fraction were evaluated by gas chromatography, as previously described (28), and the de novo lipogenesis (DNL) index was calculated as the ratio between palmitic and linoleic acid (29). The HOMA2 of insulin resistance (HOMA2-IR) was calculated using the following formula: fasting glucose (mg/dL) × fasting insulin (μU/mL)/405.

Statistical Analysis

The sample size was calculated on the primary outcome of the trial (i.e., reduction in percentage of liver fat content) (25). According to the results of a previous study on the effects of a calorie-restricted dietary intervention on PF (30), a sample size of 18 participants for each group was needed to detect a 10% difference in PF with an 80% power at a 5% significance level, assuming a 15% dropout rate.

Data are expressed as mean ± SD, unless otherwise stated. Within-group differences (8-week vs. baseline values) were evaluated by paired-samples *t* test. Differences between the two dietary groups were evaluated by *t* test for independent samples on the changes (8-week − baseline values).

To untangle the impact of changes in PF content and changes in liver fat content and body weight, between-treatment differences were evaluated by ANCOVA general linear model, taking changes in PF content (8-week − baseline values) as the dependent variable, dietary treatment as fixed factor, and changes in liver fat content and body weight as covariates.

Incremental postprandial areas under the curve (iAUC) were calculated using the trapezoidal rule after subtraction of the values under the baseline. Bivariate associations between the changes of ectopic fat (PF content and liver fat content) and postprandial glucose and insulin responses (iAUC), fasting β-hydroxybutyrate, and DNL index, were assessed by Pearson correlation. Correlations between PF content and liver fat content were also assessed. For all analyses, the level of statistical significance was set at $P < 0.05$. Statistical analysis was performed according to standard methods using the SPSS software 25.0 (SPSS/PC; IBM, Armonk, NY).

RESULTS

Anthropometrics and Metabolic Parameters

Forty-three individuals completed the study, but data on PF content were available for 39 of the participants, who were included in the analysis ($n = 21$ MUFA diet; $n = 18$ multifactorial diet) (Supplementary Fig. 2).

At baseline, participants in the two groups had a similar distribution of sex, age, anthropometrics, and metabolic parameters (Supplementary Table 2). Dietary adherence of the participants was optimal, as demonstrated by the 7 day food records they completed during the trial (25). After 8 weeks of dietary intervention, a statistically significant but very small reduction in body weight (−1.2 kg) was observed after both diets, without differences between groups. HbA_{1c} levels significantly improved after both the multifactorial diet (from 6.5 ± 0.4% [48 ± 4 mmol/mol] to 6.3 ± 0.6% [45 ± 7 mmol/mol]; $P = 0.013$) and the MUFA diet (from 6.5 ± 0.6% [48 ± 7 mmol/mol]) to 6.4 ± 0.7% [46 ± 8 mmol/mol]; $P = 0.012$) with no difference between the groups ($P = 0.763$). No significant differences in fasting plasma glucose, insulin, HOMA2-IR, and β-hydroxybutyrate concentrations between baseline and end of intervention and in their changes from baseline to end of intervention were observed between the two groups (Table 1). Conversely, the DNL index significantly decreased after the multifactorial diet (from 2.2 ± 0.8 to 1.5 ± 0.5; $P \leq 0.0001$) but did not change after the MUFA diet (from 1.9 ± 1.1 to 1.9 ± 0.9; $P = 0.949$), with a significant difference between the two groups for the changes from baseline to end of intervention (Table 1).

PF Content

At baseline, PF content was not different between the two groups. After 8 weeks of dietary intervention, it significantly decreased in the multifactorial diet (from 15.7 ± 6.5% to 14.1 ± 6.3%; $P = 0.024$),

Table 1—Anthropometrics and metabolic parameters of the participants at baseline and after the 8-week intervention

