Reducing Cholesterol and Fat Intake Improves Glucose Tolerance by Enhancing β Cell Function in Nondiabetic Subjects

Domenico Tricò,[1,2]* Silvia Trifirò,[1]* Alessandro Mengozzi,[1] Cecilia Morgantini,[1] Simona Baldi,[1] Andrea Mari,[3] and Andrea Natali[1]

[1]Department of Clinical and Experimental Medicine, University of Pisa, 56126 Pisa, Italy; [2]Institute of Life Sciences, Sant'Anna School of Advanced Studies, 56127 Pisa, Italy; and [3]Institute of Neuroscience, National Research Council, 35127 Padua, Italy

Context: A diet low in cholesterol and fat is commonly recommended to prevent metabolic and cardiovascular diseases; however, its effect on glucose tolerance is largely unknown.

Objective: We examined whether and by which mechanisms a chronic reduction of cholesterol and fat intake affects glucose tolerance in nondiabetic individuals, independently of weight changes.

Design and Participants: In this crossover, randomized clinical trial, 30 healthy subjects, including 15 with family history of type 2 diabetes (T2D) (T2D offspring), underwent a 75-g oral glucose tolerance test (OGTT) after two 14-day isocaloric high-cholesterol, high-fat (HChF) or low-cholesterol, and low-fat (LChF) diets.

Main Outcome Measures: We evaluated changes in glucose tolerance, β cell function, insulin clearance, and insulin sensitivity by modeling plasma glucose, insulin, and C-peptide levels during the OGTT.

Results: The shift from the HChF to the LChF diet was neutral on body weight but increased glucose tolerance (mean glucose −5%, $P = 0.01$) and three components of β cell function: glucose sensitivity (+17%, $P = 0.01$), insulin secretion at fasting glucose (+20%, $P = 0.02$), and potentiation (+19%, $P = 0.03$). The LChF diet improved insulin sensitivity (+7%, $P = 0.048$) only in T2D offspring, who tended to be more susceptible to the positive effect of the diet on glucose tolerance.

Conclusions: A chronic and isocaloric decrease in dietary cholesterol and fat intake improves glucose tolerance by diffusely ameliorating β cell function in nondiabetic subjects. Individuals genetically predisposed to develop T2D tend to be more susceptible to the positive effect of this dietary intervention on glucose tolerance and insulin sensitivity. (*J Clin Endocrinol Metab* 103: 622–631, 2018)

The growing prevalence of type 2 diabetes (T2D) in recent decades is closely linked to overnutrition and possibly to the relative abundance or deficiency of specific nutrients (1). In this context, large epidemiologic studies (2–10) and experimental evidence (11–18) suggest that excess cholesterol and lipid intake is a key factor in the pathogenesis of T2D (2).

Based also on these observations, a low-cholesterol, low-fat (LChF) diet is commonly prescribed for the prevention of metabolic and cardiovascular diseases. However, experimental data on the effect of physiologic reductions of dietary cholesterol and lipid on glucose tolerance are limited and inconsistent, particularly in healthy subjects (19, 20). Conflicting results of previous

studies might be explained by different investigational diets (*e.g.,* differences in dietary energy contents) and by the interaction between environmental and genetic factors, which are often neglected, because offspring of patients with T2D might be more sensitive to the deleterious effect of lipids (21, 22). Therefore, the effects of an isocaloric and physiologic reduction in dietary cholesterol and lipid intake on glucose tolerance remain largely unknown.

To fill this knowledge void, the current study examined whether and by which mechanisms a short-term manipulation of dietary cholesterol and fat content increases glucose tolerance in nondiabetic individuals with or without a family history of T2D, independently of energy intake and weight gain. In this crossover, randomized clinical trial, we compared the effects of two 14-day isocaloric dietary interventions characterized by either high or low cholesterol (250 to 300 *vs* 100 to 150 mg/day, respectively) and fat intake (40% to 45% *vs* 20% to 25% of daily calories, respectively) in healthy subjects with or without a family history of T2D. Participants underwent a 75-g oral glucose tolerance test (OGTT) at the end of each dietary intervention. The contributions of each main determinant of glucose homeostasis, namely β cell function, insulin sensitivity, and insulin clearance, were estimated by glucose, insulin, and C-peptide modeling throughout the OGTT.

Methods

Subjects

Thirty healthy subjects [men and women, age 20 to 40 years, body mass index (BMI) 20 to 30 kg/m^2] were recruited among students and fellows attending the Department of Clinical and Experimental Medicine at the University of Pisa. All participants had a detailed medical history and a complete physical examination. Subjects with a history of malignancy or hepatic, kidney, gastrointestinal, or metabolic disease, as well as those following unusual diet regimens or taking medications or supplements, were excluded. The study population included 15 subjects with family history of T2D (at least one first-degree relative affected by T2D diagnosed before age 65 years) and 15 subjects without family history of T2D. The study was designed and conducted in accordance with the principles expressed in the Helsinki Declaration of 1975 as revised in 1983. All subjects provided written informed consent before enrollment. The protocol was approved by the local human ethics committee.

Experimental design

In this crossover, randomized clinical trial, participants followed two eucaloric diet regimens: a 14-day high-cholesterol, high-fat diet (HChF) and a 14-day LChF diet. The two diets were separated by a 14-day washout period, and the order was randomized with a computer-generated random number list. Participants were asked to keep their habitual lifestyle and to maintain the same level of physical activity during the two interventions. Weekly visits were performed throughout the study for measurement of body weight and assessment of dietary compliance. At the end of each diet, subjects were admitted to our Clinical Research Unit after an overnight fast (08:00 AM) for measurement of body weight and fat mass and for arterialized blood sampling during an OGTT. For the OGTT, participants ingested 75 g glucose (150 mL of 50% dextrose solution) in 5 minutes and remained in a semiupright position for 180 minutes.

Diet interventions

All participants underwent a 45-minute individual training session at the beginning of each diet treatment. During the training session, subjects received nutritional recommendations and a dietary plan with the food composition of three typical meals (breakfast, lunch, and dinner), a list of cholesterol-rich foods to be favored or avoided, and a table of possible substitutions with variable equicaloric amounts of different foods. Meals and food variants were intended to fulfill the total daily caloric need, which was calculated by adding the basal metabolic rate to the estimated individual caloric expenditure during working and leisure physical activity (23). Dietary interventions differed for cholesterol content (HChF: 250 to 300 mg/d; LChF: 100 to 150 mg/d) and macronutrient composition (HChF: carbohydrate 30% to 35%, fat 40% to 45%, protein 20% to 25%; LChF: carbohydrate 50% to 55%, fat 20% to 25%, protein 20% to 25%). In the HChF diet, subjects were instructed to consume at least six eggs per week and cholesterol-rich food items selected from the list provided. In the LChF diet, participants were asked to avoid eggs and cholesterol-rich food and to replace them with food containing similar amounts of calories. The proposed food substitutions were designed to keep the macronutrient composition within the desired macronutrient ratio. Furthermore, the diets contained similar amounts of fiber (fruits and vegetables). Participants were asked to fill in a complete daily food record that was collected after each dietary regimen for compliance assessment (calculated as percentage of meals in which the dietary recommendations were successfully followed). For each participant, the analysis of at least two random weekday and one random weekend-day food records was performed after each dietary intervention with FoodWorks 7 (Xyris Software, Kenmore Hills, Australia).

