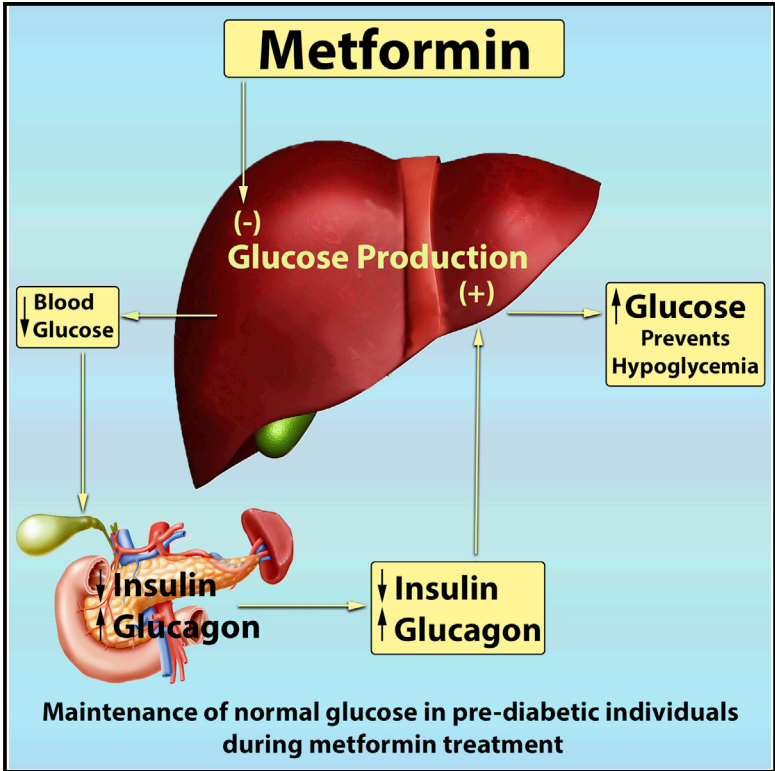# Cell Reports

Hyperglucagonemia Mitigates the Effect of Metformin on Glucose Production in Prediabetes

Graphical Abstract

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In Brief

Using a randomized, double-blinded, placebo-controlled, crossover study design in prediabetic individuals, Konopka et al. show that metformin improves fasting and postprandial glycemia without inhibiting glucagon-stimulated glucose production as reported in preclinical studies. During metformin therapy, increased glucagon and decreased glucogenic precursors may maintain glucose production to prevent hypoglycemia.

Highlights

- Metformin does not inhibit glucagon-stimulated glucose production

- Increased glucagon prevents hypoglycemia during metformin therapy

- Metformin therapy reduces glucogenic precursors

- Metformin counteracts the catabolic effects of glucagon

CellPress

Hyperglucagonemia Mitigates the Effect of Metformin on Glucose Production in Prediabetes

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SUMMARY

The therapeutic mechanism of metformin action remains incompletely understood. Whether metformin inhibits glucagon-stimulated endogenous glucose production (EGP), as in preclinical studies, is unclear in humans. To test this hypothesis, we studied nine prediabetic individuals using a randomized, placebo-controlled, double-blinded, crossover study design. Metformin increased glucose tolerance, insulin sensitivity, and plasma glucagon. Metformin did not alter average basal EGP, although individual variability in EGP correlated with plasma glucagon. Metformin increased basal EGP in individuals with severe hyperglucagonemia (>150 pg/ml). Decreased fasting glucose after metformin treatment appears to increase glucagon to stimulate EGP and prevent further declines in glucose. Similarly, intravenous glucagon infusion elevated plasma glucagon (>150 pg/ml) and stimulated a greater increase in EGP during metformin therapy. Metformin also counteracted the protein-catabolic effect of glucagon. Collectively, these data indicate that metformin does not inhibit glucagon-stimulated EGP, but hyperglucagonemia may decrease the ability of the metformin to lower EGP in prediabetic individuals.