	MUFA diet (n = 21)		Multifactorial diet (n = 18)		
	Baseline	8-week	Baseline	8-week	P value†
Body weight (kg)	84.1 (14.9)	83.1 (14.6)*	84.6 (9.3)	83.3 (9.1)*	0.749
BMI (kg/m^2)	30.8 (3.5)	30.3 (3.4)*	31.8 (3.7)	31.3 (3.7)*	0.985
Waist circumference (cm)	105 (10)	104 (10)	107 (10)	106 (10)	0.525
Plasma glucose (mmol/L)	7.2 (0.9)	7.2 (0.9)	6.8 (0.8)	6.9 (1.1)	0.995
HbA_{1c} (%)	6.5 (0.6)	6.4 (0.7)*	6.5 (0.4)	6.3 (0.6)*	0.450
HbA_{1c} (mmol/mol)	48 (7)	46 (8)*	48 (4)	45 (7)*	0.450
Plasma insulin (pmol/L)	114 (60)	120 (66)	114 (54)	96 (48)	0.118
HOMA2-IR	5.9 (3.0)	6.3 (3.6)	5.8 (2.8)	4.7 (2.4)	0.204
DNL index	1.9 (1.1)	1.9 (0.9)	2.2 (0.8)	1.5 (0.5)**	0.004
β-Hydroxybutyrate (mmol/L)	0.11 (0.08)	0.10 (0.05)	0.11 (0.05)	0.10 (0.04)	0.811

Data are means (SD) unless otherwise indicated. *$P < 0.05$, **$P < 0.0001$ vs. baseline. †Comparison of changes (8-week − baseline values) between groups.

while it did not change in the MUFA diet (from 17.1 ± 10.1% to 18.6 ± 10.6%; $P = 0.139$) (Fig. 1). Changes in PF content from baseline to the end of treatment were significantly different between the multifactorial diet and the MUFA diet both in terms of absolute values (−1.6 ± 2.6% vs. 1.5 ± 4.5%; $P = 0.014$, respectively) or percentages (−8 ± 20% vs. 10 ± 23%; $P = 0.012$, respectively) (Fig. 1). The differences in PF changes between the two diets remained statistically significant after adjusting for changes in liver fat and body weight ($P = 0.035$, ANCOVA general linear model analysis) (Fig. 1).

Postprandial Glucose and Insulin Responses

After 8 weeks of dietary intervention, plasma glucose response to the test meal was similar to baseline in both diet groups either at single time points or as iAUC (Fig. 2*A*).

Postprandial plasma insulin response did not change after the MUFA diet, while it was significantly higher at 30, 60, and 120 min after meal ($P < 0.05$ for all) after the multifactorial diet (Fig. 2*B*). Similarly, the postprandial insulin response evaluated as iAUC up to 240 min after the meal significantly increased in the multifactorial diet (from 54,115 ± 39,829 to 65,484 ± 40,448 pmol/L/min; $P = 0.041$), while it tended to decrease after the MUFA diet (from 65,769 ± 33,329 to 56,254 ± 22,786 pmol/L/min; $P = 0.083$), with a significant difference between the two diet groups ($P = 0.007$). The difference in postprandial insulin response was mainly driven by the difference observed in the first part of the curve. In fact, the insulin iAUC up to 120 min increased significantly after the multifactorial diet (from 36,340 ± 34,954 to 44,138 ± 31,878 pmol/L/min; $P = 0.037$), while it did not change after the MUFA diet (from 31,754 ± 18,446 to 26,976 ± 12,265 pmol/L/min; $P = 0.178$),

