

Postprandial lipemia induces pancreatic α cell dysfunction characteristic of type 2 diabetes: studies in healthy subjects, mouse pancreatic islets, and cultured pancreatic α cells^{1–3}

Andreas Niederwanger, Christian Ciardi, Tobias Tatarczyk, Mohammad I Khan, Martin Hermann, Christof Mittermair, Ramona Al-Zoairy, Karin Salzmann, and Michael T Pedrini

ABSTRACT

Background: Type 2 diabetes is associated with pancreatic α cell dysfunction, characterized by elevated fasting plasma glucagon concentrations and inadequate postprandial glucose- and insulin-induced suppression of glucagon secretion. The cause and the underlying mechanisms of α cell dysfunction are unknown.

Objective: Because Western dietary habits cause postprandial lipemia for a major part of a day and, moreover, increase the risk of developing type 2 diabetes, we tested the hypothesis that postprandial lipemia with its characteristic elevation of triglyceride-rich lipoproteins (TGRLs) might cause pancreatic α cell dysfunction.

Design: In a crossover study with 7 healthy volunteers, 2 experiments using 2 fat-enriched meals were performed on each volunteer; meal 1 was designed to increase plasma concentrations of both TGRLs and nonesterified fatty acids and meal 2 to increase TGRLs only. Intravenous glucose boli were injected at 0800 after an overnight fast and postprandially at 1300, 3 h after ingestion of a fat-enriched meal. Glucagon concentrations were measured throughout the days of the experiments. In addition to the study in humans, in vitro experiments were performed with mouse pancreatic islets and cultured pancreatic alpha TC 1 clone 9 (α TC1c9) cells, which were incubated with highly purified TGRLs.

Results: In humans, postprandial lipemia increased plasma glucagon concentrations and led to an inadequate glucose- and insulin-induced suppression of glucagon. There was no difference between the 2 meal types. In mouse pancreatic islets and cultured pancreatic α TC1c9 cells, purified postprandial TGRLs induced abnormalities in glucagon kinetics comparable with those observed in humans. The TGRL-induced α cell dysfunction was due to reduced γ -aminobutyric acid A receptor activation in pancreatic α cells.

Conclusion: We concluded that postprandial lipemia induces pancreatic α cell dysfunction characteristic of type 2 diabetes and, therefore, propose that pancreatic α cell dysfunction could be viewed, at least partly, as a postprandial phenomenon. *Am J Clin Nutr* 2014;100:1222–31.

INTRODUCTION

For a long time, abnormalities in glucagon secretion from pancreatic α cells have been implicated in the development of fasting and postprandial hyperglycemia in type 2 diabetes (1). In the fasting state, inappropriately high plasma glucagon con-

centrations have been observed in patients with impaired glucose tolerance and type 2 diabetes (2–4). In the postprandial state, the decrease in plasma glucagon concentrations after a glucose load is less pronounced in these patients than in healthy subjects (5–7). These abnormalities in pancreatic α cell function are evident several years before the diagnosis of impaired glucose tolerance (8). Several mechanisms have been proposed to explain these alterations in glucagon kinetics, such as insulin resistance at the level of pancreatic α cells (9), α cell desensitization by prolonged hyperglycemia (10), high-glucose stimulation of glucagon secretion (11), and loss of paracrine α cell inhibition as a consequence of the loss of the normal intra-islet oscillatory secretion pattern of insulin (12, 13).

Impaired glucose tolerance and type 2 diabetes are associated with abnormalities in the lipid profile, ie, elevated fasting and postprandial plasma concentrations of triglyceride-rich lipoproteins (TGRLs)⁴ (14, 15), elevated concentrations of nonesterified fatty acids (NEFAs), small dense LDL particles, and reduced HDL concentrations. According to the lipotoxicity concept, which was initially introduced by Roger Unger to describe the deleterious effects of excessive fatty acids on pancreatic β cell survival (16), many of the pathophysiological hallmarks of type 2

¹ From the Department of Internal Medicine I, Medical University of Innsbruck, Innsbruck, Austria (AN, CC, TT, MIK, RA-Z, KS, and MTP); KMT Laboratory, Department of Visceral, Transplant and Thoracic Surgery, Innsbruck Medical University, Innsbruck, Austria (MH); and the Clinical Department of Surgery, Hospital of Barmherzige Brüder, Salzburg, Austria (CM).

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³ Address correspondence to MT Pedrini, Department of Internal Medicine I, Medical University of Innsbruck, Anichstrasse 35, A-6020 Innsbruck, Austria. E-mail: michael.pedrini@i-med.ac.at.

⁴ Abbreviations used: AGISG, area of glucose-induced suppression of glucagon; AIR_G, acute insulin response to glucose; BSA, bovine serum albumin; GABA, γ -aminobutyric acid; GABA-A, A-type γ -aminobutyric acid receptor; GIP, glucose-dependent insulintropic polypeptide; GISG, glucose-induced suppression of glucagon; GLP-1, glucagon-like peptide 1; NEFA, nonesterified fatty acid; TGRL, triglyceride-rich lipoprotein; α TC1c9, alpha TC 1 clone 9.

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diabetes are brought about by deposition of fatty acids in non-adipose tissues leading to organ dysfunction; this so-called ectopic fat deposition was shown to occur also in heart muscle (17), skeletal muscle (18, 19), and liver (20). Despite the importance of glucagon in the regulation of glucose homeostasis, very little is known about the role of plasma lipids in the modulation of pancreatic α cell function. Previous studies addressing the effect of NEFAs on glucagon secretion in cultured pancreatic α cells or isolated pancreatic islets has led to conflicting results, with early data suggesting a glucagon-suppressing effect (21–23) and more recent reports providing evidence of a stimulatory effect (24–26).

Because people adopting Western dietary habits are in a state of postprandial lipemia for ~ 18 h/d (27) and, moreover, are at a higher risk of developing impaired glucose tolerance or type 2 diabetes (28–30), we hypothesized that postprandial lipemia might be a link between Western dietary habits and pancreatic α cell dysfunction. To test our hypothesis, we studied the effect of postprandial lipemia and its characteristic lipoproteins (ie, TGRLs) on glucagon kinetics in healthy volunteers, isolated mouse pancreatic islets, and cultured pancreatic α cells.

