Clinical and Translational Science

ARTICLE OPEN ACCESS

The Pharmacokinetic Interaction Between Metformin and the Natural Product Goldenseal Is Metformin Dose-Dependent: A Three-Arm Crossover Study in Adults With Type 2 Diabetes

James T. Nguyen[1] | Christopher M. Arian[2] | Rakshit S. Tanna[1] | Maxey G. Cherel[1] | Matthew E. Layton[3] | John R. White[4] | Kenneth E. Thummel[2,5] | Mary F. Paine[1,5]

[1]Department of Pharmaceutical Sciences, College of Pharmacy and Pharmaceutical Sciences, Washington State University, Spokane, Washington, USA | [2]Department of Pharmaceutics, School of Pharmacy, University of Washington, Seattle, Washington, USA | [3]Elson S. Floyd College of Medicine, Washington State University, Spokane, Washington, USA | [4]Department of Pharmacotherapy, College of Pharmacy and Pharmaceutical Sciences, Washington State University, Spokane, Washington, USA | [5]Center of Excellence for Natural Product Drug Interaction Research, Spokane, Washington, USA

Correspondence: Mary F. Paine (mary.paine@wsu.edu)

Received: 27 September 2024 | **Revised:** 2 December 2024 | **Accepted:** 14 December 2024

Funding: This work was supported by the National Institutes of Health National Center for Complementary and Integrative Health and the Office of Dietary Supplements, specifically the Center of Excellence for Natural Product Drug Interaction Research (U54 AT008909 and F31 AT011698), and the National Center for Advancing Translational Sciences (TL1 RR025016); Bethesda, Maryland.

Keywords: berberine | clinical study | drug interaction | goldenseal | metformin | natural product | pharmacokinetics | type 2 diabetes

ABSTRACT

Pharmacokinetic drug interactions can lead to unexpected changes in plasma concentrations of the object drug, potentially increasing the risk for adverse effects and/or decreasing therapeutic efficacy. The botanical product goldenseal was previously shown to decrease metformin systemic exposure in healthy adults. This three-arm, open-label, crossover clinical study assessed the pharmacokinetic goldenseal–metformin interaction in adults with type 2 diabetes stabilized on therapeutic doses of metformin (500–2550 mg daily). The aggregate pharmacokinetic data indicated no clinically meaningful interaction as determined by the metformin area under the plasma concentration-time curve (AUC) geometric mean ratio [90% confidence interval] of 0.93 [0.86–1.01] laying within the predefined no-effect range (0.80–1.25). However, metformin AUC decreased by ~20%, 14%, and 0% after goldenseal coadministration at low (500–750 mg), moderate (1000–1500 mg), and high (2000–2550 mg) metformin doses, respectively; renal clearance and half-life remained unchanged throughout. The exploratory pharmacodynamic endpoint, HbA1c, decreased on average from 6.8% to 6.5%, regardless of the effects of goldenseal on metformin pharmacokinetics. The decreasing effect of goldenseal on metformin systemic exposure with increasing metformin dose, coupled with no changes in renal excretion and elimination half-life, indicated that both the pharmacokinetic goldenseal–metformin interaction and the nonlinear absorption of metformin are governed by saturable, intestinal transport mechanism(s). The disconnect between changes in metformin systemic exposure and therapeutic effects emphasizes the need to evaluate clinical biomarkers to comprehensively assess drug interaction risks, particularly those involving natural products. Healthcare providers may consider cautioning patients about supplementing metformin pharmacotherapy with goldenseal to avoid risks for undesired changes in glycemic control.

Trial Registration: ClinicalTrials.gov identifier: NCT05081583

Christopher M. Arian: Contributed significantly to the completion of this work and is recognized as a co-first author.

Summary

- What is the current knowledge on the topic?
  - Coadministration of the natural product goldenseal with a subtherapeutic dose of metformin (50 mg) was previously shown to decrease metformin systemic exposure in healthy adult participants.
- What question did this study address?
  - This study investigated the pharmacokinetic goldenseal–metformin interaction, along with the potential consequent effects on clinical biomarkers of glucose control, in adults with type 2 diabetes taking therapeutic doses of metformin (500–2250 mg).
- What does this study add to our knowledge?
  - The risk of goldenseal precipitating a pharmacokinetic interaction with metformin decreased with increasing metformin dose, indicating that the interaction and the nonlinear absorption of metformin are driven by saturable transport mechanism(s). The mechanism of this presumed transporter-mediated pharmacokinetic drug interaction appears to be competitive inhibition because the magnitude diminished at increasing metformin doses. The apparent decrease in HbA1c, irrespective of the change in metformin exposure, provides a framework for future controlled studies to investigate the potential glucose lowering effects of goldenseal.
- How might this change clinical pharmacology or translational science?
  - Caution may be warranted for patients taking metformin concomitantly with goldenseal, regardless of changes in metformin systemic exposure, due to potential additive blood glucose lowering effects.

1 | Introduction

Despite groundbreaking advances in promising new pharmacotherapeutics throughout the past decade, drug–drug interactions (DDIs) remain a major public health concern worldwide [1]. Regarding the economic and health care burdens, DDIs have been estimated to account for > 70,000 emergency room visits and ~200,000 hospitalizations annually in the United States [2, 3]. DDIs are of particular concern in low- and middle-income countries due to the lack of robust pharmacovigilance programs, contributing to the disproportionate rate of poor health outcomes in these communities [4, 5]. Pharmacokinetic DDIs can lead to changes in the systemic exposure to the object drug, potentially resulting in increased off-target effects and/or decreased efficacy. These risks are further exacerbated by the fact that many pharmacotherapeutics are dose-limited by toxicities. Hence, rigorous evaluation of pharmacokinetic DDIs is critical for optimal dose selection to improve therapeutic outcomes.