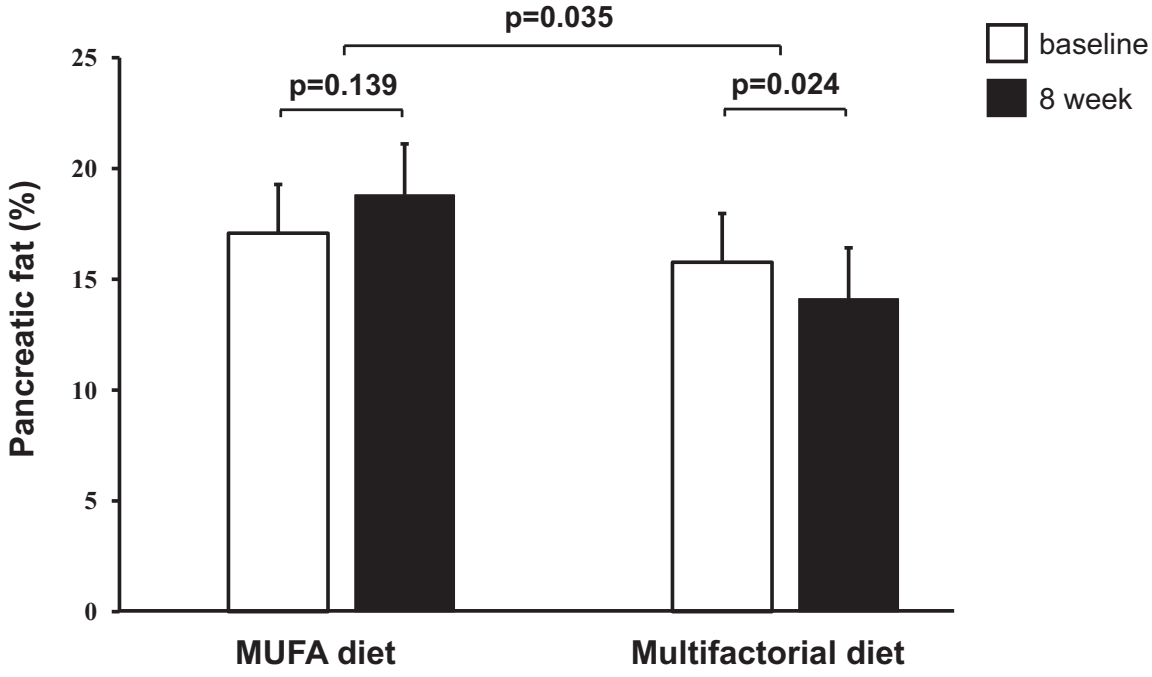

Figure 1—PF accumulation at baseline (white bar) and after the 8-week intervention (black bar) with the MUFA (n = 21) or multifactorial diet (n = 18). Data are mean ± SEM. Within-group comparisons were performed by paired-samples t test; between-treatment differences were evaluated by ANCOVA general linear model taking changes in PF content (8-week − baseline values) as dependent variable, dietary treatment as fixed factor, and changes in liver fat content and body weight as covariates.