Analytical procedures

Blood samples were obtained for blood glucose, insulin, C-peptide, and nonesterified fatty acid (NEFA) assessment 15 minutes before and immediately before glucose ingestion, then 15, 30, 60, 90, 120, 150, and 180 minutes after glucose ingestion. Plasma glucose during the OGTT was measured immediately by the glucose oxidase technique (Beckman Glucose Analyzer II; Beckman Instruments, Fullerton, CA). Blood samples for insulin, C-peptide, complete blood count, and free fatty acids were collected in tubes containing K3-EDTA. Blood samples for lipids, apolipoprotein A1, apolipoprotein B100, lipoprotein(a), transaminase, γ-glutamyltransferase, and C-reactive protein were collected in tubes containing heparin. Serum and plasma were isolated by centrifugation at 3000*g* for 15 minutes at 4°C and frozen at −20°C. Biochemical assays were performed in a single assay at the completion of the study. Insulin and C-peptide measurements were performed by electrochemiluminescence on a COBAS e411 instrument (Roche,

Indianapolis, IN). Plasma NEFAs were assayed by standard spectrophotometric methods (Synchron Clinical System CX4, Beckman Instruments).

Body height was measured to the nearest 1 mm on a stadiometer, with subjects barefoot in the free-standing position. Body weight, body composition, and basal metabolic rate were measured in the fasting state by bioelectrical impedance (TBF-300 Body Composition Analyzer; Tanita Corporation, Arlington Heights, IL) after subjects had removed shoes and heavy clothes and had emptied their pockets.

Mathematical modeling

Insulin secretion rate (ISR) was estimated by C-peptide deconvolution (24). β Cell function parameters were calculated by modeling insulin secretion and glucose concentration, as previously described (25–27). Briefly, this model describes the dependence of insulin secretion on absolute glucose concentration as a quasilinear dose–response function relating the two variables. This function is characterized by a slope and an intercept denoted as β cell glucose sensitivity and insulin secretion rate at fasting plasma glucose (ISR@FPG), respectively. The dose–response function can be modulated throughout the test by several physiological processes (*i.e.*, prolonged exposure to hyperglycemia, gastrointestinal hormones, neural modulation, nonglucose substrates), which are taken into account by a time-dependent factor called potentiation. The ratio between mean potentiation values at the end (160 to 180 minutes) and at the beginning (0 to 20 minutes) of the OGTT is calculated and expressed as the potentiation factor ratio. The dependence of insulin secretion on the rate of increase of plasma glucose concentration is described by a fourth parameter, called β cell rate sensitivity, which therefore reflects early insulin release. The time–concentration area under the curve (AUC) throughout the OGTT was calculated by the trapezoidal rule. Insulin clearance was estimated by calculating the ratio between the insulin secretion AUC and the plasma insulin AUC (28, 29). Insulin sensitivity was estimated by calculating the oral glucose insulin sensitivity (OGIS) index from the glucose and insulin concentrations at time 0, 120, and 180 minutes (30).

Statistical analysis

Based on previous data (19, 29), a sample size of 30 subjects was calculated before enrollment to provide ≥90% power to detect a 5% difference in mean glucose during the OGTT (primary outcome), deemed clinically significant, between the two experimental conditions (HChF *vs.* LChF). All metric variables were tested for normality via Kolmogorov-Smirnoff tests and normalized via logarithmic transformation before analysis when appropriate. Statistical comparisons were made with Student t tests for continuous variables and χ^2 tests for categorical variables. Within-group differences between the two diet interventions were tested by paired Student t tests. Analyses of plasma glucose, plasma insulin, ISR, and plasma NEFA profiles throughout the OGTT, as well as between-group analyses, were performed by two-way analysis of variance (ANOVA) for repeated measures. Correlations between variables were tested with Spearman rank correlations or Pearson correlations as appropriate. Statistical tests were conducted with a two-sided α level of 0.05. Data are reported as mean ± standard deviation, unless otherwise specified. Statistical analyses were performed in JMP® Pro 11.2 (SAS Institute Inc., Cary, NC).

Results

Characteristics of the study participants and diet compliance

All subjects enrolled (16 men and 14 women, age 27.2 ± 5.3 years, BMI 23.5 ± 3.3 kg/m^2) completed the two diet interventions and were included in the analysis. The main clinical and metabolic characteristics of study subjects are shown in Table 1. Self-reported daily food records were consistent with diet prescriptions and showed an adequate compliance (>90%) over each dietary intervention. Food record analysis showed a lower intake of cholesterol (124 ± 49 *vs* 333 ± 32 mg), total fat (25.1 ± 1.4 *vs* 44.8 ± 2.6% of daily energy intake), and saturated fat (6.5 ± 0.8 *vs* 15.9 ± 0.7% of daily energy intake) after the LChF diet than after the HChF diet (*P* < 0.001 for all). No differences were found between the two diets in terms of protein consumption (22.6% ± 1.7% *vs* 21.5% ± 2.9% of daily energy intake, *P* = 0.09), whereas carbohydrate intake was higher during the LChF diet (51.4% ± 5.4% *vs* 34.0% ± 1.8% of daily energy intake, *P* < 0.001).

Effects of LChF diet on body weight and plasma lipid profile

The two diets had similar daily caloric content and were neutral on body weight and BMI (Table 1), whereas body fat mass was lower after the LChF diet (*P* = 0.01). Compared with the HChF diet, the LChF diet produced a ~10% decrease in plasma total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C), without affecting triglyceride levels (Table 1). Fasting plasma NEFA concentrations were higher after the LChF diet (*P* = 0.003) and showed different profiles during the OGTT between the two diets (*P* = 0.04) (Fig. 1). Apolipoprotein A1 and B followed the drop of plasma cholesterol, whereas lipoprotein(a) was increased by the LChF diet. Markers of liver damage and inflammation were within the normal range and similar after the two diets (Table 1).