SUBJECTS AND METHODS

Study participants and study design

For this study with a crossover design, blood samples from our previous study cohort of 10 healthy male volunteers (31) were further analyzed. Of these 10 volunteers, 3 had to be excluded from analyses based exclusively on the fact that blood samples for further analysis were no longer available.

Glucagon concentrations were calculated from the mean of 2 measurements analyzed 10 and 1 min before each of the 2 intravenous glucose injections by using a bolus of 33% glucose (0.3 g/kg); the first glucose bolus was injected postabsorptively at 0800 after the subjects fasted overnight for ~ 12 h, and the second bolus postprandially at 1300 three hours after consumption of 1 of the 2 virtually isocaloric test meals (ie, at 1000). Two different experiments using meal 1 and meal 2 were performed on each study subject on 2 occasions within 3 mo as previously described (31). In a control experiment, measurements in the 7 study subjects were performed without meal ingestion.

The study protocol was reviewed and approved by the Ethic Commission of Medical University of Innsbruck, and written informed consent was obtained from all subjects. The initial recruitment date was January 2005.

Analytic procedures

The composition of meals 1 and 2 was as previously described by our group (31) and was chosen on the basis of the observation that the addition of carbohydrates to an oral fat load reduces postprandial NEFA concentrations (32). In brief, meal 1 consisted of 350 mL whipped cream, 10 g cacao powder, and 1 mL artificial sweetener and contained 11 g protein, 12 g carbohydrates, 106 g triglycerides, and 315 mg cholesterol. Of the total 1080 kcal, 4.2% was derived from protein, 4.6% from carbohydrates, and 91.2% from fat. Meal 2 consisted of 192 mL whipped cream, 10 g cacao powder, 1 mL artificial sweetener, 100 g low-fat milk powder, 10 g maltodextrin, and 160 mL water and contained 43 g protein, 65 g carbohydrates, 60 g triglycerides, and 173 mg cholesterol. Of the total 1001 kcal, 17.7% was derived from protein, 26.7% from carbohydrates, and 55.6% from fat.

Plasma glucagon was measured by using a Glucagon- I^{125} radioimmunoassay (DRG Diagnostics) and a Beckman LS analyzer. Glucose-induced suppression of glucagon (GISG) was calculated as the difference between nadir and basal glucagon concentrations after the glucose injections, and the area of glucose-induced suppression of glucagon (AGISG), ie, the infrabasal glucagon concentration during the 0–60 min after the glucose injections, was calculated by using the integration of spline areas. When upward slopes of glucagon suppression reached basal concentrations before 60 min, curves were truncated. To calculate the time period within which glucagon concentrations reached the nadir on glucose injection as well as for calculation of the nadir glucagon concentration, a polynomial regression curve was created. The acute insulin response to glucose (AIR_G) was calculated as the average suprabasal insulin concentration during the initial 2–10 min (blood samples were drawn at 2, 3, 4, 6, 8, and 10 min) after the glucose injections by using an insulin-detection kit from WAKO Chemicals. Active glucagon-like peptide 1 (GLP-1) was measured by using an enzyme immune assay kit from Yanaihara Institute Inc, and total glucose-dependent insulinotropic polypeptide (GIP) was measured by using an ELISA kit from Linco Research. Triglycerides, NEFAs, and glucose were analyzed as previously described (33). Arginine was analyzed according to the Sakaguchi reaction (34).

Murine islet isolation and cell culture

Pancreatic islets were isolated as described by Goto et al (35). Male C57Bl6 mice aged 6–8 wk served as donors. For the stationary digestion of pancreatic tissue, collagenase solution type 4 containing 5 mmol adenosine/L was used. Islet cells were purified on a discontinuous Ficoll density gradient and then handpicked. Experiments were performed immediately after isolation. Insulin was measured by using a rat/mouse insulin ELISA kit from Linco Research and glucagon by using the Glucagon- I^{125} radioimmunoassay kit from DRG Diagnostics.

All protocols involving animals were in accordance with the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals published by the NIH (publication no. 86–23, revised 1985). Experiments were approved by the Austrian Ministry of Education, Science, and Culture.

Alpha TC 1 clone 9 (α TC1c9) cells were purchased from ATCC at passage 36 and used up to passage 42. Cells were cultured by using DMEM containing 16.65 mmol glucose/L, 10% fetal calf serum, 1.5 g NaHCO₃/L, and 15 mmol HEPES/L on gelatin-coated plates.

Lipoprotein isolation

TGRLs were isolated from a healthy male individual in the postprandial state from blood withdrawn 4 h after ingestion of test meal 1. TGRLs with a Svedberg flotation rate of 20 to 400, corresponding to chylomicron remnants and VLDL/VLDL remnants, were isolated by zonal ultracentrifugation and purified further by gel filtration as previously described (33). Purity was tested by lipid electrophoresis. Lipoprotein samples were stored in the dark under nitrogen at 4°C and used within 5 d after isolation.

Analysis of lipoprotein fatty acid composition

After lipolysis of postprandial plasma, the fatty acid composition was analyzed by using gas chromatography according to the method described by Kang and Wang (36). Using known standards, we were able to identify 98% of the area under the curve of fatty acids extracted from TGRLs. Of these NEFAs, 28.6% referred to palmitate (16:0), 21.9% to oleate (18:1), and 25.3% to linoleate (18:2) as major components.

NEFA preparation

The NEFA preparation was performed as described (37). Briefly, 10 mmol palmitate, oleate, and linoleate, respectively, were dissolved in hexane at room temperature. Subsequently, 10 g celite was added and hexane was evaporated by creating a vacuum while the celite was stirred. Then, the appropriate media for pancreatic islets or α TC1c9 cells was added to the powder and further stirred for 1 h. Celite was separated by centrifugation, and the supernatant fluid was sterile filtered. NEFA concentrations were determined and adjusted to the target concentrations. The ratio in the NEFA mix used for the incubation experiments was palmitate:oleate:linoleate at 38:33:29.

