Single-Dose Metformin Enhances Bile Acid–Induced Glucagon-Like Peptide-1 Secretion in Patients With Type 2 Diabetes

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Context: Despite a position as the first-line pharmacotherapy in type 2 diabetes, the glucose-lowering mechanisms of metformin remain to be fully clarified. Gut-derived modes of action, including suppression of bile acid reabsorption and a resulting increase in glucagon-like peptide-1 (GLP-1) secretion, have been proposed.

Objective: The aim of this study was to assess the GLP-1 secretory and glucometabolic effects of endogenously released bile, with and without concomitant single-dose administration of metformin in patients with type 2 diabetes.

Design: Randomized, placebo-controlled, and double-blinded crossover study.

Setting: This study was conducted at Center for Diabetes Research, Gentofte Hospital, Denmark.

Patients: Fifteen metformin-treated patients with type 2 diabetes; all participants completed the study.

Interventions: Four experimental study days in randomized order with administration of either 1500 mg metformin or placebo in combination with intravenous infusion of cholecystokinin (0.4 pmol $\times$ kg^{-1} $\times$ min^{-1}) or saline.

Main Outcome Measure: Plasma GLP-1 excursions as measured by baseline-subtracted area under the curve.

Results: Single-dose metformin further enhanced bile acid–mediated induction of GLP-1 secretion (P = 0.02), whereas metformin alone did not increase plasma GLP-1 concentrations compared with placebo (P = 0.17). Metformin, both with (P = 0.02) and without (P = 0.02) concomitant cholecystokinin-induced gallbladder emptying, elicited reduced plasma glucose excursions compared with placebo. No GLP-1–mediated induction of insulin secretion or suppression of glucagon was observed.

Conclusions: Metformin elicited an enhancement of the GLP-1 response to cholecystokinin-induced gallbladder emptying in patients with type 2 diabetes, whereas no derived effects on insulin or glucagon secretion were evident in this acute setting. **(*J Clin Endocrinol Metab* 102: 4153–4162, 2017)**

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Abbreviations: ASBT, apical sodium-dependent bile acid transporter; bsAUC, baseline-subtracted area under curve; CCK, cholecystokinin; DPP-4, dipeptidyl peptidase-4; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide-1; TGR5, Takeda G protein–coupled receptor.

Metformin is the recommended first-line pharmacotherapy of type 2 diabetes and continues to be the most widely used glucose-lowering drug in these patients (1). The glucose-lowering mechanisms of metformin have not been fully clarified despite the fact that the drug class of biguanides was introduced >50 years ago (2). Suppression of endogenous hepatic glucose production alongside increased peripheral insulin sensitivity and glucose uptake in skeletal muscle and adipose tissue are traditionally accepted as glucose-lowering modes of action of metformin (3). However, a range of potential gut-derived glucose-lowering mechanisms of metformin has been proposed during recent years. As recently reviewed by McCreight *et al.* (4), these mechanisms include increased intestinal glucose utilization (5), adenosine monophosphate-activated protein kinase-mediated activation of vagal afferents with subsequent reduction of hepatic glucose production (6), modification of the enterohepatic bile acid circulation (7), increased glucagon-like peptide-1 (GLP-1) secretion from intestinal L cells (8–11), and/or changes in gut microbiota composition (12). The focus of the current study was the potential GLP-1–stimulatory effect of single-dose metformin itself and metformin's possible potentiation of bile acid–mediated GLP-1 secretion in the acute setting.

Metformin has been demonstrated to reduce the active reabsorption of bile acids in the terminal ileum via inhibition of the apical sodium-dependent bile acid transporter (ASBT) (7, 8, 13). The subsequent modifications of bile acid–mediated activation of the nuclear farnesoid X receptor (FXR) and the cell surface Takeda G protein–coupled receptor (TGR5) might influence GLP-1 secretion from L cells. In line with this, Trabelsi *et al.* (14) reported that bile acid–mediated FXR activation in L cells inhibited intracellular glycolytic pathways with a subsequent reduction in GLP-1 production and secretion, whereas bile acid–mediated activation of TGR5 has been demonstrated to induce GLP-1 secretion via increased adenosine triphosphate/adenosine 5′-diphosphate ratio and cyclic adenosine 5′-monophosphate levels in the L cells (15). An effect of TGR5 activation on GLP-1 secretion is supported by studies reporting increased plasma GLP-1 concentrations as a result of rectal or colonic administration of bile acids in patients with type 2 diabetes (16, 17). Furthermore, the specific ASBT inhibitors GSK2330672 and 264W94 have been reported to cause marked reductions of plasma glucose levels in humans and increased GLP-1 concentrations in rodents, respectively (18, 19).

In the current study, we extend our previous findings of GLP-1 secretion following single-dose metformin and/or cholecystokinin (CCK)-mediated gallbladder emptying in healthy young men (20) to patients with type 2 diabetes. Thus, we evaluated the isolated and combined effects of CCK-mediated gallbladder emptying and single-dose metformin on GLP-1 secretion and glucose metabolism in patients with type 2 diabetes.

Subjects and Methods

Study design

This study was a randomized, placebo-controlled, and double-blinded crossover trial with plasma GLP-1 excursion as the primary endpoint. Each of 15 participants was submitted to 4 experimental study days with administration of either single-dose 1500 mg metformin or placebo in combination with intravenous infusion of CCK or saline. Thus, on experimental days, subjects received placebo + saline, metformin + saline, placebo + CCK, or metformin + CCK, respectively. The sequence of the 4 study days was randomized, and the experimental days were performed within a period of maximum 4 months and with at least 2 weeks between individual visits. Patients were instructed to pause ongoing single-drug glucose-lowering treatment with metformin 7 days prior to each of the individual study days. Metformin treatment was resumed in the additional timespan between study days.

Study approval

This study was performed at Center for Diabetes Research, Gentofte Hospital, University of Copenhagen, after approval from the Ethics Committee of the Capital Region of Denmark (registration no. H-15007280). The study was registered at the Danish Data Protection Agency (registration no. 2012-58-0004) and ClinicalTrials.gov (ID: NCT02497313). Oral and written informed consent was received from participants prior to inclusion in the study, which was conducted in accordance with the principles of the Declaration of Helsinki (seventh revision, 2013).

Experimental procedures

At each of the 4 study days, patients arrived at the laboratory after an overnight total fast and remained fasted for nutrients throughout the 4-hour investigation period. Two cannulas were inserted in the cubital veins bilaterally for collection of blood samples and administration of saline or CCK. The forearm chosen for blood sampling was placed under a heating pad (42°C) for arterialization of blood throughout the experiment. Baseline blood samples were drawn after a 10-minute rest and heat up of the hand.