Effects of LChF diet on glucose metabolism

Fasting glucose and insulin concentrations as well as fasting ISR were not affected by the shift between the two diets (Fig. 1). Mean glucose concentrations throughout the OGTT and glucose peaks at 30 minutes were significantly lower after the LChF diet than after the HChF diet (6.1 ± 0.7 *vs* 6.4 ± 0.6 mM, *P* = 0.01; and 7.5 ± 1.0 *vs* 8.3 ± 1.4 mM, *P* = 0.005, respectively) (Fig. 1 and Table 1). Despite different glucose levels, plasma insulin

Table 1. Clinical and Metabolic Characteristics of the Study Population Stratified in Control Subjects and T2D Offspring After an HChF Diet or an LChF Diet

	Overall (n = 30)			Controls (n = 15)			T2D Offspring (n=15)		
	HChF	LChF	*P*	HChF	LChF	*P*	HChF	LChF	*P*
Clinical features									
Weight, kg	68.5 ± 13.5	68.2 ± 13.5	0.11	67.3 ± 11.1	67.1 ± 11.5	0.26	69.7 ± 15.9	69.3 ± 15.6	0.26
BMI, kg/m^2	23.4 ± 3.2	23.4 ± 3.2	0.11	22.4 ± 1.6	22.3 ± 1.7	0.21	24.0 ± 4.2	23.8 ± 4.0	0.30
Fat mass, %	20.4 ± 6.8	19.9 ± 6.7	**0.01**	19.0 ± 6.3	18.5 ± 6.3	**0.04**	21.9 ± 7.2	21.3 ± 6.9	0.08
Glucose metabolism									
Fasting plasma glucose, mmol/L	5.09 ± 0.40	5.03 ± 0.50	0.53	5.02 ± 0.34	4.94 ± 0.39	0.49	5.15 ± 0.45	5.12 ± 0.47	0.82
Mean OGTT plasma glucose, mmol/L	6.4 ± 0.6	6.1 ± 0.7	**0.01**	6.3 ± 0.5	6.1 ± 0.8	0.17	6.6 ± 0.7	6.0 ± 0.7	**0.04**
Fasting plasma insulin, pmol/L	49.7 ± 24.3	44.1 ± 20.6	0.11	48.1 ± 18.7	39.6 ± 15.0	0.28	54.4 ± 28.1	48.7 ± 24.1	0.26
Mean OGTT plasma insulin, pmol/L	245 ± 112	242 ± 109	0.44	223 ± 67	242 ± 106	0.89	268 ± 143	241 ± 116	0.15
Lipid profile									
Total cholesterol, mg/dL	168 ± 38	151 ± 37	**<0.001**	172 ± 44	151 ± 39	**0.001**	164 ± 32	150 ± 36	**0.01**
LDL-C, mg/dL	99 ± 31	88 ± 29	**<0.001**	100 ± 34	88 ± 29	**0.007**	99 ± 29	88 ± 29	**0.005**
HDL-C, mg/dL	57 ± 12	50 ± 12	**<0.001**	59 ± 13	50 ± 13	**<0.001**	55 ± 12	51 ± 12	**0.04**
Triglycerides, mg/dL	83 ± 44	77 ± 35	0.30	92 ± 56	85 ± 36	0.42	73 ± 25	69 ± 33	0.54
NEFAs, μmol/L	557 ± 200	619 ± 206	0.06	600 ± 226	751 ± 238	0.05	514 ± 168	535 ± 197	0.68
Apolipoprotein A1, mg/dL	145 ± 20	129 ± 21	**<0.001**	141 ± 17	121 ± 18	**<0.001**	150 ± 23	137 ± 22	**0.05**
Apolipoprotein B, mg/dL	67 ± 17	64 ± 19	**0.04**	68 ± 20	63 ± 17	**0.03**	66 ± 13	66 ± 21	0.72
Lipoprotein(a), mg/dL	8 ± 8	10 ± 9	**0.04**	7 ± 8	7 ± 8	0.51	10 ± 7	13 ± 10	**0.03**
Other biomarkers									
Aspartate transaminase, U/L	19 ± 9	17 ± 4	0.08	20 ± 10	18 ± 4	0.18	18 ± 8	16 ± 4	0.30
Alanine transaminase, U/L	15 ± 7	14 ± 5	0.14	16 ± 7	16 ± 5	0.45	15 ± 6	13 ± 5	0.22
γ-Glutamyltransferase, U/L	15 ± 8	13 ± 6	0.05	15 ± 10	14 ± 7	0.13	16 ± 6	13 ± 5	0.18
C-reactive protein, μg/dL	60 ± 10	46 ± 45	0.13	37 ± 22	36 ± 43	0.89	69 ± 40	59 ± 45	0.08

Statistical comparisons within groups between diet interventions were performed with paired Student *t* tests. Statistically significant *P* values are indicated in bold. Data are expressed as mean ± standard deviation.

and ISR profiles were similar after the two diets (Fig. 1). Accordingly, at the end of the LChF diet a consistent increase was observed in three major components of β cell function: β cell glucose sensitivity (+17%, *P* = 0.01), ISR@FPG (+20%, *P* = 0.02), and potentiation (+19%, *P* = 0.03) (Fig. 2). Among them, the changes in ISR@FPG (*r* = −0.47, *P* = 0.009) and potentiation (*r* = −0.48, *P* = 0.007) correlated with the changes in mean glucose concentrations between the two diets. Neither β cell rate sensitivity, a marker of early insulin secretion, nor insulin clearance was affected by the dietary shift (Fig. 2). The changes in insulin sensitivity were small and of borderline statistical significance (Fig. 2; *P* = 0.095); however, they correlated with changes in mean glucose concentrations between the two diets (*r* = −0.37, *P* = 0.045).

Impact of the genetic background

Among the 30 study participants, 50% had family history of T2D (T2D offspring). At screening, controls and T2D offspring were matched for age (25.5 ± 4.1 *vs* 28.9 ± 5.9 years, respectively; *P* = 0.07), sex (8 men and 7 women for both), and BMI (22.6 ± 1.7 *vs* 23.9 ± 3.3 kg/m^2, *P* = 0.14). Furthermore, no statistically significant differences

between groups were found in any clinical or metabolic parameter after each diet, including those related to glucose metabolism and β cell function (Table 1, Fig. 3, and Supplemental Fig. 1). Plasma NEFAs were an exception, being slightly higher in controls (*P* = 0.01). The metabolic changes induced by the shift between the two diets were not significantly different in controls and T2D offspring by the two-way ANOVA for repeated measures. However, the effect of the interaction between diet and familial diabetes was of borderline statistical significance for insulin sensitivity and β cell glucose sensitivity (*P* ≤ 0.10). Accordingly, in within-group analyses, T2D offspring displayed a tendency to a more diffuse improvement of glucose tolerance after the LChF diet compared with the HChF diet, which was associated with a significant decrease in insulin sensitivity and a smaller improvement in β cell glucose sensitivity (Fig. 3).