Glucagon and insulin secretion assays and determination of intracellular triglyceride content in murine pancreatic islets

For each condition, 10 handpicked islets were collected into incubation media (DMEM containing 5.55 mmol glucose/L, 0.25% bovine serum albumin (BSA), 1.5 g NaHCO_3 /L, and 15 mmol HEPES/L) in one well of a 96-well plate. Then, media was removed and switched either to human plasma or to low- or high-glucose incubation media including 3.89 or 13.88 mmol glucose/L, respectively, in the absence or presence of TGRLs/NEFA and incubated for 1 h. Then, aliquots of the media were removed and assayed for insulin and glucagon concentrations. Subsequently, islets were fixed in 3.7% formalin and then stained with oil red O according to Koopman et al (38). Confocal microscopy was performed by using a microlens-enhanced Nipkow disk-based confocal system UltraVIEW RS (Perkin Elmer) on an Olympus IX-70 inverse microscope with a Texas red filter. Quantification of lipid droplets was performed from a mid section through the islets by an analysis using QuantityOne (Biorad).

Glucagon secretion assay and determination of intracellular triglyceride content in α TC1c9 cells

Cells were switched from DMEM containing 16.65 mmol glucose/L, 10% fetal calf serum, 0.25% BSA, 1.5 g NaHCO_3 /L, and 15 mmol HEPES/L to starvation media (DMEM containing 5.55 mmol glucose/L, 1.5 g NaHCO_3 /L, 0.25% BSA, and 15 mmol HEPES/L) and incubated for 30 min. After starvation, media was replaced by starvation media in the absence or presence of 10 nmol γ -aminobutyric acid (GABA)/L at increasing concentrations of lipids for 1 h. Then, aliquots of the media were removed and assayed for glucagon concentrations. Subsequently, cells were fixed in 3.7% formalin and stained with Oil Red O according to Koopman et al (38). Imaging was per-

formed on an Olympus IX-70 inverse microscope with a Texas red filter.

Controls for cell viability and cytotoxicity

To test whether TGRL or NEFA preparations exert toxic effects on α TC1c9 cells, we analyzed cell viability after lipid treatment using trypan blue exclusion. Viability was found to be equal to that of nontreated cells. For all these assays, H_2O_2 was used as a positive control. Apoptosis and necrosis on incubation with lipids were excluded by an annexin V/propidium iodide stain.

Membrane isolation

After GABA and lipid incubation for 1 h, α TC1c9 cells were washed with ice-cold phosphate-buffered saline and sonified in a buffer containing 5 mmol Tris-HCl buffer/L (pH 7.4), 2 mmol EDTA/L, and various protease inhibitors. The homogenate was centrifuged for 15 min at $500 \times g$ at 4°C . The supernatant fluid was again centrifuged at $45,000 \times g$ for 15 min. The pellet was washed with the sonification buffer and resuspended in 75 mmol Tris-HCl/L (pH 7.4) containing 12.5 mmol MgCl_2 /L and 5 mmol EDTA/L.

Western blotting

Protein extracts from total cell lysates and from membrane preparations were then analyzed by Western blotting. All steps for Western blot analysis were performed as previously described (33) by using 2 different A-type GABA (GABA-A) receptor antibodies: first, a monoclonal anti GABA-A receptor β 2,3 chain antibody (clone BD17) from Millipore, and second, a rabbit polyclonal GABA-A receptor β 3 (ab4046) antibody from Abcam. As a loading control for membrane-associated GABA-A receptor, a rabbit polyclonal antibody to spectrin (α and β) from Abcam was used.

Statistical analysis

Statistical analyses were carried out by repeated-measures ANOVA with Statistica version 8.0 (StatSoft). When differences were detected by univariate analysis, a Bonferroni post hoc test was used. Otherwise, univariate *P* values are shown. All data are presented as means \pm SDs, and significance was assumed at *P* < 0.05.

RESULTS

Effect of the test meals on plasma triglyceride, NEFA, insulin, glucose, and glucagon concentrations in humans

We first conducted a study in a crossover design with 7 healthy volunteers to assess the effect of postprandial lipemia on glucagon kinetics. Two different isocaloric fat-enriched test meals were designed, such that meal 1 increased both plasma triglyceride and NEFA concentrations, and meal 2 increased triglyceride concentrations only. Meal 1 and meal 2 were administered at 1000 to our study subjects on separate days to study the role of plasma triglyceride and NEFA concentrations in the regulation of plasma glucagon concentrations. A comparison of



the triglyceride concentrations between the postabsorptive state at 0800 and the postprandial state at 1300 showed a significant increase from 1.00 ± 0.28 to 2.19 ± 0.74 mmol/L with meal 1 ($P < 0.001$) and from 1.03 ± 0.36 to 2.08 ± 0.61 mmol/L with meal 2 ($P = 0.003$) without a statistical difference between the 2 meals (Figure 1A). On the other hand, NEFA concentrations rose from 0.50 ± 0.16 to 0.76 ± 0.14 mmol/L with ingestion of meal 1 ($P = 0.05$), but decreased from 0.47 ± 0.24 to 0.27 ± 0.14 mmol/L with ingestion of meal 2 ($P = 0.309$), leading to a highly significant difference of postprandial NEFA concentrations ($P < 0.001$) between the 2 meal types (Figure 1B). Insulin concentrations increased from 7.39 ± 3.21 at 0800 to 12.39 ± 4.97 mU/L at 1300 with meal 1 ($P = 0.736$) and from 5.84 ± 2.17 to 14.50 ± 8.68 mU/L with meal 2 ($P = 0.018$); however, the change was significant only with meal 2, which contained a higher portion of carbohydrates than did meal 1 (Figure 1C). In regard to glucose concentrations, no significant difference was found between the postabsorptive and the postprandial states with meal

1 (5.59 ± 0.83 compared with 5.48 ± 0.76 mmol/L; $P = 0.74$) and meal 2 (5.77 ± 0.77 compared with 5.22 ± 0.85 mmol/L; $P = 0.5$) (Figure 1D).

Plasma glucagon concentrations rose significantly from 0800 to 1300 with either of the 2 meals—with meal 1 from 80.7 ± 18.2 to 104.2 ± 17.5 ng/L ($P = 0.017$) and with meal 2 from 86.1 ± 16.2 to 106.8 ± 30.1 ng/L ($P = 0.028$)—without a significant difference between the meals (Figure 1E).