INTRODUCTION

The biguanide metformin is the most commonly prescribed oral anti-hyperglycemic agent, consumed annually by over 150 million people worldwide. Despite metformin's efficacy in lowering blood glucose and decreasing the incidence of type 2 diabetes mellitus (T2DM) (Knowler et al., 2002), its mechanisms of action remain incompletely understood. In T2D individuals, metformin lowers blood glucose by decreasing endogenous glucose production (EGP) (DeFronzo et al., 1991; Hundal et al., 2000; Musi et al., 2002; Stumvoll et al., 1995). Subsequent work demonstrated that metformin acted to inhibit EGP by activating AMP-activated

protein kinase (AMPK) (Shaw et al., 2005; He et al., 2009). However, metformin reduced EGP in AMPK knockout mice, challenging the notion that AMPK is required for decreased EGP by metformin (Foretz et al., 2010). However, these authors utilized supra-pharmacologic doses of metformin, and Cao et al. (2014) subsequently demonstrated that pharmacologic doses of metformin could indeed inhibit hepatic gluconeogenesis. Metformin was also recently discovered to decrease glucagon-induced glucose production (Miller et al., 2013) and diminish the use of gluconeogenic metabolites for glucose production by altering mitochondrial glycerophosphate dehydrogenase and the cellular redox status in the liver (Madiraju et al., 2014). Moreover, metformin was recently shown to impart decreased fasting glucose and hepatic glucose production through the intestines (Duca et al., 2015; Buse et al., 2016). Therefore, several lines of evidence suggest that metformin lowers EGP by independent or perhaps combined mechanisms that change rate-limiting gluconeogenic enzyme levels (He et al., 2009; Foretz et al., 2010), decrease glucagon action (Miller et al., 2013), or limit the conversion of gluconeogenic substrates (e.g., lactate, alanine, amino acids [AAs]) to glucose (DeFronzo et al., 1991; Madiraju et al., 2014; Stumvoll et al., 1995).

Although preclinical models have provided clues regarding how metformin may elicit its therapeutic effect, translating these mechanisms to the clinical situation has been difficult because many studies have used supra-pharmacologic dosing schemes and biguanide derivatives contraindicated for human use (He et al., 2009; He and Wondisford, 2015). Furthermore, metformin may also influence glucogenic precursors and insulin sensitivity through its influence on amino acid kinetics, a possibility that has yet to be explored in humans. Therefore, we investigated whether metformin, at therapeutic doses, would inhibit glucagon-stimulated EGP and AA kinetics in humans. We conducted a randomized, placebo-controlled, double-blinded crossover study in prediabetic individuals and measured EGP and AA kinetics using stable isotope methodology under basal, glucagon-deficient, and glucagon-stimulated conditions.

RESULTS AND DISCUSSION

Nine participants completed the study (physical characteristics are shown in Table S1). Seven had a family history of T2DM, and eight were metformin-naive. One participant had previously

used metformin but discontinued it more than 2 years before the study commenced. Some participants were taking antidepressant medications (n = 5), statins (n = 3), β-blockers (n = 1), or diuretics (n = 1) through the entire study, and these participants did not differ in their response to metformin therapy.

Metformin and a placebo were prescribed at a dose of 500 mg twice daily during the first week and 1,000 mg twice daily during the second week. On the basis of returned pill counts, subjects adhered to the prescribed doses with a compliance rate of 99% and 94% during week 1 and 96% and 94% during week 2 for metformin and the placebo, respectively. Four participants reported gastrointestinal discomfort, three of which occurred during metformin treatment. Body weight and composition remained unchanged during the 2-week study (Table S1).

Compared with the placebo, metformin-treated patients had lower mean fasting plasma glucose, insulin, and c-peptide levels but markedly higher mean plasma glucagon levels (Figure 1; Table 1). The insulin-to-glucagon ratio and the homeostasis model assessment of insulin resistance (HOMA-IR) were decreased by metformin (Figures 1A and 1B).

Whole-body insulin sensitivity, β cell responsivity, and disposition index (DI) were modeled following consumption of a mixed meal (600 kcal; 30% carbohydrate, 55% fat, 15% protein) (Figure S1). Insulin sensitivity (S_I) was significantly improved by metformin (Figure 1C). Although β cell responsivity (Φ, Φ_D, Φ_S) was unaltered (Figure 1D), total, dynamic, and static Dis) (DI_D; DI_S, -the product of β cell responsivity and S_I; Figure 1E) were significantly improved by metformin. The 2-fold increase in whole-body insulin sensitivity was markedly greater than the decrease in HOMA-IR, which may suggest that the effects of metformin are more robust in response to the physiological challenge of a high-fat mixed meal. After the meal, metformin decreased (p < 0.01) the area above baseline for glucose, c-peptide, and insulin (Figures 1F–1H). In addition to increasing postabsorptive plasma glucagon, metformin increased (p < 0.01) postprandial plasma glucagon at 120, 180, and 240 min (Figure 1I).