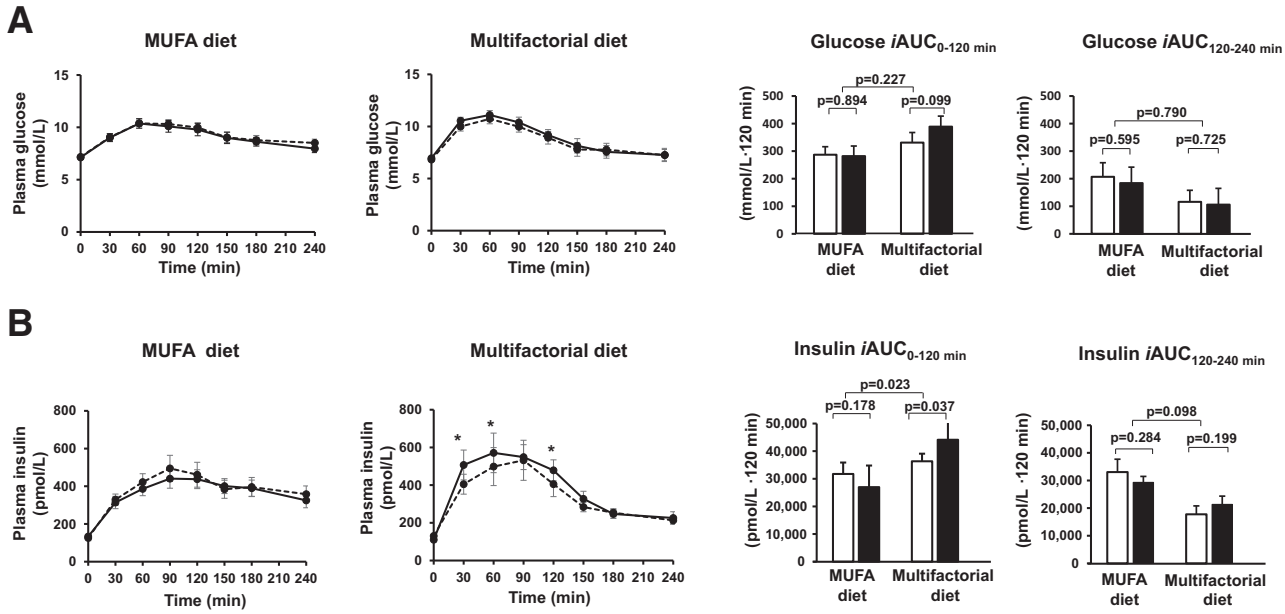

Figure 2—Plasma glucose (*A*) and insulin responses (*B*) to a test meal at baseline (dashed lines and white bars) and after the 8-week intervention (solid lines and black bars) with the MUFA or multifactorial diet. Data are mean ± SEM. Within-group comparisons were performed by paired-samples *t* test; between-group comparisons of diet-induced changes (8-week − baseline values) were performed by *t* test for independent samples. Insulin data were available on 35 participants (n = 18 in MUFA group; n = 17 in multifactorial group).

with a significant difference between the two diet groups ($P = 0.023$) (Fig. 2*B*). Conversely, the 120–240-min postprandial insulin iAUC did not show any difference between the two diet groups (Fig. 2*B*).

Correlation analyses were performed pooling the two groups of participants. Changes in PF content from baseline to the end of intervention were inversely correlated to the changes in the early postprandial insulin response ($iAUC_{0–120}$) ($r = -0.383$; $P = 0.023$) (Fig. 3), while no correlation was observed with changes in the late response expressed as iAUC 120–240 min ($r = -0.189$; $P = 0.276$), DNL index ($r = 0.006$; $P = 0.973$), or β-hydroxybutyrate ($r = 0.239$; $P = 0.154$).

The changes in PF content were not significantly correlated with those in liver fat ($r = 0.163$; $P = 0.323$). The changes in liver fat were not correlated with early postprandial insulin response ($iAUC_{0–120}$) ($r = -0.174$; $P = 0.309$) and fasting β-hydroxybutyrate ($r = 0.101$; $P = 0.550$), while directly correlated with changes in DNL index ($r = 0.436$; $P = 0.007$).

CONCLUSIONS

In this clinical trial, we show that a multifactorial isocaloric diet, naturally rich in different beneficial dietary components, significantly reduced PF by ∼20% in overweight/obese patients with T2D, independently of body weight loss, as compared with a diet with a similar content of protein, carbohydrate, and fat but different for the quality of these nutrients. Importantly, our results confirm and expand the beneficial effects of this diet on ectopic fat accumulation already shown for the liver (25). Moreover, our study demonstrates that the reduction in PF content is associated with a significant increase in insulin response during the early postprandial phase, which is a marker of good functionality of the pancreatic β-cells. This plays an important role in the regulation of glucose homeostasis, as it may contribute to prevent the failure of metabolic control in the long term and, possibly, to delay the onset of T2D in individuals at risk (31). In this respect, a similar reduction of PF was obtained after a substantial weight loss in the DIRECT Study and was paralleled by the remission of diabetes (32).

In view of the increasing scientific and clinical interest on PF, attention on the appropriate treatment of the ectopic fat deposition in this organ is warranted. Weight loss achieved by different approaches might represent the main therapeutical goal to treat PF as well as other ectopic fat (14). Both bariatric surgery and substantial weight loss achieved through a very-low-calorie diet are associated with a statistically significant and clinically relevant decrease in PF. Bariatric surgery, with its subsequent relevant weight loss, reduced PF content in patients with T2D (15,16), in which also an improvement in the first-phase insulin secretion has been reported (15). Similarly, very-low-calorie diets (6,17–20), alone or in combination with physical activity (30,33,34), reduced PF content and improved β-cell function (17,34).