At time 0 minutes, 1500 mg study drug (metformin/placebo) was administered alongside a 100 ml watery mixture of 1.500 mg acetaminophen for indirect assessment of gastric emptying. Subsequently, a 60-minute intravenous 40 ml infusion of saline or CCK (0.4 pmol sulfated CCK-8 $\times$ kg^{-1} $\times$ min^{-1}) was initiated. A lead time of 5 minutes was applied between oral ingestion of study drug and initiation of infusion. Blood samples were drawn 30, 15, and 0 minutes before and 10, 20, 30, 45, 60, 75, 90, 120, 150, 180, 210, and 240 minutes (plasma glucose alone at times 75, 150, and 210 minutes) after initiation of infusion. Plasma glucose concentrations were measured bedside using a YSI 2300 STAT PLUS analyzer (YSI, Yellow Springs, OH). Radioimmunoassays were applied for measurements of total plasma GLP-1, glucagon, and CCK.

Serum C-peptide was measured using a two-sided electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). Plasma acetaminophen was measured using Johnson & Johnson Vitros 5.1 FS (Ortho Clinical Diagnostics, Raritan, NJ). Gallbladder measurements were performed by ultrasound (GE Health Care, Milwaukee, WI): twice at baseline and again 15, 30, 45, 60, 90, 120, 180, and 240 minutes after start of infusion. Height, width, and length of the gallbladder were estimated for calculation of gallbladder volume by the ellipsoid method. Resting energy expenditure was assessed by indirect calorimetry (Medgraphics, Saint Paul, MN) during 10-minute periods at baseline, and again immediately following stop of infusion at time 60 minutes.

Peptides and materials

Metformin 500 mg (Orifarm, Odense, Denmark) and matching placebo tablets (Pharmacy of the Capital Region, Herlev, Denmark) were applied as study drugs. A total of 1500 mg Pinex® (acetaminophen) effervescent tablets (Actavis, Dublin, Ireland) was used for indirect assessment of gastric emptying. Synthetic sulfated CCK-8 (Bachem, Weil am Rhein, Germany) was formulated for infusion (Pharmacy of the Capital Region, Herlev, Denmark) using 2% human albumin (Statens Serum Institut, Copenhagen, Denmark) and sterilized water with subsequent sterile filtration and testing for microbiological contamination before experimental use.

Calculations and statistical analysis

Results are reported as median values with interquartile range, unless otherwise stated. Basal values of glucose and hormones are reported as the mean of three consecutive measurements (−30, −15, and 0 minutes) prior to administration of study drug and start of infusion. The trapezoidal rule was applied for calculation of area under curve values and presented as baseline-subtracted area under curve (bsAUC), unless otherwise stated. Insulin secretion rate values were calculated by deconvolution of measured C-peptide concentrations and application of population-based parameters for C-peptide kinetics. GraphPad Prism version 7.0 (GraphPad Software, La Jolla, CA) was used for statistical analyses. One-way repeated measures analysis of variance with Fisher's least significant difference *post hoc* test was used to test for variations and differences with a two-tailed P value <0.05 considered statistically significant. Log transformation of non-Gaussian distributed data was performed prior to statistical analysis. No adjustments for multiple comparisons have been performed for analyses of the metabolic parameters outlined above.

Results

Subject characteristics

This study included metformin-treated male (n = 12) and postmenopausal female patients (n = 3) with type 2

Table 1. Metabolic Effects of Metformin and CCK

	Baseline	$bsAUC_{0–240\ min}$
Plasma glucose	mmol/L	mmol/L × min
(a) Pla + saline	8.5 [8.0; 10.2]	−144 [−284; −33][b,d]
(b) Met + saline	9.6 [7.9; 10.4]	−210 [−249; −152][a,c]
(c) Pla + CCK	9.6 [8.2; 10.3]	−145 [−249; −32][b,d]
(d) Met + CCK	9.4 [7.8; 10.6]	−195 [−365; −83][a,c]
rmANOVA	$P = 0.59$	$P = 0.01$
Plasma GLP-1	pmol/L	pmol/L × min
(a) Pla + saline	9.7 [7.0; 12.3]	−19.2 [−259.2; 219.2][c,d]
(b) Met + saline	9.3 [6.3; 10.7]	246.7 [36.7; 421.7][d]
(c) Pla + CCK	9.3 [6.3; 11.7]	129.2 [−1.7; 860.8][a,d]
(d) Met + CCK	7.7 [7.0; 11.7]	678.3 [517.5; 1192.0][a,b,c]
rmANOVA	$P = 0.76$	$P < 0.0001$
Insulin secretion rate	pmol/L × kg^{-1} × min^{-1}	pmol/L × kg^{-1}
(a) Pla + saline	2.3 [1.9; 2.6]	7.6 [−30.1; 52.1]
(b) Met + saline	2.4 [1.7; 2.8]	13.8 [−37.5; 42.1]
(c) Pla + CCK	2.2 [1.9; 2.8]	−10.1 [−44.4; 24.4]
(d) Met + CCK	2.4 [1.9; 3.0]	−18.6 [−71.6; 24.3]
rmANOVA	$P = 0.46$	$P = 0.49$
Serum C-peptide	pmol/L	pmol/L × min
(a) Pla + saline	858.7 [627.7; 962.3]	7755 [−9932; 18,765]
(b) Met + saline	879 [580; 1035]	5937 [−10,270; 16,830]
(c) Pla + CCK	827.3 [667.0; 999.7]	−2455 [−9238; 19,900]
(d) Met + CCK	856.3 [642.0; 1123.0]	3290 [−12,200; 10,815]
rmANOVA	$P = 0.63$	$P = 0.86$
Plasma glucagon	pmol/L	pmol/L × min
(a) Pla + saline	11.3 [7.7; 16.3]	−227.5 [−409.2; −94.2]
(b) Met + saline	15.0 [8.7; 18.0]	−306.7 [−546.7; −64.2]
(c) Pla + CCK	13.3 [8.3; 20.0]	−186.7 [−548.3; −77.5]
(d) Met + CCK	13.7 [8.0; 18.0]	−141.7 [−240.0; 128.3]
rmANOVA	$P = 0.10$	$P = 0.31$

Values are presented as medians with interquartile ranges. Abbreviations: Met, metformin; Pla, placebo; rmANOVA, repeated-measures analysis of variance.

Superscript letters represent P values < 0.05 (rmANOVA *post hoc* test) for individual study days (a-d).

diabetes [median (interquartile range in parentheses) age 66.0 years (61.0; 69.0), bodyweight 89.6 kg (85.0; 100.4), body mass index 30.0 kg/m^2 (26.7; 31.0), duration of type 2 diabetes 7.0 years (4.0; 10.0), fasting plasma glucose 8.4 mmol/L (7.7; 9.0), and HbA1c 50.0 mmol/mol (44.0; 56.0)]. The reported fasting plasma glucose and HbA1c concentrations were measured during ongoing metformin treatment at a screening visit prior to inclusion in the study. Key exclusion criteria included any liver or gastrointestinal disease, hypo- or hyperthyroidism, or nephropathy. Ongoing metformin treatment comprised immediate release formulation in all included patients, and the majority of these were treated with 1000 mg twice daily.

Glucose excursions

No significant differences in baseline plasma glucose concentrations were evident between the 4 experimental study days (Table 1). On all 4 study days, these fasting plasma glucose concentrations were significantly increased ($P < 0.05$) compared with the on-treatment baseline concentration reported above, which confirms an effect of metformin washout. Metformin single dose 1500 mg, both with ($P = 0.02$) and without ($P = 0.02$) concomitant CCK-induced gallbladder emptying, elicited reduced plasma glucose excursions as measured by bsAUC compared with placebo (Table 1; Fig. 1). No isolated effects of CCK infusion on plasma glucose concentrations were observed (Table 1).