Although metformin improved glucose regulation and whole-body insulin sensitivity based on the oral minimal model, its effect on basal EGP was variable depending on the basal glucagon level (Figures 2A–2C). Subtle changes in glucagon have been shown to have profound effects on hepatic glucose metabolism (Roden et al., 1996). Of interest, participants with basal glucagon levels <150 pg/ml (n = 6 of 9 experienced a decrease; p = 0.01, Cohen's d = 0.61) in EGP, whereas participants with basal glucagon levels >150 pg/ml (n = 3 of 9) experienced an increase (p = 0.02, Cohen's d = 1.26) in EGP after metformin treatment (Figure 2C). This accounts for the lack of overall change in mean EGP by metformin in our study (Figure 2A). It is possible that the improvement in glucose tolerance and insulin sensitivity occurred because of other mechanisms of action, such as the influence of metformin in the gut (Duca et al., 2015; Buse et al., 2016), because this study has not identified the mechanism of action by which metformin improves indices of insulin action. Previous reports that administered metformin over a longer period have indicated that metformin lowers basal EGP in T2DM (Hundal et al., 2000; Stumvoll et al., 1995), but we found that, in prediabetic humans, the decline in EGP was prevented by a compensatory increase in glucagon levels. These findings

suggest that a compensatory increase in glucagon antagonizes metformin's inhibitory effect on EGP and may be involved in the delayed or lack of response to metformin by some patients. Studies combining metformin therapy with glucagon-like peptide 1 (GLP1) agonists or dipeptidyl peptidase 4 (DPP4) inhibitors have demonstrated a synergetic effect in achieving glycemic control (Zander et al., 2001; DeFronzo et al., 2009; Solis-Herrera et al., 2013) in T2DM, but it remains to be determined whether such a combination prevents the compensatory glucagon increase and increases the odds of hypoglycemic episodes in pre-diabetic individuals.