Our study indicates that, beyond reduced caloric intake and weight loss, the composition of the diet might impact on PF content, as also shown for liver fat. Previous studies evaluated the effects on PF of diets with different compositions in individuals of very-low-calorie diets, showing a significant and relevant weight loss. To this regard, a recent trial showed that a carbohydrate-reduced high-protein diet (providing 30% of energy as carbohydrate, 30% as protein, and 40% as fat) reduced the PF content less than a conventional diet in patients with T2D, in contrast to its effects on liver fat (20). This result was obtained in parallel with a significant weight loss (−5.8 kg) that was similar in the test diet and the control diet, but likely contributing to the observed effects. Our study, instead, clearly demonstrates that dietary composition per se is effective in influencing PF content since the weight reduction

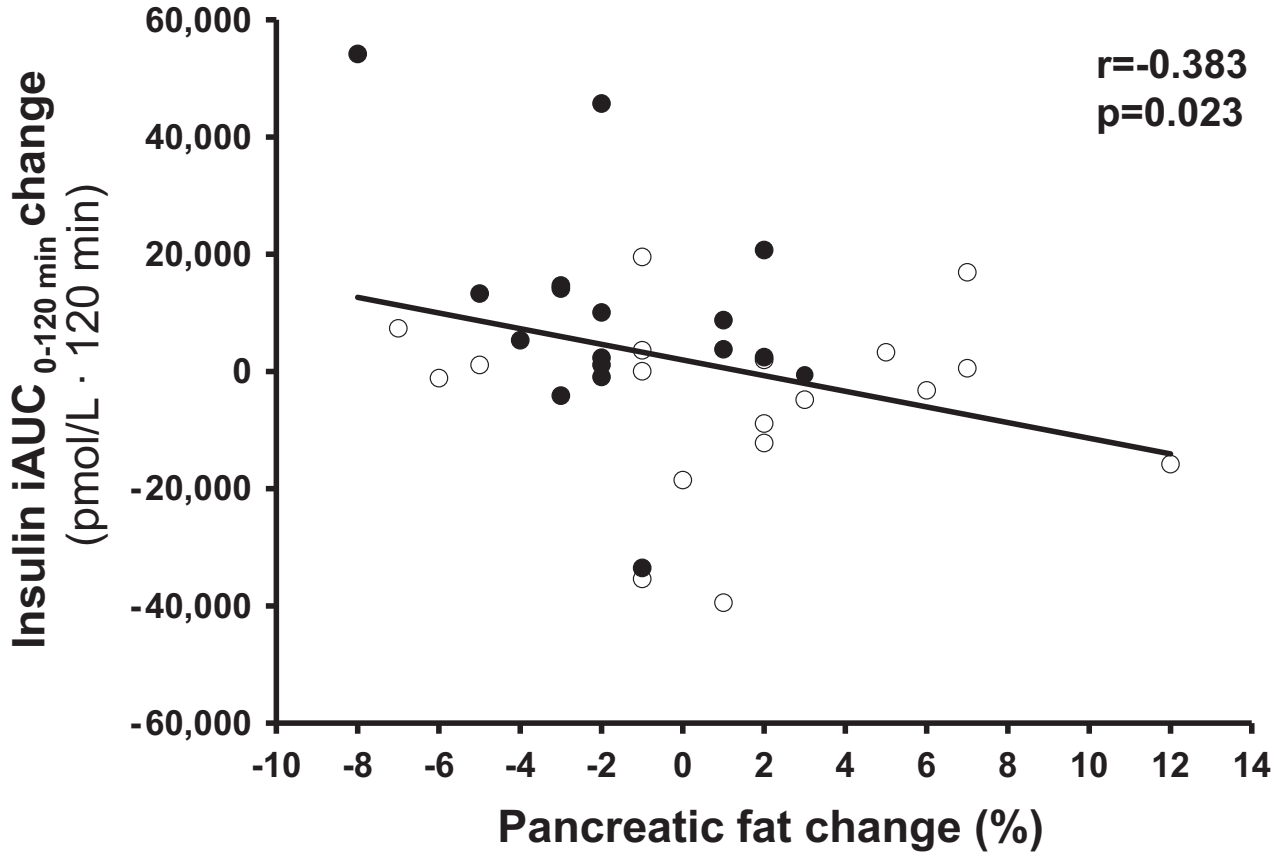

Figure 3—Correlation between changes (8-week − baseline) in PF content and early plasma insulin response ($iAUC_{0–120\ min}$) in the participants (n = 35) in the MUFA (white circles) or multifactorial group (black circles).