To assess whether the plasma concentrations of regulators of glucagon secretion are modulated by postprandial lipemia, we analyzed plasma concentrations of arginine which stimulates glucagon secretion and of the 2 incretins GIP and GLP-1. GIP stimulates glucagon release from α cells, whereas GLP-1 has been shown to inhibit it. These analyses showed a postprandial increase in GIP concentrations, whereas plasma concentrations of GLP-1 and arginine showed no change postprandially (Table 1). To rule out circadian changes in glucagon concentrations, we measured glucagon concentrations at 0800 and at 1300 without

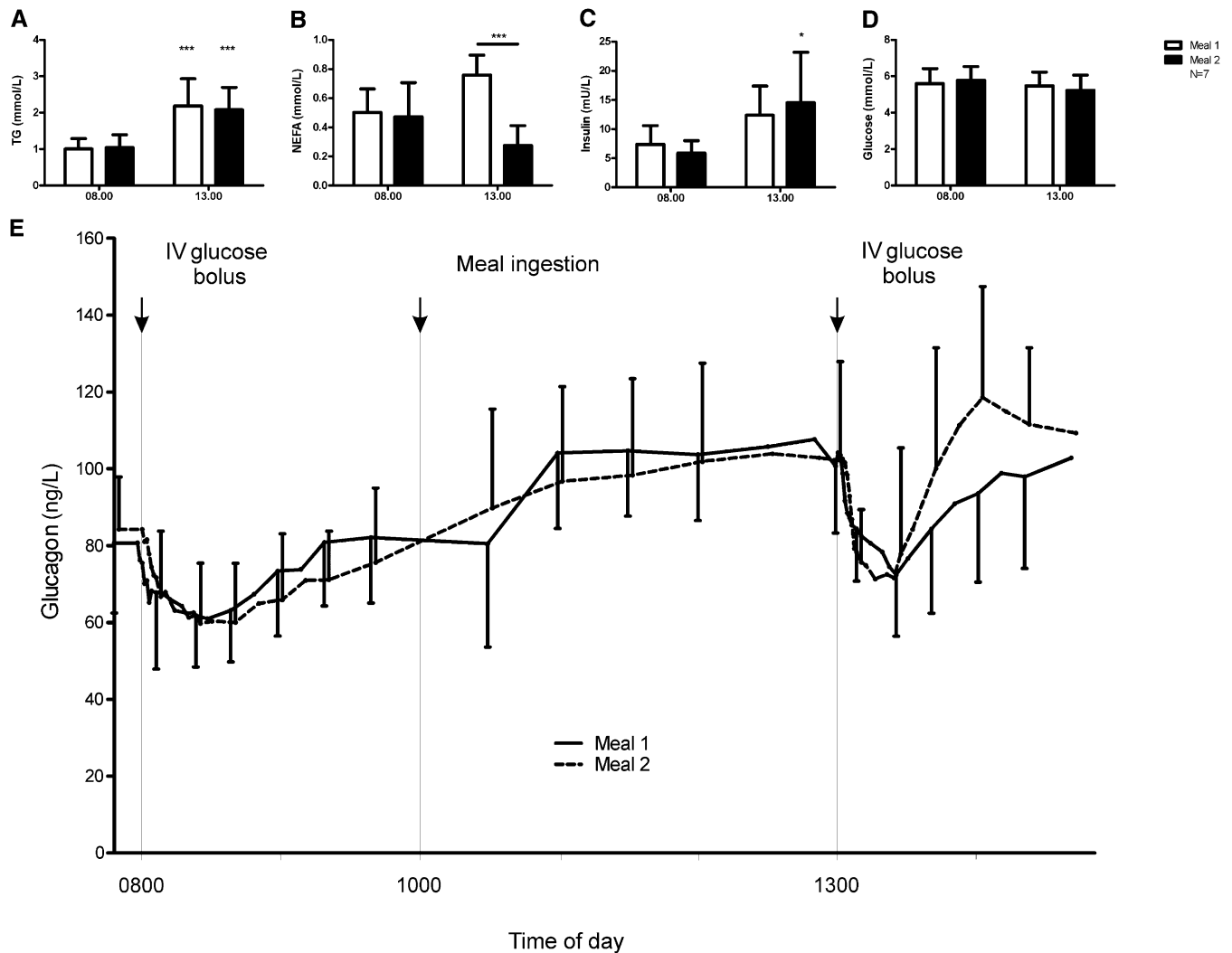


FIGURE 1. Mean (\pm SD) plasma concentrations of triglycerides, NEFAs, insulin, glucose, and glucagon on the days of the experiments. Plasma triglycerides, NEFAs, insulin, and glucose were measured postabsorptively before the first glucose bolus at 0800 and before the second glucose bolus at 1300 for meal 1 and meal 2. Plasma profiles of glucagon are shown for meal 1 (continuous line) and meal 2 (broken line). The glucose boli (IV) and meals were provided at the times indicated. ****Significantly different from the respective meal type postabsorptively at 0800 (repeated-measures ANOVA): * $P < 0.05$, *** $P < 0.001$. The time-by-treatment interaction was significant for each of the variables. IV, intravenous; NEFA, nonesterified fatty acid; TG, triglycerides.



TABLE 1

GISG, AGISG, and concentrations of GIP, GLP-1, arginine, and the nadir of glucagon on the days of the experiments¹

Variable	Postabsorptive state at 0800	Postprandial state at 1300	<i>P</i> value ²
GIP (ng/mL)			
Meal 1	33 ± 15	357 ± 156	<0.001
Meal 2	36 ± 14	256 ± 124	<0.001
GLP-1 (pg/L)			
Meal 1	6.88 ± 0.77	7.11 ± 0.65	0.83
Meal 2	6.66 ± 0.55	6.80 ± 0.44	0.74
Arginine (μmol/L)			
Meal 1	601 ± 149	492 ± 154	0.48
Meal 2	506 ± 189	440 ± 128	0.48
Nadir of glucagon (ng/mL)			
Meal 1	60.5 ± 13.2	73.9 ± 14.7	<0.001
Meal 2	58.7 ± 17.7	72.4 ± 18.8	0.009
GISG (ng/mL)			
Meal 1	23.7 ± 9.9	34.2 ± 7.2	0.49
Meal 2	31.2 ± 5.1	39.5 ± 13.1	0.49
AGISG (ng · min/L)			
Meal 1	923 ± 539	1254 ± 395	0.62
Meal 2	1252 ± 368	967 ± 376	0.62

¹ All values are means ± SDs. Plasma concentrations of GIP, GLP-1, and arginine and the nadir of glucagon were measured postabsorptively before the first glucose bolus at 0800 and postprandially before the second glucose bolus at 1300 in the 7 study subjects. GISG and AGISG were calculated as described in Subjects and Methods. AGISG, area of glucose-induced suppression of glucagon; GIP, glucose-dependent insulinotropic polypeptide; GISG, glucose-induced suppression of glucagon concentration; GLP-1, glucagon-like peptide 1.