We also examined the effect of metformin on whole-body protein metabolism because amino acids and their metabolites may affect insulin sensitivity (Everett et al., 1981; Nair et al., 1992; Newgard, 2012) and play a key role in determining gluconeogenesis through regulating substrate availability (Felig et al., 1975). To study amino acid metabolism, we used di-labeled leucine (1-^{13}C, ^{15}N leucine) to comprehensively assess leucine carbon and nitrogen flux, transamination, reamination, and oxidation (Table 1). During the basal period, metformin increased leucine transamination (6%, p = 0.046) and reamination (15%, p = 0.02) but did not alter leucine carbon flux (representing leucine appearance from endogenous protein degradation), nitrogen flux, or oxidation (Table 1). These results corroborate previous reports that leucine transamination is influenced by insulin treatment and glycemic control in diabetic individuals (Halvatsiotis et al., 2002; Nair et al., 1995). Such anabolic effects reduce the availability of amino moieties for synthesis of glucose precursors (e.g., alanine and glutamine) (Galim et al., 1980; Haymond and Miles, 1982). In addition to measuring amino acid kinetics, we quantitatively profiled systemic AA metabolites in plasma. There were 16 metabolites influenced by metformin, including elevated metabolites of the urea pathway (e.g., arginine and citrulline [p < 0.001] and ornithine [p = 0.003]). These results suggest that metformin treatment affects amino acid metabolites involved in ammonia disposal. The targeted amino metabolite profiling also revealed a decline in gluconeogenic AAs (p = 0.011) and specific metabolites such as glutamic acid, proline, alanine, isoleucine, alloisoleucine, and α-amino-N-butyric acid (p < 0.05), which are all involved in subsequent transamination and gluconeogenic pathways (Table S2). We also noted that metformin did not affect concentrations of essential AAs, which are derived from whole-body protein degradation in the fasted state. These findings are supported by our results showing that metformin did not affect leucine carbon flux derived from endogenous protein degradation. Previous findings in a pre-clinical model indicated that metformin lowers basal EGP by diminishing the use of gluconeogenic substrates (Madiraju et al., 2014). Conversely, we show that metformin decreases glucose precursors, likely from increased utilization to preserve EGP in prediabetic individuals. An advantage of metformin over other glucose-lowering agents is the minimal risk of hypoglycemia. The prediabetic model allowed us to study individuals at high risk of developing T2D but without very high glucose levels compared with those with overt T2D. This gave us an opportunity to identify decreased glucogenic precursors and increased plasma glucagon as likely mechanisms by which the glucose-lowering effect of metformin does not result in hypoglycemia.
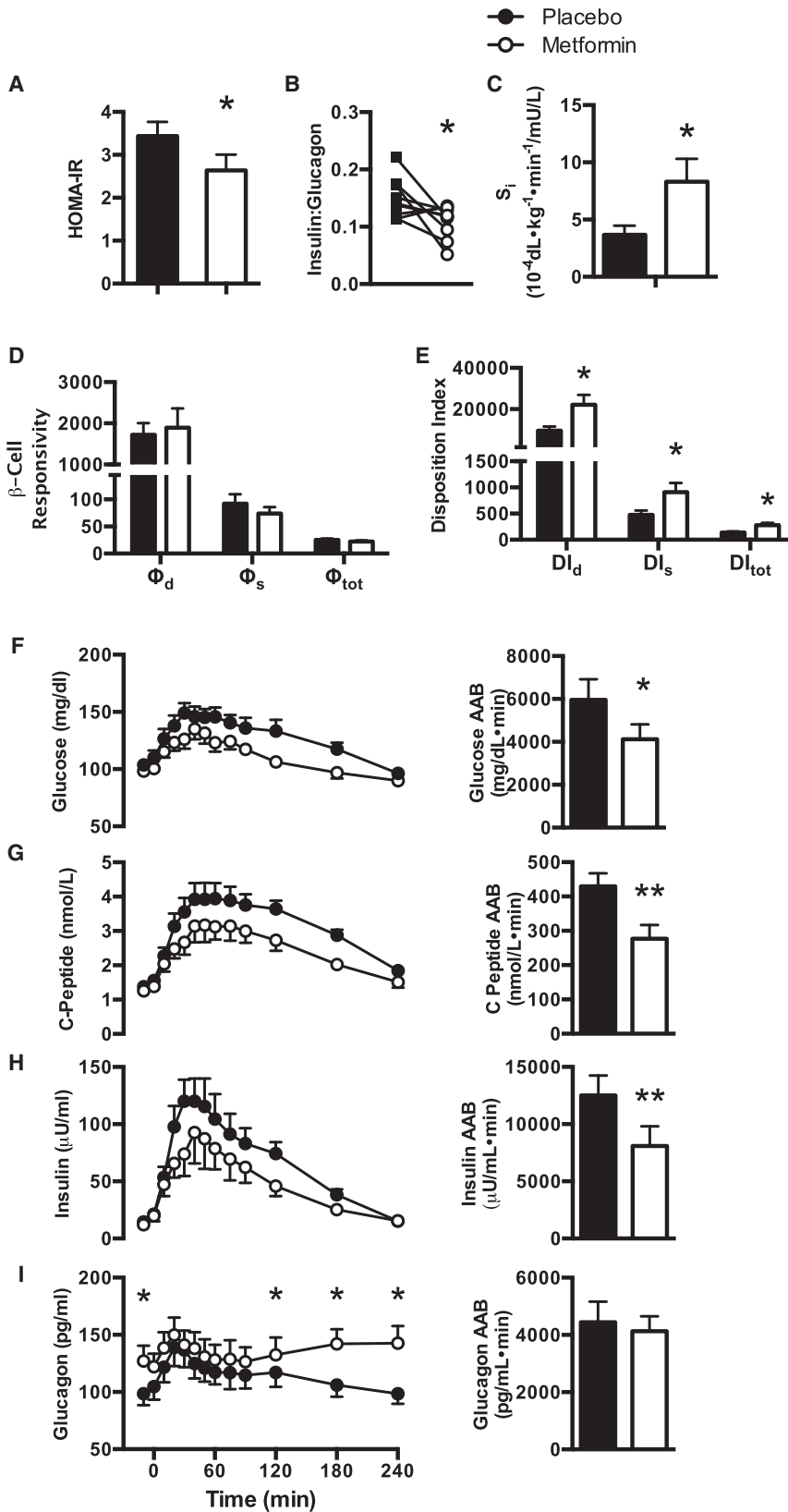
Figure 1. Metformin Improves Fasting and Postprandial Indices of Insulin Sensitivity

(A and B) HOMA-IR (A) and insulin-to-glucagon ratio (B).