observed with both diets was similarly minimal and, therefore, unlikely to have any clinical and metabolic effect. A weight reduction of at least 5–10% of the initial body weight is needed to significantly modify the metabolic status in people with diabetes or abnormal glucose regulation (35). To the best of our knowledge, the only study that evaluated the effects of diet composition per se, independently of changes in body weight, on PF content in T2D showed that a low-carbohydrate high-protein diet reduced PF content more than a conventional diet (36). However, as long-term compliance to low-carbohydrate diets may be poor, the outcomes of this study may have limited applicability in clinical practice. Conversely, our study used a diet that, being multifactorial, did not require any major change in the amounts of the various dietary components. The increased amounts of fiber, polyphenols, and vitamins were achieved by using food items largely present in the habitual diet all over the world and, in particular, in the Mediterranean diet, a dietary pattern not only healthy, but also gastronomically appealing.

The mechanisms by which the composition of our multifactorial diet affected PF content are not clearly understood (37). Although it could be expected that the same mechanisms leading to the accumulation of fat in other districts, namely liver, could also operate in pancreas, the mechanisms for PF accumulation seem to be more complex and partly discordant from those operative for liver fat (4). In our study, through the use of indirect indices, we evaluated two of the mechanisms possibly regulating the amount of PF (i.e., DNL and fat oxidation). DNL decreased significantly with the multifactorial diet, and this change was significantly related to the decrease in liver fat (38). No change was observed for the index of fat oxidation. The lack of correlations between changes in liver fat content and PF, and between changes in PF and DNL, suggests that different mechanisms may explain the dietary effects on hepatic and PF responses and that DNL, relevant for liver fat, may not be involved in PF accumulation.

In consideration of the multiple features of the multifactorial diet, it is likely that its efficacy depends on the synergy of various dietary components. So far, there are no studies investigating the possible mechanisms by which the different dietary components might impact PF; we can speculate that the intake of polyphenols, other antioxidants, fiber, and PUFA could play a more relevant role since they have been shown to influence ectopic fat accumulation in other districts by different mechanisms, including changes in gut microbiota composition, inflammation, and oxidative stress (23,24).

Our multifactorial diet was also able to increase early postprandial insulin response. Interestingly, the reduction of PF and the increase in postprandial insulin response

were inversely correlated, supporting the hypothesis of a causal relationship. The plausible mechanisms by which the reduction of PF might improve postprandial insulin response are still unclear. The reduction in intrapancreatic fat, which was measured in the current study, may have reversed the effects of the excess of adipose tissue in the pancreas. The adipocytes within the pancreas may lead to a release of free fatty acids with apoptosis of β-cells and lowering of insulin secretion (39); moreover, they might favor an inflammatory milieu and promote the release of cytokines, chemokines, and further metabolites that could worsen β-cell function by paracrine pathways (4). Finally, although intrapancreatic adipocytes account for most of PF content (4), the possible detrimental effect on insulin secretion may relate to the accumulation of triglycerides within the β-cell. This might reduce the activity of glucokinase, the key enzyme involved in insulin secretion, as reported in the few studies performed in vitro and in animals (40).

Strengths of our study are the optimal adherence of the participants to the dietary interventions and the randomized and controlled design of sufficiently long duration for the evaluation of the effects of the dietary intervention on the PF content. Of course, the study also presents some limitations. First, the study population only included patients with T2D in optimal blood glucose control, and, therefore, our findings cannot be generalized to patients with a more severe form of the disease or, on the other side, those with metabolic syndrome/prediabetes. Another limitation is that the insulin secretion was not evaluated by gold-standard methods, such as the hyperglycemic clamp or the frequent-sampling intravenous glucose tolerance test evaluated by the minimal model. However, the insulin response to the test meal used in our trial is a more physiological and clinically relevant approach.

Conclusion

In summary, we show for the first time in this study that a multifactorial isocaloric diet, naturally rich in different beneficial dietary components, already shown to be effective in reducing liver fat content, is able per se, independently of changes in body weight, to reduce PF in overweight/obese patients with T2D. The reduction in PF content was associated to a significant increase in early postprandial insulin response, which in the long term might contribute to the preservation of glucose control, thus delaying the secondary failure of the hypoglycemic treatment.

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