² Significance was assumed at *P* < 0.05 (repeated-measures ANOVA).

prior meal ingestion and found no significant difference (84.7 ± 20.4 compared with 70.6 ± 20.0 ng/L; *P* = 0.28).

Glucagon suppression in relation to insulin concentrations is inadequate during postprandial lipemia in humans

With the intravenous glucose injections at 0800 and 1300, insulin concentrations showed a sharp rise, and AIR_G was significantly higher in the postprandial state at 1300 than postabsorptively at 0800 with either of the 2 meals (*P* < 0.001); the AIR_G rose from 63 ± 27 to 139 ± 91 mU/L with meal 1 and from 43 ± 18 to 121 ± 81 mU/L with meal 2.

The glucagon concentrations immediately decreased with glucose injections, whereby the nadirs of glucagon concentrations were higher in the postprandial state than in the postabsorptive state with either of the 2 meals (Figure 1E, Table 1). GISG and AGISG showed no differences between the postabsorptive and postprandial states of either of the 2 meal types (Table 1).

TGRLs elevate glucagon secretion and blunt insulin-induced glucagon suppression in mouse pancreatic islets

Mouse pancreatic islets were incubated with postabsorptive and postprandial whole human plasma at a glucose concentration of 4.72 mmol/L. Plasma was isolated from one of the study subjects after an overnight fast and 4 h after ingestion of meal 1. The triglyceride concentration in postabsorptive plasma was 1.11 mmol/L and in postprandial plasma was 2.80 mmol/L,

which closely corresponded to the mean triglyceride concentrations measured in humans. In accordance with the observations in humans, incubation with postprandial plasma increased glucagon secretion from α cells compared with postabsorptive plasma (556 ± 157 compared with 421 ± 132 pg/min per islet; *P* = 0.005; Figure 2A). The insulin concentration in incubation media did not change on incubation with postprandial plasma (1.21 ± 0.40 with postabsorptive compared with 1.08 ± 0.47 pg/min per islet with postprandial plasma; Figure 2B).

Then, islets were incubated in the absence or presence of highly purified TGRLs at a triglyceride concentration of 2.80 mmol/L. We also studied the effect of those NEFAs shown by gas chromatography to be quantitatively predominant in our TGRL fraction (NEFA mix, ie, palmitate, oleate, and linoleate) at a final concentration of 0.8 mmol/L. In accordance with the experiments using whole human plasma, glucagon secretion rose in the presence of TGRLs from 447 ± 63 to 602 ± 112 pg/min per islet (*P* = 0.012) (Figure 2C; second white bar). The NEFA mix increased glucagon secretion from 447 ± 63 to 545 ± 89 pg/min per islet (*P* = 0.044; Figure 2C; rightmost white bar).

To simulate the intravenous glucose injections performed in humans, islets were incubated with media, the glucose concentration of which was adjusted to 13.88 mmol/L to correspond with the mean maximum glucose concentration achieved on injection of the glucose boli in humans (Figure 2C; black bars). Glucagon concentrations were suppressed by high-glucose media in the absence of lipids (447 ± 63 compared with 295 ± 94 pg/min per islet; *P* = 0.031). However, when islets were incubated in the presence of TGRLs, glucagon concentrations increased with high-glucose media (602 ± 112 compared with 905 ± 123 pg/min per islet; *P* < 0.001). Incubation with the NEFA mix showed similar effects as TGRL (545 ± 89 compared with 840 ± 135 pg/min per islet; *P* < 0.001).

Analyzing insulin concentrations, we found that incubation of islets with high glucose led to the expected increase in insulin secretion (Figure 2D). In the absence of lipids, insulin secretion rose from 0.91 ± 0.30 to 1.94 ± 0.37 pg/min per islet (*P* < 0.001). With TGRL incubation, insulin secretion increased from 1.26 ± 0.58 to 2.36 ± 0.56 pg/min per islet (*P* < 0.001) and with the NEFA mix from 1.21 ± 0.42 to 2.49 ± 0.59 pg/min per islet (*P* < 0.001).

TGRLs elevate glucagon secretion and blunt GABA-induced glucagon suppression in cultured αTC1c9 cells

Cultured αTC1c9 cells were then used to validate our data obtained from pancreatic islets and to elucidate the underlying molecular mechanism. Because αTC1c9 cells are known to lose their responsiveness to insulin as a result of the increasing passage of the cell line, we had to use the chemical transmitter GABA to assess the effect of lipids on glucagon secretion. GABA is co-secreted with insulin and also suppresses glucagon secretion (39).