(C) Whole-body S_I.

(D and E) β Cell responsivity (D) and disposition index (E) modeled after a mixed-meal challenge.

(F–I) Basal and postprandial plasma values and area above baseline (AAB) after 2 weeks of metformin compared with placebo for glucose (F), C-peptide (G), insulin (H), and glucagon (I). Postprandial glucagon was decreased with metformin at 120, 180, and 240 min.

$*p < 0.05$, $**p < 0.01$, $***p < 0.001$ metformin versus placebo. Data are presented as mean ± SEM.

Table 1. Hormones, Leucine Kinetics, and Resting Energy Expenditure

	Placebo			Metformin		
	Basal	Somatostatin	Glucagon	Basal	Somatostatin	Glucagon
Glucose (mg/dl)	108 ± 3	103 ± 7[a]	214 ± 8[b]	93 ± 3[c]	85 ± 10[a,c]	212 ± 10[b]
Glucagon (pg/ml)	90.7 ± 10.0	54.3 ± 7.5[a]	157.1 ± 7.7[b]	119.1 ± 13.5	60.2 ± 6.9[a]	156.6 ± 11.5[b]
Insulin (µU/ml)	13.2 ± 1.4	0.53 ±0.06[a]	5.4 ± 1.4[b]	11.4 ± 1.5[c]	0.46 ± 0.05[a]	4.7 ± 0.5
C-peptide (nmol/l)	1.31 ± 0.09	0.33 ± 0.02[a]	0.46 ± 0.03[b]	1.13 ± 0.08[c]	0.28 ± 0.02[a,c]	0.38 ± 0.03[b,c]
Leucine C flux	95.0 ± 4.2	89.6 ± 3.5[a]	85.5 ± 4.0[b]	93.0 ± 1.7	90.2 ± 2.0[a]	87.6 ± 2.4
Leucine oxidation	19.9 ± 1.2	16.5 ± 1.2[a]	23.7 ± 1.7[b]	18.3 ± 0.8	16.4 ± 0.7	19.8 ± 1.3[b,c]
Leucine protein synthesis	75.1 ± 3.8	73.2 ± 3.5[a]	61.8 ± 3.5[b]	74.7 ± 2.0	73.8 ± 2.0[a]	67.8 ± 2.0[b,c]
Leucine N flux	155.0 ± 6.3	197.9 ± 7.5[a]	215.1 ± 12.5[b]	159.3 ± 6.2	204.7 ± 6.1[a]	230.4 ± 9.4[b]
Leucine transamination	79.9 ± 4.2	124.8 ± 7.1[a]	149.8 ± 10.2[b]	84.5 ± 4.7[c]	130.9 ± 4.6[a]	165.0 ± 7.8[b]
Leucine reamination	60.0 ± 4.2	108.3 ± 4.2[a]	126.1 ± 10.0[b]	66.3 ± 4.7[c]	114.5 ± 4.6[a]	145.2 ± 7.3[b,c]
% Flux oxidized	21.0 ± 1.3	18.5 ± 1.5[a]	27.9 ± 2.0[b]	19.7 ± 1.0	18.2 ± 0.8[a]	22.5 ± 1.1[b,c]
REE (kcal/day)	1669 ± 138	1682 ± 122	1925 ± 139[b]	1727 ± 103	1719 ± 93	1858 ± 97
RER	0.82 ± 0.01	0.75 ± 0.02	0.78 ± 0.02	0.82 ± 0.01	0.74 ± 0.02	0.75 ± 0.02

Data are expressed as micromoles per kilogram of FFM per hour for whole-body leucine kinetics. Data are represented as mean ± SE.

[a] $p < 0.05$ somatostatin versus basal.

[b] $p < 0.05$ glucagon versus somatostatin.

[c] $p < 0.05$ metformin versus placebo.