Discussion

In this study, we measured the effects of a large but physiologic decrease in dietary cholesterol and fat intake on glucose tolerance and its major determinants and we examined the role of the interaction with an unfavorable
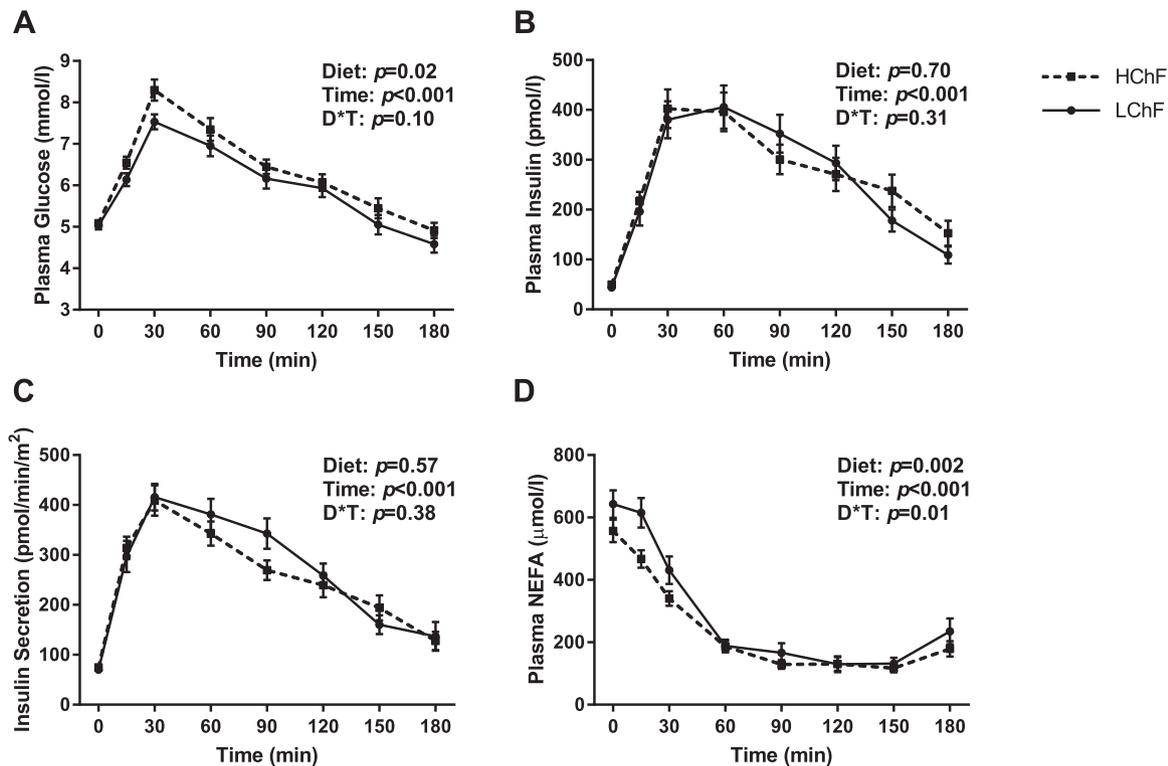
Figure 1. (A) Plasma glucose, (B) plasma insulin, (C) ISR, and (D) plasma NEFAs during a 75-g OGTT after a 14-day HChF diet (dashed line) or a 14-day isocaloric LChF diet (continuous line) in nondiabetic subjects (n = 30). Repeated-measure analyses were performed by two-way ANOVA. P values are shown for diet, time, and diet × time interaction (D*T) effects. Data are reported as mean ± standard error of the mean.

genetic background in nondiabetic subjects. Although neutral on body weight, the shift from an HChF diet to an isocaloric LChF diet, mimicking the shift from an unhealthy to a healthy Western diet, improved oral glucose tolerance without modifying plasma insulin and total insulin secretion rate. Accordingly, different components of the β cell function were improved by the LChF diet, including β cell glucose sensitivity, ISR@FPG, and potentiation. The beneficial effect of the LChF diet on glucose tolerance and particularly on insulin sensitivity tended to be more evident in individuals genetically predisposed to develop T2D.

Our aim was to test the hypothesis that a diet low in cholesterol and fat improves glucose tolerance, independently of energy intake and weight gain. The cholesterol content of the two diets was designed to explore the entire physiologic range, being similar to the median spontaneous cholesterol intake of the first (131 mg/d) and the fifth (273 mg/d) quintile of a large observational study (2). Consistent with our results, in this study higher cholesterol intake was associated with an increased risk of T2D after a follow-up of 14 years (relative risk 1.36; 95% confidence interval, 1.17 to 1.59) (2). By focusing particularly on the cholesterol content of the food, our dietary intervention might have elicited effects that depend on the variations in plasma cholesterol levels. However, given the minor changes observed in the lipid profile and

the expected divergent effects of LDL-C and HDL-C on glucose metabolism (16, 31–33), it is unlikely that the small and concurrent changes in the two lipoproteins between the diets can explain our observations. In addition, regression analysis did not show correlations between changes in lipoproteins and variations of glucose tolerance or β cell function.