As with pancreatic islets, TGRL incubation increased glucagon secretion from αTC1c9 cells; in the absence of GABA, significance was observed at 1.79 mmol/L TGRL triglycerides and in the presence of GABA at 0.90 mmol/L (Figure 3A). As expected, GABA stimulation reduced glucagon secretion from αTC1c9 cells. In accordance with our observations in pancreatic islets, GABA-induced glucagon suppression was blunted in

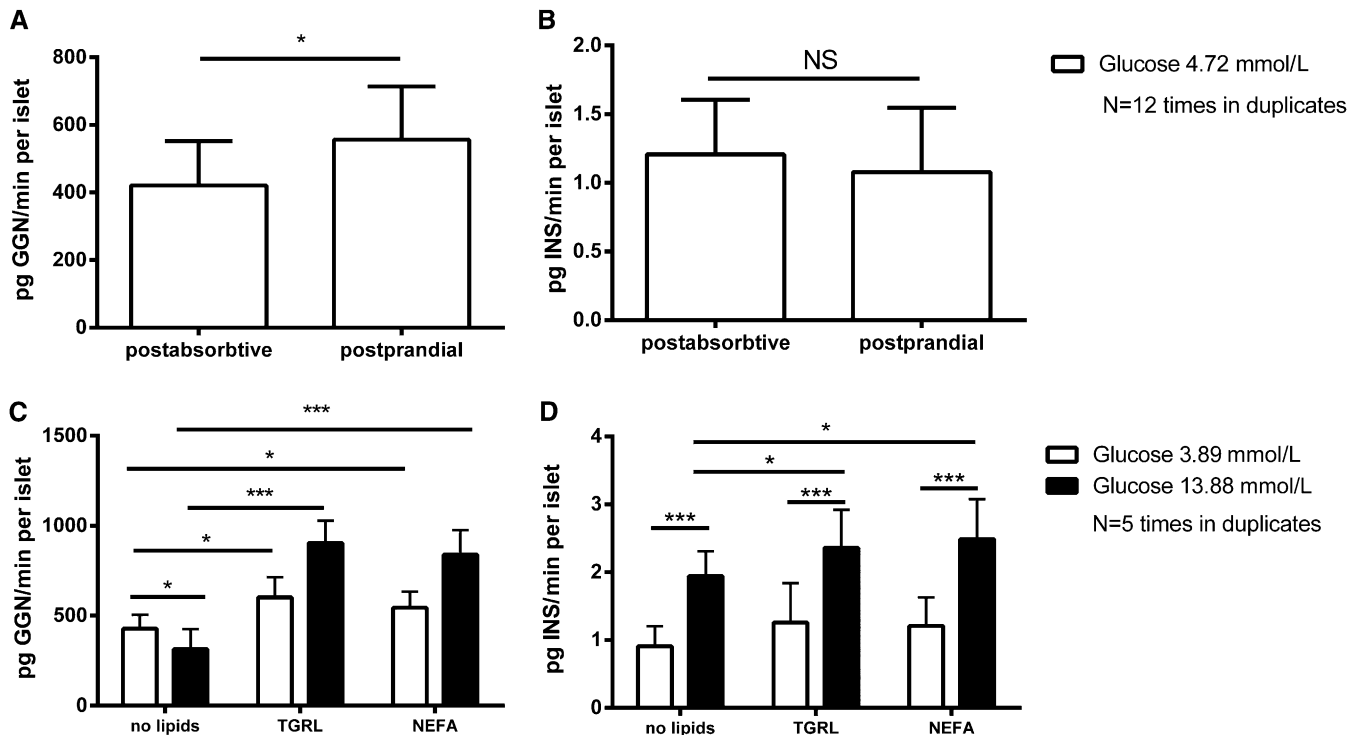


FIGURE 2. Effect of human plasma, TGRLs, and the NEFA mix on insulin and glucagon secretion in mouse pancreatic islets. Islets were incubated with postabsorptive and postprandial whole human plasma at a glucose concentration of 4.72 mmol/L for 1 h (A and B) and in subsequent experiments with low glucose (3.89 mmol/L) and high glucose (13.88 mmol/L) incubation media in the absence or presence of lipids (TGRLs, NEFA mix) for 1 h (C and D). Subsequently, glucagon and insulin concentrations were analyzed. Data are expressed as means \pm SDs. Significant differences (repeated-measures ANOVA): * $P < 0.05$, *** $P < 0.001$. INS, insulin; GGN, glucagon; NEFA, nonesterified fatty acid; TGRL, triglyceride-rich lipoprotein.

the presence of TGRLs, even at a concentration of 0.22 mmol/L TGRL triglycerides (Figure 3A). Incubation with the NEFA mix led to effects comparable with those of TGRLs (Figure 3B). When cells were incubated with the NEFAs individually, the blunting of GABA-induced glucagon suppression was observed with each of the 3 NEFAs at a concentration of 0.125 mmol/L, whereas the increase in glucagon secretion in the absence of GABA was observed only with oleate (Figure 3, C, D, and E).

TGRLs reduce GABA-A receptor activation in cultured α TC1c9 cells

Next, we sought to elucidate the molecular mechanisms of TGRL-induced pancreatic α cell dysfunction. For this purpose, we studied the effect of TGRLs and the NEFA mix on the translocation of GABA-A receptor to the cell membrane in α TC1c9 cells. As shown in Figure 4A, lipid incubations significantly reduced GABA-A receptor translocation in both the absence and presence of GABA, whereas the total cellular content of GABA-A receptors did not change with lipid incubations.

TGRLs increase intracellular triglyceride content in pancreatic islets and α TC1c9 cells

Confocal microscopy was used to assess the intracellular triglyceride content in mouse islets. As shown in Figure 4B, incubations with TGRLs and the NEFA mix led to an increase in

intracellular triglycerides. Accordingly, lipid incubation resulted in triglyceride accumulation in α TC1c9 cells (data not shown).

DISCUSSION

Here we report on a potential cause and on the underlying molecular mechanism by which the abnormalities in glucagon kinetic characteristic of type 2 diabetes may develop. Our data strongly suggest that postprandial TGRLs are capable of causing these abnormalities by reducing GABA-A receptor activation in pancreatic α cells.

In the transition from a fasting state to a state of postprandial lipemia, plasma glucagon concentrations rose to a comparable degree with ingestion of either of the 2 test meals by our study population. The increase in postprandial glucagon concentration could not be accounted for by postprandial GLP-1 concentrations, arginine concentrations, glucose/insulin concentrations, or by diurnal variation of plasma glucagon concentrations. On the other hand, the postprandial increase in GIP concentrations may be a potential explanation for the increase in postprandial glucagon concentrations. Alternatively, lipids might have caused this increase (24–26). Because the 2 meals induced sharply contrasting plasma NEFA profiles, we concluded that plasma NEFA had no major role in elevating postprandial glucagon concentrations. This pointed to TGRLs, the plasma concentrations of which were elevated to a comparable degree after ingestion of meals 1 and 2.