To further investigate the interaction between metformin treatment and glucagon, we continuously infused somatostatin for 2 hr to suppress endogenous glucagon and insulin secretions (Figure S1). Following a period of low glucagon and insulin levels, we added a continuous infusion of glucagon to the somatostatin infusion for 3 hr to raise plasma glucagon levels to near those observed in participants with basal hyperglucagonemia (>150 pg/ml). Somatostatin was infused to prevent insulin secretion in response to glucagon administration. This study design allowed us to test the hypothesis that metformin antagonizes glucagon-mediated glucose production as reported in preclinical studies. Glucagon and insulin plasma concentrations during the somatostatin and somatostatin + glucagon infusions were not different between metformin and placebo treatments, whereas c-peptide levels were marginally lower ($p < 0.05$) with metformin (Table 1). The plasma metformin concentration was 1.74 ± 0.12 µM during glucagon infusions. By design, EGP was lower ($p < 0.05$) under glucagon-deficient and higher ($p < 0.001$) under glucagon-stimulated conditions compared with the basal state (Figure 2A). EGP was not different with metformin during glucagon-deficient stages. The primary effect of metformin on EGP occurred during glucagon infusion. Specifically, the change in EGP from baseline was greater with metformin compared with the placebo (1.3 ± 0.4 µmol/kg of fat-free mass [FFM]/min, $p = 0.017$) (Figure 2D). A prevailing hypothesis is that metformin improves glucose regulation by inhibiting glucagon-stimulated glucose production. In support, studies in hepatocytes and mice indicate that biguanides inhibit glucagon signaling to mitigate hepatic glucose production (Miller et al., 2013). However, these preclinical models included phenformin and supratherapeutic doses of metformin to inhibit glucagon action. This study included prediabetic adults consuming therapeutic doses of the only Food and

Drug Administration-approved biguanide. Our results indicate that metformin does not inhibit glucagon-stimulated EGP in patients with prediabetes.

In addition to EGP, leucine AA kinetics were largely altered by somatostatin and further changed by the addition of glucagon infusion (Table 1). Importantly, somatostatin infusion with the accompanying decline in insulin and glucagon reduced leucine oxidation (absolute and percent of leucine flux). However, increasing glucagon levels increased leucine oxidation, transamination, and reamination while decreasing protein synthesis. These effects of glucagon are congruent with our previous data in healthy individuals (Nair et al., 1987) and those with type 1 diabetes (Charlton and Nair, 1998). The glucagon-induced increase in leucine oxidation ($p = 0.01$) and decrease in protein synthesis ($p = 0.03$) were attenuated by metformin treatment (Table 1). During placebo treatment, glucagon, as previously reported, increased resting energy expenditure (Nair et al., 1984), but the glucagon effect on resting energy expenditure was not observed during metformin treatment (Table 1). These data suggest that metformin counteracts the catabolic effects of glucagon by attenuating both the increase in leucine oxidation and energy expenditure and the decrease in protein synthesis. Glucagon secretion is well known to increase to prevent hypoglycemia, and plasma glucagon levels are high in the diabetic states (Felig et al., 1976). Thus, these results show that the potential adverse catabolic effects of the increase in glucagon levels (Nair et al., 1987; Pain et al., 1983) seem to be mitigated by metformin.

We used a randomized double-blind crossover study design to test the prevailing hypothesis that metformin would inhibit glucagon-mediated substrate metabolism in individuals with prediabetes. Although a sample size of nine prediabetic individuals not currently on glucose-lowering medications may appear to be a limitation of this study, this study design allowed
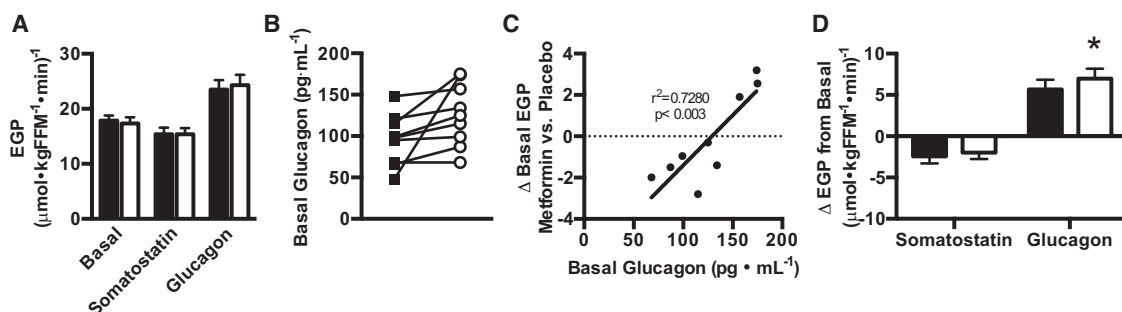
Figure 2. Endogenous Glucose Production after Metformin Treatment in Prediabetic Individuals
(A) EGP under basal, glucagon-deficient (somatostatin), and glucagon-stimulated conditions (glucagon).
(B) Fasted plasma glucagon.
(C) Relationship between the change in basal EGP to basal, fasting glucagon after metformin treatment.
(D) The difference in EGP (Δ EGP) under glucagon-deficient (somatostatin) and glucagon-stimulated (glucagon) conditions versus basal EGP.
*$p < 0.05$ metformin versus placebo. Data are presented as mean ± SEM.