GLP-1

We observed no significant differences in baseline plasma GLP-1 concentrations between the study days (Table 1). A significant increase in bsAUC for GLP-1 was evident following CCK infusion ($P = 0.02$), whereas isolated administration of metformin did not meet statistical significance in terms of GLP-1 bsAUC compared with the day with placebo and saline ($P = 0.17$) (Table 1; Fig. 1). Interestingly, the combination of metformin and CCK significantly enhanced GLP-1 secretion with significantly higher bsAUC for GLP-1 compared with the days with metformin ($P < 0.01$) and CCK alone ($P = 0.02$) (Table 1; Fig. 1).

Glucagon, C-peptide, and insulin secretion rate

Baseline plasma glucagon and serum C-peptide concentrations did not differ between the 4 experimental

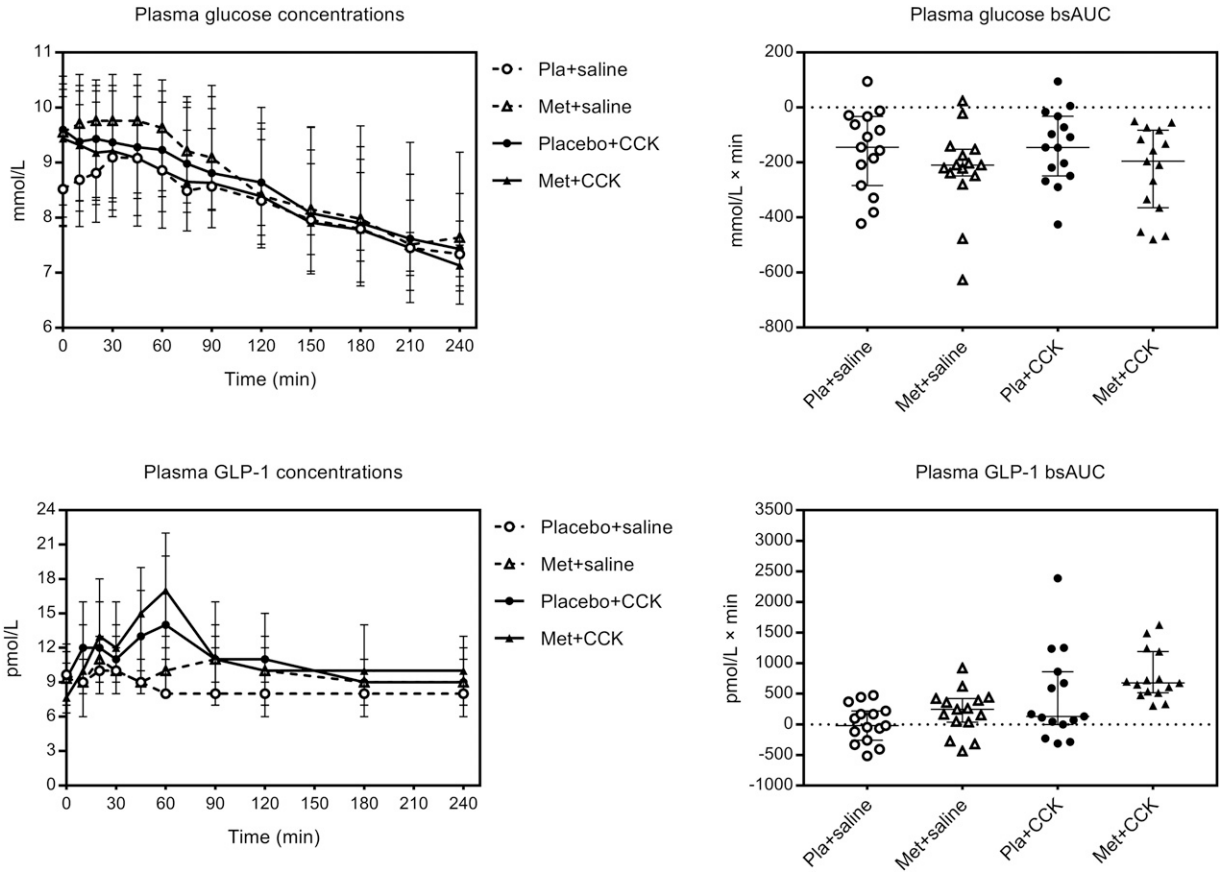

Figure 1. The graphs on top illustrate plasma glucose concentrations (medians with interquartile ranges) and bsAUC$_{240\ min}$ scatter plots (bars represent median and interquartile range) for plasma glucose on the 4 different study days. The graphs below show plasma GLP-1 concentrations (median with interquartile range) and bsAUC$_{240\ min}$ scatter plots (bars represent median and interquartile range) for plasma GLP-1 on the 4 different study days. Met, metformin; Pla, placebo. See Table 1 for information on differences between the 4 days.

study days (Table 1). No metformin or CCK-mediated effects on plasma glucagon excursions were evident (Table 1; Fig. 2). Furthermore, we observed no metformin or CCK-mediated effects on C-peptide excursions or insulin secretion rates (Table 1; Fig. 2).

CCK and gallbladder dynamics

No differences in baseline gallbladder volumes were observed between the experimental study days (Table 2). Infusion of CCK increased the bsAUC for this hormone compared with saline, whereas no effect of metformin on CCK excursions was observed (Table 2). Along these lines, significant gallbladder contraction in terms of reduced minimum volume and bsAUC was evident on the 2 days with CCK infusion compared with saline days (Table 2). Figure 3 provides an overview on plasma CCK concentrations and gallbladder dynamics.

Gastric emptying

Infusion of CCK caused a delay in gastric emptying. Thus, significantly reduced maximum concentrations and a trend toward increased time to maximum plasma concentration of acetaminophen were observed after CCK compared with saline (Table 2; Fig. 4). No effect of metformin on gastric emptying compared with placebo was evident.

Resting energy expenditure

We observed no significant differences in baseline values for resting energy expenditure between the study days. In addition, no differences in δ values (time 0 minutes to time 60 minutes) were evident with estimates (kJ/24 hours) of 49 [−8; 180], 120 [10; 235], 43 [−69; 229], and 91 [−110; 267] for days with placebo + saline, metformin + saline, placebo + CCK, and metformin + CCK, respectively.

Discussion

In this study, we show that CCK-induced gallbladder emptying in patients with type 2 diabetes elicits a significant increase in plasma GLP-1 excursion that can be further enhanced by concomitant administration of single-dose metformin, whereas metformin alone had a minor effect on the increase in plasma GLP-1 concentrations.