On the other hand, the decrease in fat intake (~45% *vs* ~25% of total daily calories), which was, in quantitative terms, within the range of what is expected when a low-cholesterol diet is prescribed, is probably a main determinant of the observed effects. It is known that the acute (2 to 6 hours) and short-term (3 to 7 days) infusion or ingestion of lipid affects glucose tolerance by reducing insulin sensitivity, increasing insulin secretion, and possibly inhibiting insulin clearance (22, 34–44). The extent of these effects (but not the direction) is influenced by the length of exposure and the quality of fat (36), the subject's ethnicity (35), and the genetic predisposition to develop T2D (22). Data on more prolonged dietary fat manipulations in healthy subjects are conflicting and very limited. In 24 obese nondiabetic patients, a 2-week high-fat (~50% of total daily calories) weight-maintaining diet produced a decrease in oral glucose tolerance (OGTT glucose AUC +8%) and β cell function associated with a not statistically significant decline in insulin sensitivity and a major decrease in minimal model glucose
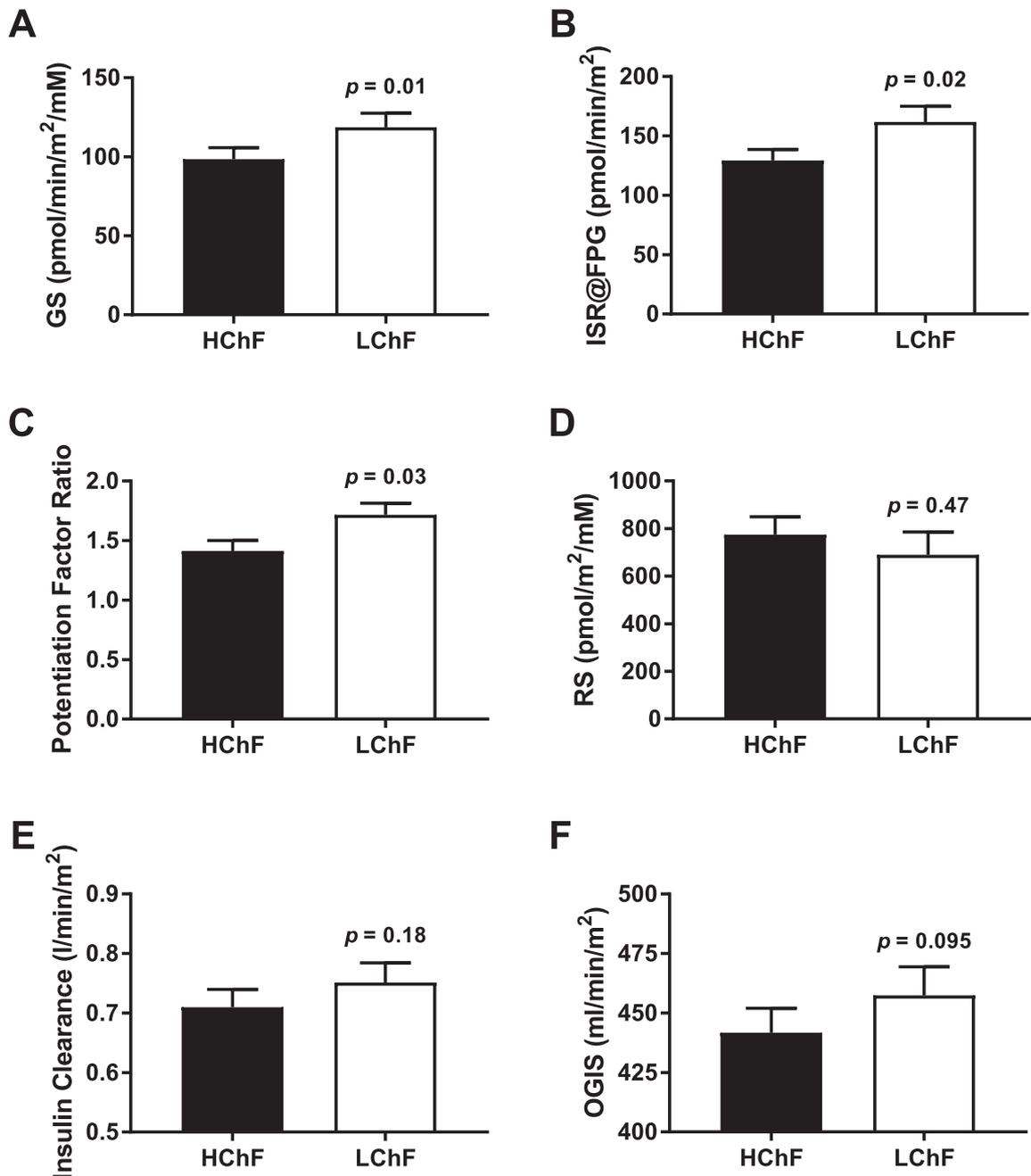
Figure 2. (A) β cell glucose sensitivity (GS), (B) ISR@FPG, (C) potentiation factor ratio, (D) β cell rate sensitivity (RS), (E) insulin clearance, and (F) OGIS index estimated by mathematical modeling of plasma glucose, insulin, and C-peptide during a 75-g OGTT after either a 14-day HChF diet (black bars) or a 14-day isocaloric LChF diet (white bars) in nondiabetic subjects (n = 30). Differences between the two diet interventions were tested by paired Student *t* tests. Data are reported as mean ± standard error of the mean.

effectiveness (*i.e.*, the ability of plasma glucose *per se* to increase tissue glucose uptake and inhibit endogenous glucose production) (19). In eight nondiabetic subjects, a 3-week high-fat diet (~50% of total daily calories) did not affect fasting glucose or fasting insulin levels, but glucose tolerance and insulin secretion were not evaluated (20). Furthermore, the same intervention was neutral on insulin sensitivity, despite a mean weight gain of 0.4 kg (20). In our study, we found a significant improvement in glucose tolerance after the shift from the HChF to the

LChF diet (~5% mean glucose decrease). Although this positive effect was small in quantitative terms, its clinical relevance is not negligible because it occurred in healthy nonobese subjects after a short intervention, which focused only on the quality of nutrients and not on total energy intake. In fact, we might expect a much greater effect on glucose homeostasis after long-term and more extreme changes in dietary habit and in patients with altered glucose tolerance. Our study demonstrated in humans a nonselective inhibitory effect of a prolonged
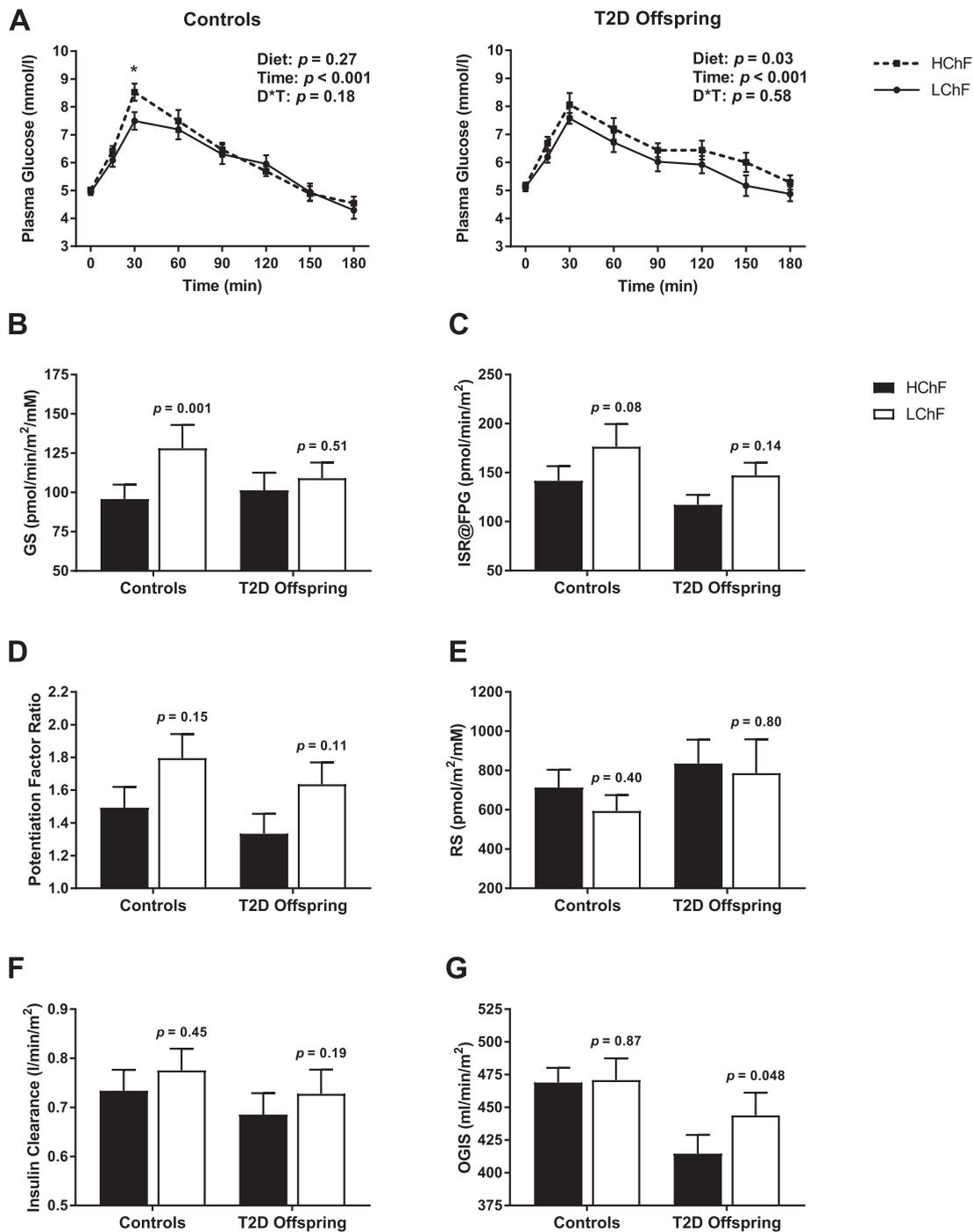
Figure 3. (A) Plasma glucose, (B) β cell glucose sensitivity (GS), (C) ISR@FPG, (D) potentiation factor ratio, (E) β cell rate sensitivity (RS), (F) insulin clearance, and (G) OGIS index during a 75-g OGTT after a 14-day HChF diet or a 14-day isocaloric LChF diet in nondiabetic subjects without (controls, n = 15) or with family history of T2D (T2D offspring, n = 15). For plasma glucose profiles, repeated-measure analyses were performed by two-way ANOVA, and P values are shown for diet, time, and diet $\times$ time interaction (D*T) effects. For other variables, P values are shown for within-group differences between the two diet interventions tested by paired Student t tests. Data are reported as mean $\pm$ standard error of the mean. *$P < 0.05$ for peak glucose.