The experiments on the suppressibility of glucagon concentrations by the intravenous glucose injections showed that GISG

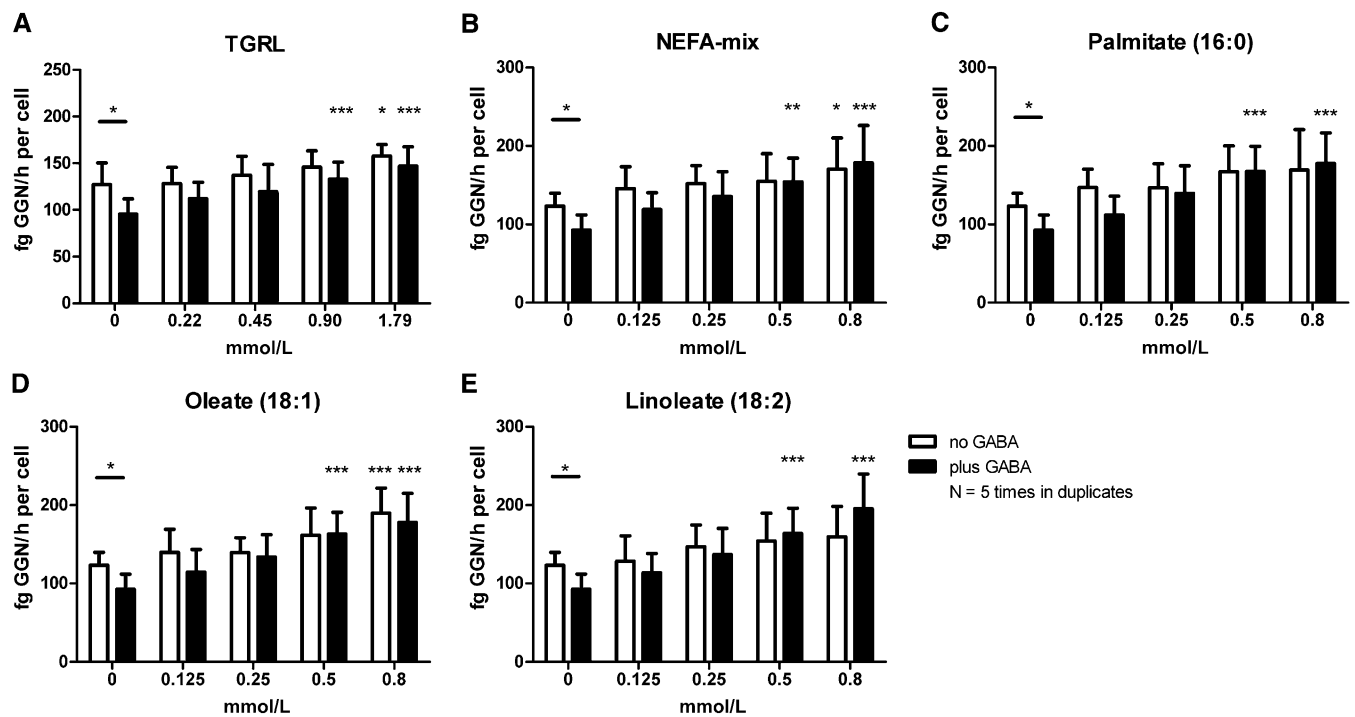


FIGURE 3. Effect of TGRLs, NEFA mix, palmitate, oleate, and linoleate on glucagon secretion in cultured α TC1c9 cells. α TC1c9 cells were incubated with incubation media in the absence or presence of GABA at increasing lipid concentrations for 1 h. Subsequently, glucagon concentrations were analyzed. A: α TC1c9 cells were incubated at increasing TGRL triglyceride concentrations as indicated. B: α TC1c9 cells were incubated with NEFA mix at increasing concentrations as indicated. C-E: α TC1c9 cells were incubated with palmitate, oleate, and linoleate at increasing concentrations, as indicated. Data are expressed as means \pm SDs. Significantly different from the respective condition in the absence of lipids (repeated-measures ANOVA): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. fg, fasting; GABA, γ -aminobutyric acid; GGN, glucagon; NEFA, nonesterified fatty acid; TGRL, triglyceride-rich lipoprotein; α TC1c9, alpha TC 1 clone 9.

and AGISG were statistically not different between the post-absorptive and postprandial states. However, because the increase in AIR_G was \sim 2-fold higher in the postprandial state than in the postabsorptive state with either of the 2 meals, we concluded that the suppression of glucagon during postprandial lipemia might be inadequate. Inadequate insulin-induced suppression of glucagon in relation to the prevailing insulin concentration has been observed in type 2 diabetes and impaired glucose tolerance (40–42).

Taken together, these experiments in humans pointed to postprandial TGRLs as a potential cause of the postprandial increase in glucagon concentrations and, furthermore, suggested an inadequate postprandial insulin-induced suppression of glucagon concentrations brought about by TGRLs. However, because the experiments on the suppressibility of glucagon concentrations by intravenous glucose showed differences only in light of plasma insulin concentrations, and this study in humans did not rule out other causative factors—such as GIP, neural regulation, impaired paracrine regulation, or a memory effect—brought about by application of 2 consecutive glucose boli in the dysregulation of glucagon kinetics, we initiated a series of in vitro experiments by using isolated mouse pancreatic islets and cultured pancreatic α TC1c9 cells to study the putative role of TGRLs in altering glucagon kinetics.

Incubation of mouse pancreatic islets with postprandial human plasma and, subsequently, with highly purified TGRLs revealed that lipid incubations cause an increase in glucagon secretion and, moreover, not only impair insulin-induced suppression of glucagon secretion, but even increase it, supporting our conclusions from the in vivo study. Because TGRL

may interfere with glucagon secretion by pancreatic α cells through whole lipoprotein particle uptake or through NEFAs after hydrolysis of the triglyceride component in the lipoprotein particle by endothelial lipoprotein lipase, we also studied the effect of those NEFAs that are quantitatively predominant in our TGRL fraction. This NEFA mix elicited effects comparable, but weaker, to those of whole lipoprotein particles in mouse islets. Supporting the validity of the in vitro system used, our experiments with mouse pancreatic islets showed an accentuated increase of glucose-induced insulin secretion in the presence of TGRL/NEFA—a phenomenon noted in vivo in humans (43). Experiments using pancreatic α TC1c9 cells strengthened our conclusions drawn from the experiments with mouse pancreatic islets.