The marked and statistically significant increase in plasma GLP-1 concentrations after CCK infusion and concomitant metformin compared with the 3 days with

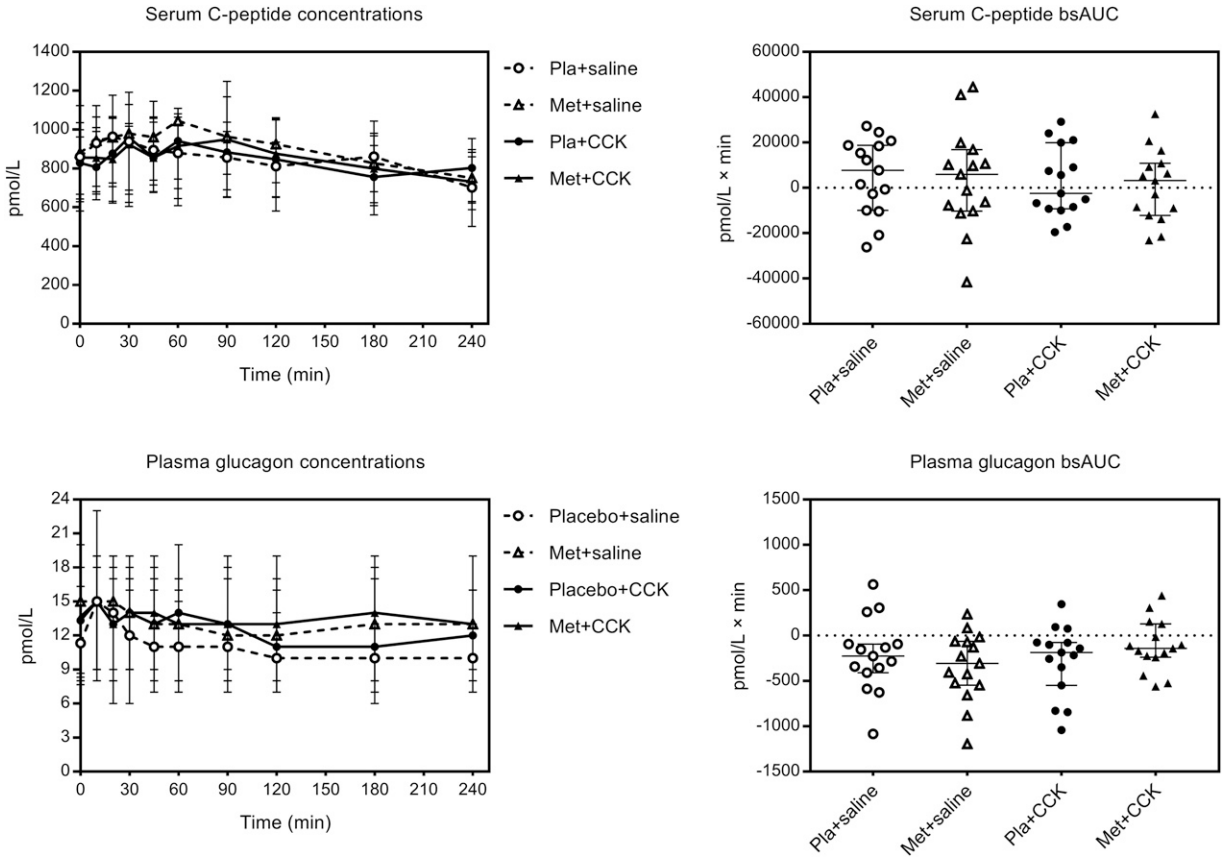

Figure 2. The graphs on top show serum C-peptide concentrations and $bsAUC_{240\ min}$ scatter plots (bars represent median and interquartile range) for serum C-peptide on the 4 different study days. The graphs below illustrate plasma glucagon excursions in relation to the various interventions (median with interquartile range) and $bsAUC_{240\ min}$ scatter plots (bars represent median and interquartile range) for plasma glucagon. Met, metformin; Pla, placebo. See Table 1 for calculations on differences between the 4 days.

Table 2. CCK, Gallbladder Dynamics, and Gastric Emptying

CCK	Baseline (pmol/L)	$bsAUC_{0–240\ min}$ (pmol/L × min)		
(a) Pla + saline	1.2 [0.8; 1.4]	−54 [−87; 10][c,d]		
(b) Met + saline	1.5 [0.7; 1.6]	2 [−42; 69][c,d]		
(c) Pla + CCK	1.3 [1.1; 1.5]	693 [623; 824][a,b]		
(d) Met + CCK	1.1 [0.9; 1.6]	817 [765; 868][a,b]		
rmANOVA	$P = 0.30$	$P < 0.0001$		
Gallbladder Volume	**Baseline (mL)**	**$bsAUC_{0–240\ min}$ (mL × min)**	**V_{min} (min)**	
(a) Pla + saline	43.7 [35.3; 52.2]	797 [−195; 1556][c,d]	36 [30; 42][b,c,d]	
(b) Met + saline	40.7 [36.2; 45.6]	1039 [−401; 1695][c,d]	31 [23; 38][a,c,d]	
(c) Pla + CCK	40.9 [36.0; 45.8]	−2270 [−2749; −1254][a,b]	3 [3; 5][a,b]	
(d) Met + CCK	40.6 [38.5; 50.0]	−2747 [−3582; −1111][a,b]	5 [2; 7][a,b]	
rmANOVA	$P = 0.42$	$P < 0.0001$	$P < 0.0001$	
Acetaminophen	**Baseline (mmol/L)**	**$bsAUC_{0–240\ min}$ (mmol/L × min)**	**C_{max} (mmol/L)**	**T_{Cmax} (min)**
(a) Pla + saline	0.0 [0.0; 0.0]	18 [14; 22]	0.20 [0.17; 0.25][c,d]	20 [10; 20]
(b) Met + saline	0.0 [0.0; 0.0]	17 [14; 21]	0.19 [0.14; 0.22][c,d]	20 [10; 20]
(c) Pla + CCK	0.0 [0.0; 0.0]	15 [15; 21]	0.15 [0.12; 0.17][a,b]	30 [10; 45]
(d) Met + CCK	0.0 [0.0; 0.0]	16 [14; 21]	0.15 [0.10; 0.16][a,b]	45 [10; 90]
rmANOVA	$P = 0.65$	$P = 0.19$	$P < 0.0001$	$P = 0.16$

Values are presented as medians with interquartile ranges. Abbreviations: C_{max}, maximum concentration; Met, metformin; Pla, placebo; rmANOVA, repeated-measures analysis of variance; T_{Cmax}, time to maximum concentration; V_{min}, time to minimum volume.