the detection of meaningful changes in glucose and glucagon while using gold standard approaches to assess glucogenic metabolites and endogenous glucose production. Additional participant groups including obese and normoglycemic individuals and patients with overt T2D may have allowed further comparisons with people with normal glucose levels and severe hyperglycemia. Future studies might consider further addressing the relationship between metformin, glucagon, and glycemic control using larger sample sizes and diverse participant populations.

CONCLUSION

With the prospect of developing alternative or improved therapies to counter T2D worldwide, understanding the therapeutic action of metformin in humans is critical. This study offers insight into the effects of metformin in prediabetic individuals. We demonstrated that metformin may affect glucagon-mediated AA metabolism and energy expenditure, but, in contrast to prevailing hypotheses, we found no inhibitory effect of metformin on glucagon-induced EGP. In this study, EGP during hyperglucagonemia was actually greater with metformin compared with the placebo. Therefore, the effect of metformin on EGP depends on plasma glucagon levels so that any inhibitory effect of metformin on EGP was neutralized at high basal glucagon levels or during glucagon infusion studies. This study provides insight, in humans, that high glucagon levels antagonize the ability of metformin to suppress EGP, disputing a previous report in preclinical models (Miller et al., 2013). These findings provide the impetus for further study to determine whether glucagon may be a biomarker used during metformin therapy to improve precision medicine to stop the progression of prediabetes to overt T2D.

EXPERIMENTAL PROCEDURES

This study was approved by the Mayo Clinic Institutional Review Board, and all subjects gave written, informed consent. This study was conducted at the Mayo Clinic from December 2013 to December 2014 and is registered at ClinicalTrials.gov (NCT01956929).

Study Participants
Inclusion criteria were as follows: obesity (BMI >30 kg/m^2), sedentary (<1 hr of structured activity per week), nonsmoking, and not taking any medication to control blood glucose. Qualifying participants (n = 33) had a medical history and physical examination, including a blood test, after an overnight fast to measure glucose, hemoglobin A1c, and chemistry profile. As recommended by the American Diabetes Association in 2014 (American Diabetes Association, 2014), participants with plasma glucose between 100–125 mg/dl or hemoglobin A1c of 5.7%–6.4% (5.93 ± 1.16) were considered prediabetic and were eligible for the study.

Randomization and Study Intervention
The study was a randomized double-blind crossover trial with a >6-week washout period between interventions. Participants were randomized for a two-treatment, two-period crossover design using permuted block randomization. Block sizes of two and eight were used to assign participants to a treatment sequence of placebo followed by metformin or vice versa. Assignment of treatment sequences to participants was accomplished through the central research pharmacy to preserve the blind for study investigators. Participants were instructed to not change their diet, physical activity, or medications for the entirety of the study. Participants were directed to take the study medication with the morning and evening meals (week 1, 500 mg twice daily; week 2, 1,000 mg twice daily). The 1,000-mg, twice daily dose was continued during the inpatient study days (Figure S1). Metformin and placebo capsules were matched for size and color. Participants received extra capsules so that returned pills could be counted to determine compliance. Resting energy expenditure (REE) and body composition were measured during an outpatient visit before the three consecutive inpatient study days in the clinical research unit (CRU). Inpatient visits were repeated after the second intervention.

Diet and Inpatient Study Preparation
Participants were provided a weight-maintaining diet with a standardized macronutrient composition (50% carbohydrate, 30% fat, and 20% protein) for 3 days before and during the inpatient study days. Participants were admitted to the CRU the evening before each inpatient study day and consumed their evening meal and medication by 19:00 (Figure S1).