Next, we sought to elucidate the underlying molecular mechanisms of pancreatic α cell dysfunction. Insulin induces activation of GABA-A receptors in α cells by receptor translocation via an Akt kinase-dependent pathway leading to membrane hyperpolarization in α cells and, ultimately, to suppression of glucagon secretion (44). Defects in this pathway have been postulated to contribute to diabetic hyperglycemia (6, 10, 44, 45). Our experiments showed that TGRL and NEFA incubations reduced translocation of GABA-A receptors to the cell membrane, providing a plausible explanation for the increased glucagon concentrations with lipid incubations.

We proposed the accumulation of intracellular triglycerides as a cause of the abnormalities in pancreatic α cell function induced by TGRLs, because many studies have shown that the accumulation of intracellular triglycerides and their metabolites

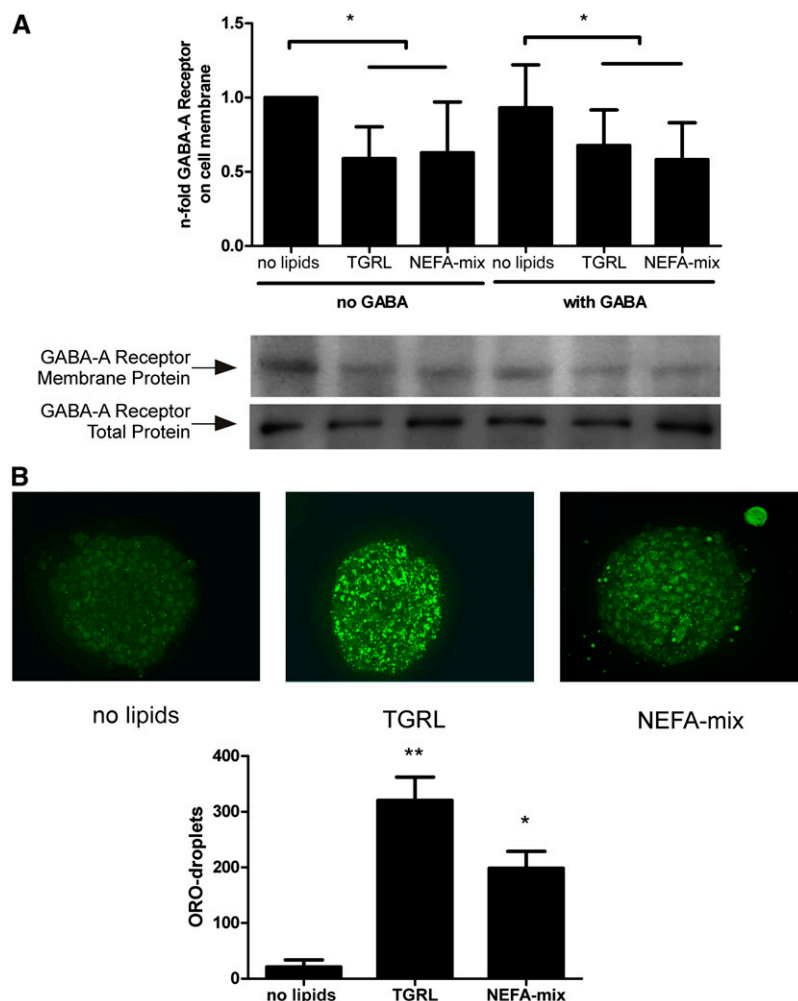


FIGURE 4. Reduced GABA-A receptor translocation to the cell membrane in α TC1c9 cells and increased triglyceride content in mouse pancreatic islets on lipid incubation. A: α TC1c9 cells were incubated with incubation media with or without GABA in the absence or presence of lipids (TGRLs or the NEFA mix). Western blot analysis with antibodies to the GABA-A receptor was then performed in membrane preparations (top) and in total cell lysates (bottom). The bar graph shows the quantification of GABA-A receptors in cell membrane preparations, and the bars represent the means \pm SDs for 5 independent experiments performed in duplicates. Significant differences (repeated-measures ANOVA): * $P < 0.05$. B: The triglyceride content of mouse islets cells was measured on staining with ORO in the absence of lipids (TGRLs or the NEFA mix). The bar graph shows the quantification of ORO droplets in mouse islets, and significant differences are indicated (repeated-measures ANOVA): * $P < 0.05$, ** $P < 0.01$. GABA, γ -aminobutyric acid; GABA-A, A-type γ -aminobutyric acid receptor; NEFA, nonesterified fatty acid; ORO, oil red O; TGRL, triglyceride-rich lipoprotein; α TC1c9, alpha TC 1 clone 9.

results in cellular dysfunction (46, 47), presumably by inducing oxidative stress (48).

Although the physiologic significance and quantitative contribution of postprandial TGRLs to the abnormalities of glucagon regulation in type 2 diabetes remain to be established, the current study supports our hypothesis that, besides obesity, postprandial lipemia could be viewed as a link between Western dietary habits and the pathophysiological changes characteristic of impaired glucose tolerance and type 2 diabetes. This hypothesis is strengthened by previous studies from our group and others on the effect of TGRLs on skeletal muscle insulin sensitivity (33), hepatic insulin sensitivity (49), and pancreatic β cell function (50) and by studies on insulin resistance during postprandial lipemia (31, 51). Because of the concept that atherosclerosis is a postprandial phenomenon (52), it is tempting to speculate that postprandial TGRLs represent a common factor in the etiology of the diverse defects characteristic of impaired glucose tolerance and type 2 diabetes.

The authors' responsibilities were as follows—AN and MTP: designed the research and drafted the manuscript; AN, CC, TT, MH, CM, RA-Z, KS, MIK, and MTP: performed the research; AN, MIK, and MTP: analyzed the data; and MTP: had primary responsibility for the final content. None of the authors had a conflict of interest regarding any aspect of this research.

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