Superscript letters represent P values < 0.05 (rmANOVA *post hoc* test) for individual study days (a-d).

placebo, metformin, and CCK, respectively, is in line with the findings by Rohde *et al.* (20) in subjects with normal glucose tolerance. Furthermore, a range of studies has reported increased GLP-1 excursions following continuous metformin treatment in both healthy subjects and patients with type 2 diabetes, submitted to standardized meal test or oral glucose load (8–11, 21). In contrast to the study by Rohde *et al.* (20), in which single-dose metformin was reported to induce GLP-1 secretion in healthy subjects, no significant isolated effect of metformin on GLP-1 concentrations was observed in the current study, including patients with type 2 diabetes. This apparent discrepancy might be due to inadequate metformin washout or a result of the established difference in bile acid pool composition between healthy subjects and patients with type 2 diabetes (22). The latter holds potential implications for FXR- and TGR5-mediated GLP-1 secretion (14, 15). In addition, genetic variations in metformin-transporting proteins could be speculated to have influenced the GLP-1 secretory effect of isolated metformin in the current study (23). However, all included patients had acceptable glycemic control with metformin as single-drug glucose-lowering therapy, which points to sufficient absorption capacity in these patients. Our findings are consistent with previous studies by Mannucci *et al.* and Lindsay *et al.* (11, 24) that found no acute metformin-mediated induction of GLP-1 secretion in patients with type 2 diabetes following an oral glucose load and in the fasting state, respectively. The GLP-1 excursions observed in the current study did reveal a tendency to a rise after metformin (particularly toward the end of the experimental days), thereby suggesting a latent onset metformin-induced GLP-1 secretion in line with an adenosine monophosphate–activated protein kinase–mediated GLP-1 secretory effect of metformin previously proposed by Duca *et al.* (6). Studies have demonstrated no changes in fasting plasma GLP-1 concentrations following dipeptidyl peptidase-4 (DPP-4) inhibition or gastric bypass surgery (25, 26). In addition, DPP-4 inhibition was reported to elicit a 5 to 10 pmol/L increase in meal-induced plasma concentrations of intact GLP-1 (25), whereas a 10-fold increase in postprandial total GLP-1 concentrations was observed following gastric bypass surgery in patients with type 2 diabetes (26). However, a comparison with the GLP-1 increments observed in the current study should be performed with caution due to the fasting setting and measurement of only total GLP-1.

The glucose-lowering effect of continuous treatment with metformin is well established (27), whereas the acute metformin-mediated glucometabolic effects in patients with type 2 diabetes have been examined to a much lesser extent. We observed single-dose administration of 1500 mg metformin to elicit statistically significant placebo-corrected reductions in plasma glucose excursions, both alone and in combination with CCK-induced gallbladder emptying, whereas no glucose-lowering effect of isolated CCK infusion was observed. We observed no concomitant metformin-mediated effects on C-peptide or glucagon excursions, which points to no GLP-1–mediated

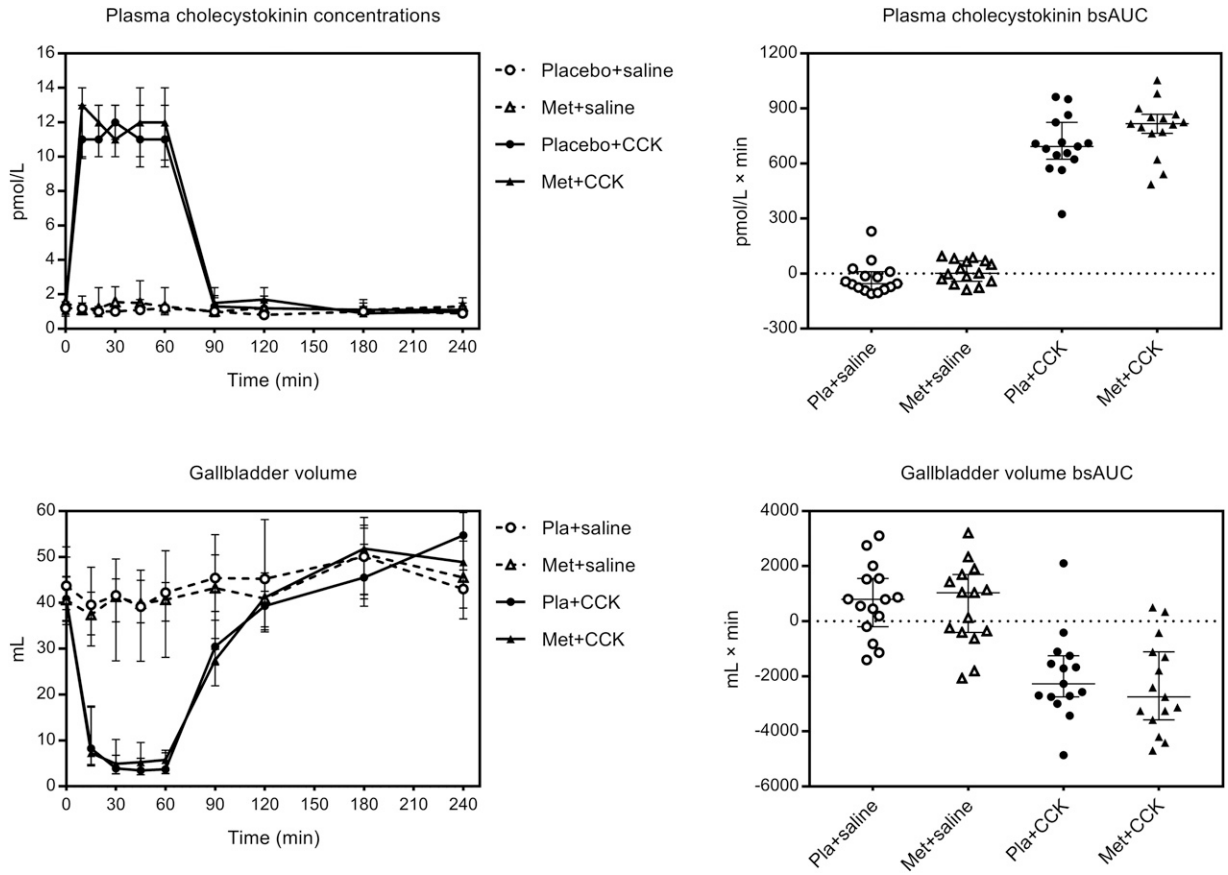

Figure 3. The graphs on top show plasma CCK concentrations and $bsAUC_{240\ min}$ scatter plots (bars represent median and interquartile range) for plasma CCK on the 4 different study days. The graphs below display gallbladder dynamics (median with interquartile range) and $bsAUC_{240\ min}$ scatter plots (bars represent median and interquartile range) for gallbladder volumes. Met, metformin; Pla, placebo. See Table 2 for calculations on differences between the 4 days.

effects of metformin on fasting plasma glucose in the acute setting. In studies by Lindsay *et al.* and Sambol *et al.* (24, 28), there was no acute glucose-lowering effect of metformin in the fasting state in patients with type 2 diabetes, which contrasts our results. However, the first study applied a lower metformin dose of 1000 mg and did not examine the effects of metformin during concomitant intestinal appearance of bile acids (24). In addition, in the study by Sambol *et al.* (28), an immediate improvement of postprandial plasma glucose excursions was observed, which is in accordance with the glucose-lowering effect of combined metformin and CCK observed in our study during conditions resembling postprandial bile acid circulation.