and physiologic exposure to lipids on β cell function. Indeed, the HChF diet produced an impairment in insulin secretion that extended to three out of four β cell functions, which were restored by the LChF diet. This finding is a proof of concept for the paradigm of β cell lipotoxicity (*i.e.*, an accumulation of lipid intermediates

leading to β cellular dysfunction and eventually death), which is based on *in vitro* and animal evidence and on short-term human studies (45). Although diffuse, the β cell dysfunction did not extend to rate sensitivity, which reflects early insulin release, suggesting that the readily available insulin pool is not sensitive to this dietary
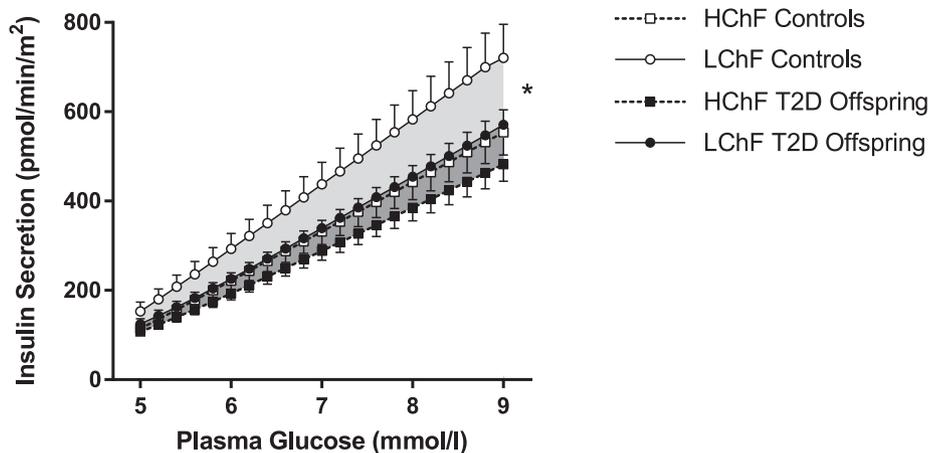
Figure 4. Plasma glucose/insulin secretion dose response curve after a 14-day HChF diet or a 14-day isocaloric LChF diet in nondiabetic subjects without (controls, n = 15) or with family history of T2D (T2D offspring, n = 15). Within-group differences between the two diet interventions were tested by paired Student t tests. Data are reported as mean ± standard error of the mean. $*P < 0.05$ for the slope of the curve (β cell glucose sensitivity).

manipulation in healthy subjects. Although acute and short-term elevations in plasma lipid have been shown to reduce hepatic insulin clearance (22, 41–44), particularly in T2D offspring (22), insulin clearance was not affected by the shift between the two diets.

The positive effects of the LChF diet tended to be more evident in T2D offspring, in whom this intervention produced a significant increase in glucose tolerance and insulin sensitivity in the within-group analysis. This finding is consistent with the hypothesis that offspring of patients with T2D are more prone to the deleterious effect of lipids on glucose tolerance and on insulin action (22) and potentially more sensitive to the benefit from lowering dietary lipids. A previous study did not observe a reduction in insulin sensitivity in healthy T2D offspring after a 4-day lipid infusion; however, subjects enrolled presented at baseline with a lower insulin sensitivity compared with matched controls without a family history of diabetes, so the authors hypothesized that the "lipotoxic" effect of elevated plasma lipid could not further impair a fully established alteration in insulin sensitivity in those subjects (22). In our study, the improvement in β cell glucose sensitivity after the LChF diet was significant only in subjects without a family history of T2D, who acquired the β cell phenotype of T2D offspring when exposed to the HChF diet and recovered after the LChF diet (Fig. 4). In accordance with previous evidence (46), this observation suggests a subclinical and potentially irreversible impairment in β cell function in subjects genetically predisposed to develop glucose intolerance, which may contribute to the increased risk of T2D in these individuals.

Because the two diets differed in carbohydrate intake (~51% vs ~34% of daily energy intake, $P < 0.001$), besides cholesterol and fat content, it is not possible to exclude the contribution of a higher carbohydrate consumption in the observed metabolic changes. As previously reviewed in detail (47, 48), both high-carbohydrate, low-fat diets and low-carbohydrate, high-fat diets have been reported to improve glucose control, often in combination with a restriction in energy intake and a drop in body weight; thus, to date the evidence is inconclusive for determining whether and to what extent a different carbohydrate intake affects glucose homeostasis independent of body weight. Also, the duration of our diet interventions was short; therefore, a more sustained effect of an HChF diet or an LChF diet remains to be determined.

In conclusion, we demonstrated that lowering dietary cholesterol and fat intake improves glucose tolerance in nondiabetic subjects by diffusely enhancing major β cell functions. Subjects genetically predisposed to develop T2D tend to be more susceptible to the beneficial effect of the diet on insulin sensitivity and glucose tolerance.