Study Outcomes
The primary study outcomes were basal and glucagon-stimulated EGP. Secondary outcomes included AA kinetics and results from the mixed meal tolerance test.

Mixed Meal Tolerance Test
Insulin sensitivity (S$_I$); total, dynamic, and static β cell responsivity (Φ, Φ$_D$, and Φ$_S$); and DIs (DI$_D$ and DI$_S$) were modeled after a mixed meal (600 kcal; 30% carbohydrate, 55% fat, and 15% protein) (Cobelli et al., 2014).

Glucose and AA Metabolism

EGP and whole-body AA kinetics (i.e., flux, transamination, reamination, and oxidation) were determined on day 2 during a 2-hr basal period, a 2-hr period of somatostatin infusion (93 ng/kg of FFM/min to suppress pancreatic hormone secretion), and a 3-hr period of continuously glucagon (3 ng/kg of FFM/min to elevate plasma glucagon) plus somatostatin, as previously described (Charlton and Nair, 1998; Charlton et al., 1996; Nair et al., 1987). EGP and AA kinetics were measured by an isotope-dilution technique using a priming bolus of [^{13}C]sodium bicarbonate concomitant with primed-continuous infusions of [6,6-^{2}H$_2$]glucose and [1-^{13}C,^{15}N]leucine (Charlton et al., 1996; Charlton and Nair, 1998; Nair et al., 1995). Whole-body REE and respiratory exchange ratio (RER) were assessed by indirect calorimetry for 20 min during each period. Arterialized venous blood and expired air were collected hourly and every 10 min during the last 30 min of each period.

Measurement of Hormones and Metabolites

Plasma glucose, insulin, c-peptide, and glucagon concentrations were measured as previously described (Lalia et al., 2015). Plasma enrichment of the infused stable isotopes and [^{13}C]α-ketoisocaproic acid, the transaminated product of leucine, was measured using gas chromatography-mass spectrometry (GC-MS). The isotope enrichment of ^{13}CO$_2$ in expired air was measured by isotope-ratio mass spectrometry (Charlton and Nair, 1998; Charlton et al., 1996; Nair et al., 1987, 1995). AA metabolites in plasma were analyzed using liquid chromatography-tandem mass spectrometry and GC-MS (Lanza et al., 2010).

Sample Size and Statistical Analysis

A modified intention-to-treat analysis was conducted for this study. Although 12 participants were randomized, only 11 participants initiated the study. Two participants did not complete the crossover design, and their samples were not analyzed along with other participants because of cost constraints. To avoid assay batch effects, a decision was made to not include these data in the final analysis. As such, data on the nine completing subjects were analyzed using SAS (version 9.4) according to the principles of a two-period, two-treatment crossover study with no assumed carryover effect. Models with a period effect were found to not suggest a significant period effect, so final results without a period effect are presented. In addition to testing for differences at each of the three time points (basal, somatostatin infusion, and glucagon infusion), the incremental change in values (i.e., somatostatin–basal and glucagon–basal at each treatment visit) was tested to address the change in baseline values during the two study visits. Data are presented as mean with 95% confidence intervals or SE with p values provided. Statistical significance was set at $p < 0.05$.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.04.024.

AUTHOR CONTRIBUTIONS

Conceptualization, A.R.K., R.R.E., M.M.R., M.L.J., I.R.L., and K.S.N.; Methodology, A.R.K., M.M.R., M.L.J., R.E.C., I.R.L., and K.S.N.; Validation, A.R.K., R.R.E., I.R.L., K.S.N.; Formal Analysis, A.R.K., R.E.C., M.S., and C.C.; Investigation, A.R.K., R.R.E., M.M.R., and M.L.J.; Resources, A.R.K., R.R.E., R.E.C., C.C., and K.S.N.; Data Curation, A.R.K., R.E.C., M.S., and C.C.; Writing – Original Draft, A.R.K.; Writing – Review & Editing, M.M.R., M.L.J., R.E.C., F.E.C., I.R.L., and K.S.N.; Visualization, A.R.K., R.R.E., I.R.L., and K.S.N., Project Administration, A.R.K., R.R.E., and K.S.N.; Funding Acquisition, K.S.N.

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