The mechanisms behind metformin-mediated potentiation of GLP-1 secretion from enteroendocrine L cells remain to be clarified (29). A metformin-mediated induction of the L cell secretory response rather than a suppression of DPP-4 activity is supported by the increase in total GLP-1 (intact + metabolites) observed in the current study (21, 29, 30). A recent study has reported metformin to reduce the rate of glucose absorption along the proximal parts of the small intestine, which was suggested to hold implications for GLP-1 secretion due to an intensified glucose-mediated stimulation of the large population of L cells in the more distal part of the ileum (31). However, the relevance of this proposed mechanism seems negligible in the current study design with no intake of nutrients. A range of *in vitro* studies has examined the direct effects of metformin on GLP-1 secretion and reported contradicting results (6, 29, 32, 33). Interestingly, in a study of GLUTag cells, Kappe *et al.* (33) reported no direct GLP-1 secretory effect of metformin, but found a metformin-mediated sensitizing effect on L cells with increased nutrient-induced GLP-1 secretion following metformin treatment. This L cell–sensitizing effect of metformin appears to be in accordance with the metformin-mediated potentiation of bile acid–induced GLP-1 secretion observed in our study. In a recent review, Bahne *et al.* (34) suggested the metformin-induced potentiation of GLP-1 secretion to involve modulation of enterohepatic bile acid circulation. Along these lines, a metformin-induced inhibition of ASBT could be speculated to elicit a beneficial balance between postabsorptive activation of the nuclear FXR and the basolaterally located TGR5 in terms of GLP-1 secretory effects, which

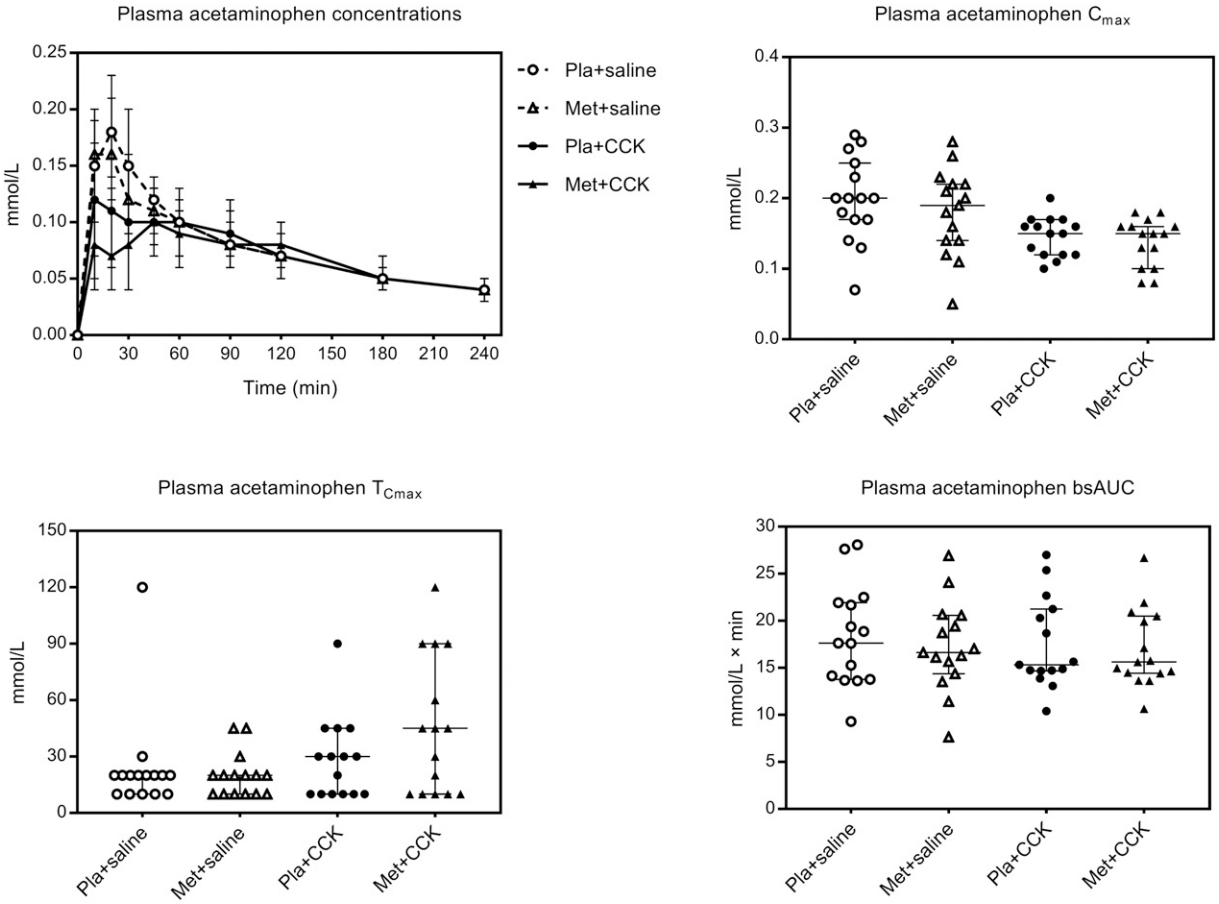

Figure 4. The graphs display plasma acetaminophen concentrations and scatter plots (bars represent median and interquartile range) for maximum acetaminophen concentration (C_{max}), time to maximum acetaminophen concentration (T_{Cmax}), and $bsAUC_{240\ min}$ for plasma acetaminophen on the 4 different study days. Met, metformin; Pla, placebo. See Table 2 for information on differences between the 4 days.

would require a concomitant, sustained stimulation of TGR5 and a decrease in FXR activation (14, 15, 35). The simultaneous peak GLP-1 concentrations at time 60 minutes on the 2 days with CCK alone and concomitant metformin administration would be in accordance with such a mechanism. A sustained increase in GLP-1 concentrations was evident after the combination of CCK and metformin compared with placebo [also seen in the study by Rohde *et al.* (20)], thereby raising the possibility of a bile acid–induced stimulation of L cells in the more distal parts of the gastrointestinal tract as an explanation for the metformin-mediated potentiation of GLP-1 secretion, which has previously been suggested in relation to specific ASBT inhibition (19). Nevertheless, the mechanisms behind the metformin-mediated potentiation of bile acid–induced GLP-1 secretion remain speculative at this point.

We do not suspect that the CCK infusion has direct effects on GLP-1 secretion in the present setting with twofold peak CCK plasma concentrations compared with the normal postprandial rises (36). Thus, a study by Hansen and Holst (37) demonstrated no induction of GLP-1 secretion in a perfused porcine ileum model following application of CCK in a concentration 10 to 50 times normal postprandial levels. The observed placebo-corrected increase in bsAUC for plasma GLP-1 following CCK-induced gallbladder emptying is in agreement with previous studies investigating bile acid–mediated effects on GLP-1 secretion in patients with type 2 diabetes (16, 38).

We observed no effects of either metformin administration or CCK infusion on resting energy expenditure, and the single-dose approach makes glucose-lowering effects caused by changes in bile acid or gut microbiota compositions implausible in the present setting. No clear associations were evident between the GLP-1–inducing effects and insulin secretion or plasma glucose levels, which might have been due to an impaired β cell responsiveness to GLP-1 in patients with type 2 diabetes (39). GLP-1–independent mechanisms involving direct hepatic effects and increased splanchnic glucose utilization might have contributed to the observed acute glucose-lowering impact of metformin (3, 5). Nevertheless, the metformin-mediated potentiation of GLP-1 secretion might prove beneficial in a postprandial setting via potential delay of gastric emptying and satiety-inducing effects.