Acknowledgments

insulin clearance; supervised statistical data analyses; and revised the manuscript critically. A.N. conceived and designed the study, analyzed and interpreted the data, and revised the manuscript. All authors critically revised the manuscript and approved the final version of the article. D.T. and A.N. are responsible for the integrity of the data and the accuracy of the data analysis.

Current Affiliation: C. Morgantini's current affiliation is Integrated Cardio Metabolic Centre, Department of Medicine, Karolinska Institutet, 141 52 Huddinge, Sweden.

Correspondence and Reprint Requests: Domenico Tricò, MD, Department of Clinical and Experimental Medicine, University of Pisa, Via Roma 67, 56126 Pisa, Italy. E-mail: domenico.trico@for.unipi.it.

References

1. Nolan CJ, Damm P, Prentki M. Type 2 diabetes across generations: from pathophysiology to prevention and management. *Lancet.* 2011;**378**(9786):169–181.
2. Salmerón J, Hu FB, Manson JE, Stampfer MJ, Colditz GA, Rimm EB, Willett WC. Dietary fat intake and risk of type 2 diabetes in women. *Am J Clin Nutr.* 2001;**73**(6):1019–1026.
3. Dotevall A, Johansson S, Wilhelmsen L, Rosengren A. Increased levels of triglycerides, BMI and blood pressure and low physical activity increase the risk of diabetes in Swedish women. A prospective 18-year follow-up of the BEDA study. *Diabet Med.* 2004;**21**(6):615–622.
4. Tirosh A, Shai I, Bitzur R, Kochba I, Tekes-Manova D, Israeli E, Shochat T, Rudich A. Changes in triglyceride levels over time and risk of type 2 diabetes in young men. *Diabetes Care.* 2008;**31**(10):2032–2037.
5. Pankow JS, Duncan BB, Schmidt MI, Ballantyne CM, Couper DJ, Hoogeveen RC, Golden SH; Atherosclerosis Risk in Communities Study. Fasting plasma free fatty acids and risk of type 2 diabetes: the Atherosclerosis Risk in Communities study. *Diabetes Care.* 2004;**27**(1):77–82.
6. Abbasi A, Corpeleijn E, Gansevoort RT, Gans RO, Hillege HL, Stolk RP, Navis G, Bakker SJ, Dullaart RP. Role of HDL cholesterol and estimates of HDL particle composition in future development of type 2 diabetes in the general population: the PREVEND study. *J Clin Endocrinol Metab.* 2013;**98**(8):E1352–E1359.
7. Besseling J, Kastelein JJ, Defesche JC, Hutten BA, Hovingh GK. Association between familial hypercholesterolemia and prevalence of type 2 diabetes mellitus. *JAMA.* 2015;**313**(10):1029–1036.
8. Drew BG, Duffy SJ, Formosa MF, Natoli AK, Henstridge DC, Penfold SA, Thomas WG, Mukhamedova N, de Courten B, Forbes JM, Yap FY, Kaye DM, van Hall G, Febbraio MA, Kemp BE, Sviridov D, Steinberg GR, Kingwell BA. High-density lipoprotein modulates glucose metabolism in patients with type 2 diabetes mellitus. *Circulation.* 2009;**119**(15):2103–2111.
9. von Eckardstein A, Schulte H, Assmann G. Risk for diabetes mellitus in middle-aged Caucasian male participants of the PROCAM study: implications for the definition of impaired fasting glucose by the American Diabetes Association. Prospective Cardiovascular Münster. *J Clin Endocrinol Metab.* 2000;**85**(9):3101–3108.
10. Wilson PW, D'Agostino RB, Fox CS, Sullivan LM, Meigs JB. Type 2 diabetes risk in persons with dysglycemia: the Framingham Offspring Study. *Diabetes Res Clin Pract.* 2011;**92**(1):124–127.
11. Hao M, Head WS, Gunawardana SC, Hasty AH, Piston DW. Direct effect of cholesterol on insulin secretion: a novel mechanism for pancreatic beta-cell dysfunction. *Diabetes.* 2007;**56**(9):2328–2338.
12. Kruit JK, Kremer PH, Dai L, Tang R, Ruddle P, de Haan W, Brunham LR, Verchere CB, Hayden MR. Cholesterol efflux via ATP-binding cassette transporter A1 (ABCA1) and cholesterol uptake via the LDL receptor influences cholesterol-induced impairment of beta cell function in mice. *Diabetologia.* 2010;**53**(6):1110–1119.
13. Brunham LR, Kruit JK, Verchere CB, Hayden MR. Cholesterol in islet dysfunction and type 2 diabetes. *J Clin Invest.* 2008;**118**(2):403–408.
14. de Haan W, Karasinska JM, Ruddle P, Hayden MR. Hepatic ABCA1 expression improves β-cell function and glucose tolerance. *Diabetes.* 2014;**63**(12):4076–4082.
15. Giacca A, Xiao C, Oprescu AI, Carpentier AC, Lewis GF. Lipid-induced pancreatic β-cell dysfunction: focus on in vivo studies. *Am J Physiol Endocrinol Metab.* 2011;**300**(2):E255–E262.
16. Natali A, Baldi S, Bonnet F, Petrie J, Trifirò S, Tricò D, Mari A; RISC Investigators. Plasma HDL-cholesterol and triglycerides, but not LDL-cholesterol, are associated with insulin secretion in non-diabetic subjects. *Metabolism.* 2017;**69**:33–42.
17. Samuel VT, Petersen KF, Shulman GI. Lipid-induced insulin resistance: unravelling the mechanism. *Lancet.* 2010;**375**(9733):2267–2277.
18. Seghieri M, Tricò D, Natali A. The impact of triglycerides on glucose tolerance: lipotoxicity revisited. *Diabetes Metab.* 2017;**43**(4):314–322.
19. Swinburn BA, Boyce VL, Bergman RN, Howard BV, Bogardus C. Deterioration in carbohydrate metabolism and lipoprotein changes induced by modern, high fat diet in Pima Indians and Caucasians. *J Clin Endocrinol Metab.* 1991;**73**(1):156–165.
20. Borkman M, Campbell LV, Chisholm DJ, Storlien LH. Comparison of the effects on insulin sensitivity of high carbohydrate and high fat diets in normal subjects. *J Clin Endocrinol Metab.* 1991;**72**(2):432–437.
21. Unger RH. Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. *Diabetes.* 1995;**44**(8):863–870.
22. Kashyap S, Belfort R, Gastaldelli A, Pratipanawatr T, Berria R, Pratipanawatr W, Bajaj M, Mandarino L, DeFronzo R, Cusi K. A sustained increase in plasma free fatty acids impairs insulin secretion in nondiabetic subjects genetically predisposed to develop type 2 diabetes. *Diabetes.* 2003;**52**(10):2461–2474.
23. Tricò D, Filice E, Trifirò S, Natali A. Manipulating the sequence of food ingestion improves glycemic control in type 2 diabetic patients under free-living conditions. *Nutr Diabetes.* 2016;**6**(8):e226.
24. Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes.* 1992;**41**(3):368–377.
25. Mari A, Schmitz O, Gastaldelli A, Oestergaard T, Nyholm B, Ferrannini E. Meal and oral glucose tests for assessment of beta-cell function: modeling analysis in normal subjects. *Am J Physiol Endocrinol Metab.* 2002;**283**(6):E1159–E1166.
26. Mari A, Tura A, Gastaldelli A, Ferrannini E. Assessing insulin secretion by modeling in multiple-meal tests: role of potentiation. *Diabetes.* 2002;**51**(suppl 1):S221–S226.
27. Mari A, Ferrannini E. Beta-cell function assessment from modelling of oral tests: an effective approach. *Diabetes Obes Metab.* 2008;**10**(suppl 4):77–87.
28. Tricò D, Filice E, Baldi S, Frascerra S, Mari A, Natali A. Sustained effects of a protein and lipid preload on glucose tolerance in type 2 diabetes patients. *Diabetes Metab.* 2016;**42**(4):242–248.
29. Tricò D, Baldi S, Tulipani A, Frascerra S, Macedo MP, Mari A, Ferrannini E, Natali A. Mechanisms through which a small protein and lipid preload improves glucose tolerance. *Diabetologia.* 2015;**58**(11):2503–2512.
30. Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. *Diabetes Care.* 2001;**24**(3):539–548.

31. Kruit JK, Brunham LR, Verchere CB, Hayden MR. HDL and LDL cholesterol significantly influence beta-cell function in type 2 diabetes mellitus. *Curr Opin Lipidol.* 2010;**21**(3):178–185.

32. Fryirs MA, Barter PJ, Appavoo M, Tuch BE, Tabet F, Heather AK, Rye KA. Effects of high-density lipoproteins on pancreatic beta-cell insulin secretion. *Arterioscler Thromb Vasc Biol.* 2010;**30**(8):1642–1648.

33. Abderrahmani A, Niederhauser G, Favre D, Abdelli S, Ferdaoussi M, Yang JY, Regazzi R, Widmann C, Waeber G. Human high-density lipoprotein particles prevent activation of the JNK pathway induced by human oxidised low-density lipoprotein particles in pancreatic beta cells. *Diabetologia.* 2007;**50**(6):1304–1314.

34. Stettler R, Ith M, Acheson KJ, Décombaz J, Boesch C, Tappy L, Binnert C. Interaction between dietary lipids and physical inactivity on insulin sensitivity and on intramyocellular lipids in healthy men. *Diabetes Care.* 2005;**28**(6):1404–1409.

35. Bakker LE, van Schinkel LD, Guigas B, Streefland TC, Jonker JT, van Klinken JB, van der Zon GC, Lamb HJ, Smit JW, Pijl H, Meinders AE, Jazet IM. A 5-day high-fat, high-calorie diet impairs insulin sensitivity in healthy, young South Asian men but not in Caucasian men. *Diabetes.* 2014;**63**(1):248–258.

36. Lopez S, Bermudez B, Ortega A, Varela LM, Pacheco YM, Villar J, Abia R, Muriana FJ. Effects of meals rich in either mono-unsaturated or saturated fat on lipid concentrations and on insulin secretion and action in subjects with high fasting triglyceride concentrations. *Am J Clin Nutr.* 2011;**93**(3):494–499.

37. Numao S, Kawano H, Endo N, Yamada Y, Konishi M, Takahashi M, Sakamoto S. Short-term low carbohydrate/high-fat diet intake increases postprandial plasma glucose and glucagon-like peptide-1 levels during an oral glucose tolerance test in healthy men. *Eur J Clin Nutr.* 2012;**66**(8):926–931.

38. Hernández EA, Kahl S, Seelig A, Begovatz P, Irmler M, Kupriyanova Y, Nowotny B, Nowotny P, Herder C, Barosa C, Carvalho F, Rozman J, Neschen S, Jones JG, Beckers J, de Angelis MH, Roden M. Acute dietary fat intake initiates alterations in energy metabolism and insulin resistance. *J Clin Invest.* 2017;**127**(2):695–708.

39. Manco M, Bertuzzi A, Salinari S, Scarfone A, Calvani M, Greco AV, Mingrone G. The ingestion of saturated fatty acid tri-acylglycerols acutely affects insulin secretion and insulin sensitivity in human subjects. *Br J Nutr.* 2004;**92**(6):895–903.

40. Brøns C, Jensen CB, Storgaard H, Hiscock NJ, White A, Appel JS, Jacobsen S, Nilsson E, Larsen CM, Astrup A, Quistorff B, Vaag A. Impact of short-term high-fat feeding on glucose and insulin metabolism in young healthy men. *J Physiol.* 2009;**587**(Pt 10):2387–2397.

41. Carpentier A, Mittelman SD, Bergman RN, Giacca A, Lewis GF. Prolonged elevation of plasma free fatty acids impairs pancreatic beta-cell function in obese nondiabetic humans but not in individuals with type 2 diabetes. *Diabetes.* 2000;**49**(3):399–408.

42. Svedberg J, Björntorp P, Smith U, Lönnroth P. Free-fatty acid inhibition of insulin binding, degradation, and action in isolated rat hepatocytes. *Diabetes.* 1990;**39**(5):570–574.

43. Wiesenthal SR, Sandhu H, McCall RH, Tchipashvili V, Yoshii H, Polonsky K, Shi ZQ, Lewis GF, Mari A, Giacca A. Free fatty acids impair hepatic insulin extraction in vivo. *Diabetes.* 1999;**48**(4):766–774.

44. Hennes MM, Dua A, Kissebah AH. Effects of free fatty acids and glucose on splanchnic insulin dynamics. *Diabetes.* 1997;**46**(1):57–62.

45. Del Prato S. Role of glucotoxicity and lipotoxicity in the pathophysiology of type 2 diabetes mellitus and emerging treatment strategies. *Diabet Med.* 2009;**26**(12):1185–1192.

46. Stadler M, Pacini G, Petrie J, Luger A, Anderwald C; RISC Investigators. Beta cell (dys)function in non-diabetic offspring of diabetic patients. *Diabetologia.* 2009;**52**(11):2435–2444.

47. Wheeler ML, Dunbar SA, Jaacks LM, Karmally W, Mayer-Davis EJ, Wylie-Rosett J, Yancy WS, Jr. Macronutrients, food groups, and eating patterns in the management of diabetes: a systematic review of the literature, 2010 [published correction appears in Diabetes Care. 2012;35(6):1395–1395]. *Diabetes Care.* 2012;**35**(2):434–445.

48. 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**(3):505–516.