The marked delaying effect on gastric emptying caused by increased plasma CCK concentrations on study days

with CCK infusion is in agreement with previous studies (40). However, Rohde *et al.* (20) applied a similar lead time between gastric tube administration of study drug and initiation of CCK infusion, with no significant CCK-mediated impact on acetaminophen absorption (20). Similar acetaminophen pharmacokinetics was expected in the present setting due to oral administration of a small volume watery mixture. We suspect no pharmacodynamic consequences of the observed effect on gastric emptying in the current setting due to similar bsAUC for acetaminophen on the 2 study days with metformin administration. Nonetheless, the observed differences in maximum acetaminophen concentration and time to maximum acetaminophen concentration on the 2 days including metformin might be speculated to have influenced metformin pharmacokinetics within the gastrointestinal tract.

In conclusion, we found single-dose administration of metformin to enhance bile acid–induced GLP-1 secretion in patients with type 2 diabetes, whereas no significant isolated effect of metformin on GLP-1 secretion was observed. The metformin-mediated reductions in plasma glucose excursions (with and without CCK) without concomitant changes in C-peptide and glucagon excursions point to no acute GLP-1–mediated effect of metformin on fasting plasma glucose. The underlying mechanisms of metformin-induced GLP-1 secretion remain speculative, but might involve metformin-mediated modulation of bile acid circulation with potential subsequent implications for bile acid receptor activation in the GLP-1–secreting L cells, as well as sensitization of these enteroendocrine cells by metformin.

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References

1. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR. Management of hyperglycaemia in type 2 diabetes: a patient-centered approach. Position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetologia*. 2012;**55**(6):1577–1596.
2. Pernicova I, Korbonits M. Metformin–mode of action and clinical implications for diabetes and cancer. *Nat Rev Endocrinol*. 2014;**10**(3):143–156.
3. Bailey CJ, Turner RC. Metformin. *N Engl J Med*. 1996;**334**(9):574–579.
4. McCreight LJ, Bailey CJ, Pearson ER. Metformin and the gastrointestinal tract. *Diabetologia*. 2016;**59**(3):426–435.
5. Gontier E, Fourme E, Wartski M, Blondet C, Bonardel G, Le Stanc E, Mantzarides M, Foehrenbach H, Pecking AP, Alberini JL. High and typical 18F-FDG bowel uptake in patients treated with metformin. *Eur J Nucl Med Mol Imaging*. 2008;**35**(1):95–99.
6. Duca FA, Côté CD, Rasmussen BA, Zadeh-Tahmasebi M, Rutter GA, Filippi BM, Lam TK. Metformin activates a duodenal Ampk-dependent pathway to lower hepatic glucose production in rats. *Nat Med*. 2015;**21**(5):506–511.
7. Scarpello JH, Hodgson E, Howlett HC. Effect of metformin on bile salt circulation and intestinal motility in type 2 diabetes mellitus. *Diabet Med*. 1998;**15**(8):651–656.
8. Napolitano A, Miller S, Nicholls AW, Baker D, Van Horn S, Thomas E, Rajpal D, Spivak A, Brown JR, Nunez DJ. Novel gut-based pharmacology of metformin in patients with type 2 diabetes mellitus [published correction appears in PLoS One. 2014;9(8):e106594]. *PLoS One*. 2014;**9**(7):e100778.
9. Mannucci E, Ognibene A, Cremasco F, Bardini G, Mencucci A, Pierazzuoli E, Ciani S, Messeri G, Rotella CM. Effect of metformin on glucagon-like peptide 1 (GLP-1) and leptin levels in obese nondiabetic subjects. *Diabetes Care*. 2001;**24**(3):489–494.
10. Thondam SK, Cross A, Cuthbertson DJ, Wilding JP, Daousi C. Effects of chronic treatment with metformin on dipeptidyl peptidase-4 activity, glucagon-like peptide 1 and ghrelin in obese patients with type 2 diabetes mellitus. *Diabet Med*. 2012;**29**(8):e205–e210.
11. Mannucci E, Tesi F, Bardini G, Ognibene A, Petracca MG, Ciani S, Pezzatini A, Brogi M, Dicembrini I, Cremasco F, Messeri G, Rotella CM. Effects of metformin on glucagon-like peptide-1 levels in obese

patients with and without type 2 diabetes. *Diabetes Nutr Metab*. 2004;**17**(6):336–342.
12. Wu H, Esteve E, Tremaroli V, Khan MT, Caesar R, Mannerås-Holm L, Ståhlman M, Olsson LM, Serino M, Planas-Fèlix M, Xifra G, Mercader JM, Torrents D, Burcelin R, Ricart W, Perkins R, Fernàndez-Real JM, Bäckhed F. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nat Med*. 2017;**23**(7):850–858.
13. Carter D, Howlett HCS, Wiernsperger NF, Bailey C. Effects of metformin on bile salt transport by monolayers of human intestinal Caco-2 cells. *Diabetes Obes Metab*. 2002;**4**(6):424–427.
14. Trabelsi M-S, Daoudi M, Prawitt J, Ducastel S, Touche V, Sayin SI, Perino A, Brighton CA, Sebti Y, Kluza J, Briand O, Dehondt H, Vallez E, Dorchies E, Baud G, Spinelli V, Hennuyer N, Caron S, Bantubungi K, Caiazzo R, Reimann F, Marchetti P, Lefebvre P, Bäckhed F, Gribble FM, Schoonjans K, Pattou F, Tailleux A, Staels B, Lestavel S. Farnesoid X receptor inhibits glucagon-like peptide-1 production by enteroendocrine L cells. *Nat Commun*. 2015;**6**:7629.
15. Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, Macchiarulo A, Yamamoto H, Mataki C, Pruzanski M, Pellicciari R, Auwerx J, Schoonjans K. TGR5-mediated bile acid sensing controls glucose homeostasis. *Cell Metab*. 2009;**10**(3):167–177.
16. Adrian TE, Gariballa S, Parekh KA, Thomas SA, Saadi H, Al Kaabi J, Nagelkerke N, Gedulin B, Young AA. Rectal taurocholate increases L cell and insulin secretion, and decreases blood glucose and food intake in obese type 2 diabetic volunteers. *Diabetologia*. 2012; **55**(9):2343–2347.
17. Adrian TE, Ballantyne GH, Longo WE, Bilchik AJ, Graham S, Basson MD, Tierney RP, Modlin IM. Deoxycholate is an important releaser of peptide YY and enteroglucagon from the human colon. *Gut*. 1993;**34**(9):1219–1224.
18. Nunez DJ, Yao X, Lin J, Walker A, Zuo P, Webster L, Krug-Gourley S, Zamek-Gliszczynski MJ, Gillmor DS, Johnson SL. Glucose and lipid effects of the ileal apical sodium-dependent bile acid transporter inhibitor GSK2330672: double-blind randomized trials with type 2 diabetes subjects taking metformin. *Diabetes Obes Metab*. 2016;**18**(7):654–662.
19. Chen L, Yao X, Young A, McNulty J, Anderson D, Liu Y, Nystrom C, Croom D, Ross S, Collins J, Rajpal D, Hamlet K, Smith C, Gedulin B. Inhibition of apical sodium-dependent bile acid transporter as a novel treatment for diabetes. *Am J Physiol Endocrinol Metab*. 2012;**302**(1):E68–E76.
20. Rohde U, Sonne DP, Christensen M, Hansen M, Brønden A, Toräng S, Rehfeld JF, Holst JJ, Vilsbøll T, Knop FK. Cholecystokinin-induced gallbladder emptying and metformin elicit additive glucagon-like peptide-1 responses. *J Clin Endocrinol Metab*. 2016;**101**(5):2076–2083.
21. Migoya EM, Bergeron R, Miller JL, Snyder RNK, Tanen M, Hilliard D, Weiss B, Larson P, Gutierrez M, Jiang G, Liu F, Pryor KA, Yao J, Zhu L, Holst JJ, Deacon C, Herman G, Thornberry N, Amatruda J, Williams-Herman D, Wagner JA, SinhaRoy R. Dipeptidyl peptidase-4 inhibitors administered in combination with metformin result in an additive increase in the plasma concentration of active GLP-1. *Clin Pharmacol Ther*. 2010;**88**(6):801–808.
22. Haeusler RA, Astiarraga B, Camastra S, Accili D, Ferrannini E. Human insulin resistance is associated with increased plasma levels of 12α-hydroxylated bile acids. *Diabetes*. 2013;**62**(12):4184–4191.
23. Shu Y, Sheardown SA, Brown C, Owen RP, Zhang S, Castro RA, Ianculescu AG, Yue L, Lo JC, Burchard EG, Brett CM, Giacomini KM. Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. *J Clin Invest*. 2007;**117**(5): 1422–1431.
24. Lindsay JR, Duffy NA, McKillop AM, Ardill J, O'Harte FPM, Flatt PR, Bell PM. Inhibition of dipeptidyl peptidase IV activity by oral metformin in type 2 diabetes. *Diabet Med*. 2005;**22**(5):654–657.
25. Herman GA, Bergman A, Stevens C, Kotey P, Yi B, Zhao P, Dietrich B, Golor G, Schrodter A, Keymeulen B, Lasseter KC, Kipnes MS, Snyder K, Hilliard D, Tanen M, Cilissen C, De Smet M, de Lepeleire I, Van Dyck K, Wang AQ, Zeng W, Davies MJ, Tanaka W, Holst JJ, Deacon CF, Gottesdiener KM, Wagner JA. Effect of single oral doses of sitagliptin, a dipeptidyl peptidase-4 inhibitor, on incretin and plasma glucose levels after an oral glucose tolerance test in patients with type 2 diabetes. *J Clin Endocrinol Metab*. 2006;**91**(11):4612–4619.
26. Jørgensen NB, Jacobsen SH, Dirksen C, Bojsen-Møller KN, Naver L, Hvolris L, Clausen TR, Wulff BS, Worm D, Lindqvist Hansen D, Madsbad S, Holst JJ. Acute and long-term effects of Roux-en-Y gastric bypass on glucose metabolism in subjects with type 2 diabetes and normal glucose tolerance. *Am J Physiol Endocrinol Metab*. 2012;**303**(1):E122–E131.
27. Hirst JA, Farmer AJ, Ali R, Roberts NW, Stevens RJ. Quantifying the effect of metformin treatment and dose on glycemic control. *Diabetes Care*. 2012;**35**(2):446–454.
28. Sambol NC, Chiang J, O'Conner M, Liu CY, Lin ET, Goodman AM, Benet LZ, Karam JH. Pharmacokinetics and pharmacodynamics of metformin in healthy subjects and patients with noninsulin-dependent diabetes mellitus. *J Clin Pharmacol*. 1996; **36**(11):1012–1021.
29. Mulherin AJ, Oh AH, Kim H, Grieco A, Lauffer LM, Brubaker PL. Mechanisms underlying metformin-induced secretion of glucagon-like peptide-1 from the intestinal L cell. *Endocrinology*. 2011; **152**(12):4610–4619.
30. Hinke SA, Kühn-Wache K, Hoffmann T, Pederson RA, McIntosh CHS, Demuth H-U. Metformin effects on dipeptidylpeptidase IV degradation of glucagon-like peptide-1. *Biochem Biophys Res Commun*. 2002;**291**(5):1302–1308.
31. Wu T, Xie C, Wu H, Jones KL, Horowitz M, Rayner CK. Metformin reduces the rate of small intestinal glucose absorption in type 2 diabetes. *Diabetes Obes Metab*. 2017;**19**(2):290–293.
32. Kim M-H, Jee J-H, Park S, Lee M-S, Kim K-W, Lee M-K. Metformin enhances glucagon-like peptide 1 via cooperation between insulin and Wnt signaling. *J Endocrinol*. 2014;**220**(2):117–128.
33. Kappe C, Patrone C, Holst JJ, Zhang Q, Sjöholm A. Metformin protects against lipoapoptosis and enhances GLP-1 secretion from GLP-1-producing cells. *J Gastroenterol*. 2013;**48**(3):322–332.
34. Bahne E, Hansen M, Brønden A, Sonne DP, Vilsbøll T, Knop FK. Involvement of glucagon-like peptide-1 in the glucose-lowering effect of metformin. *Diabetes Obes Metab*. 2016;**18**(10):955–961.
35. Brighton CA, Rievaj J, Kuhre RE, Glass LL, Schoonjans K, Holst JJ, Gribble FM, Reimann F. Bile acids trigger GLP-1 release predominantly by accessing basolaterally located G protein-coupled bile acid receptors. *Endocrinology*. 2015;**156**(11):3961–3970.
36. Sonne DP, Rehfeld JF, Holst JJ, Vilsbøll T, Knop FK. Postprandial gallbladder emptying in patients with type 2 diabetes: potential implications for bile-induced secretion of glucagon-like peptide 1. *Eur J Endocrinol*. 2014;**171**(4):407–419.
37. Hansen L, Holst JJ. The effects of duodenal peptides on glucagon-like peptide-1 secretion from the ileum: a duodeno–ileal loop? *Regul Pept*. 2002;**110**(1):39–45.
38. Hansen M, Scheltema MJ, Sonne DP, Hansen JS, Sperling M, Rehfeld JF, Holst JJ, Vilsbøll T, Knop FK. Effect of chenodeoxycholic acid and the bile acid sequestrant colesevelam on glucagon-like peptide-1 secretion. *Diabetes Obes Metab*. 2016; **18**(6):571–580.
39. Kjems LL, Holst JJ, Vølund A, Madsbad S. The influence of GLP-1 on glucose-stimulated insulin secretion: effects on beta-cell sensitivity in type 2 and nondiabetic subjects. *Diabetes*. 2003;**52**(2): 380–386.
40. Sayegh AI. The role of cholecystokinin receptors in the short-term control of food intake. *Prog Mol Biol Transl Sci*. 2013;**114**: 277–316.