People with diabetes often experience polypharmacy due to uncontrolled blood glucose and/or comorbidities, greatly increasing the risk for adverse DDIs. Drug interactions, which are typically perceived to result from concomitant drugs, can also be precipitated by botanical dietary supplements and other natural products [6]. Grapefruit juice and St. John's wort are two textbook examples of natural products that precipitate clinically significant pharmacokinetic drug interactions. Grapefruit juice can greatly increase systemic exposure to certain drugs by inhibiting the prominent drug-metabolizing enzyme cytochrome P450 (CYP) 3A4, potentially leading to drug toxicity. In contrast, grapefruit juice can decrease systemic drug exposure by inhibiting the apically localized intestinal uptake transporters organic anion transporting polypeptides (OATPs), potentially leading to reduced drug efficacy [7]. St. John's wort also can reduce drug systemic exposure by inducing CYP3A4 expression. Despite these well-known examples, natural products remain relatively understudied and overlooked based on the common (mis)perception that they are safe because they are derived from natural sources. Concerns for natural product–drug interactions are further compounded as more and more patients seek natural alternatives to self-treat a variety of medical complications, including type 2 diabetes [8, 9], contributing to the exponential growth in sales of these ubiquitous and readily accessible products [10].

An established but lesser-known natural product precipitant of pharmacokinetic drug interactions is goldenseal [*Hydrastis canadensis* L. (Ranunculaceae)], which is commonly used to self-treat the common cold, indigestion, and allergic rhinitis [11, 12]. Goldenseal also has been used for glucose control because berberine, the predominant phytoconstituent present in goldenseal products [13], reportedly has glucose lowering effects in both rodent models with diabetes and adults with type 2 diabetes [14–16]. Like grapefruit juice, goldenseal is a CYP3A4 inhibitor and has been shown to increase systemic exposure to susceptible drugs [17, 18]. More recently, our group observed an ~25% decrease in metformin systemic exposure after coadministration of a subtherapeutic dose (50 mg) of metformin with goldenseal to 16 healthy adults [19]. This minor pharmacokinetic interaction may be clinically relevant as incremental changes in metformin exposure have been shown to affect glucose control [20]. The working hypothesis is that goldenseal inhibited intestinal uptake transporters involved in metformin absorption because the drug does not readily permeate the intestinal epithelial lining (P_{app}: $1.4–5.0 \times 10^{-7}$ cm/s) due to its hydrophilicity (log *D*), −3.41 (pH 7.4), pK_a (12.4), and lack of metabolism [21–23]. Subsequent mechanistic studies confirmed that goldenseal is an inhibitor of various intestinal uptake transporters (e.g., OCT1/3, PMAT, and ThTR2) both in HEK293 cells overexpressing select transporters and in mice [24].

Although this first documented clinical pharmacokinetic interaction involving metformin and a natural product generated novel information, several questions remain: (i) Is this interaction relevant to people with diabetes taking therapeutic doses of metformin? (ii) Does chronic goldenseal administration increase interaction risk? (iii) Does the pharmacokinetic interaction affect glucose control? Accordingly, the aims of the present study were to evaluate the effects of a well-characterized goldenseal product on metformin pharmacokinetics in adults with type 2 diabetes, after both acute and chronic goldenseal exposure, and assess for potential changes in clinical biomarkers of glucose control (i.e., HbA1c, glycated albumin %, HOMA-IR). Although a metformin dose-dependent pharmacokinetic interaction was observed that did not lead to anticipated changes in clinical biomarkers, goldenseal appeared to affect HbA1c independent of the

pharmacokinetic interaction. Results highlight a potential disconnect between a pharmacokinetic natural product–drug interaction and anticipated changes in therapeutic effects.

2 | Methods

2.1 | Participant Eligibility and Enrollment

Adults with type 2 diabetes who were stabilized on metformin (immediate or extended-release formulations), with doses ranging from 500 to 2550 mg daily, were recruited to participate in a 3-arm, open-label, crossover pharmacokinetic study (Figure S1). The clinical protocol was approved by the Washington State University (WSU) Institutional Review Board (IRB registration #18889) and registered in the ClinicalTrials.gov database (NCT #05081583). All activities took place at the WSU Human Research Clinic on the Health Sciences Campus in accordance with 45 CFR 46 and adherence to Good Clinical Practice guidelines. Signed written informed consent and Health Insurance Portability and Accountability Act compliance forms were obtained from all potential participants prior to screening, which consisted of medical history assessment, physical exam, and clinical laboratory testing (complete blood count with differentials and platelets, complete metabolic panel, HbA1c, and urinalysis). Participation eligibility was based on screening evaluation and inclusion/exclusion criteria (Table S1). A compiled list of concomitant medications is included in the supplemental information; none of these drugs are known to affect metformin pharmacokinetics.

2.2 | Clinical Study Design

On the morning of each inpatient study day, participants were provided a standardized meal (~500 kcal; ~37 g fat, 16 g carbohydrate, 22 g protein) immediately prior to metformin administration. During Arm 1 (baseline), they consumed their entire daily intake of metformin as a single oral dose (Figure 1). During Arm 2 (acute goldenseal exposure), they were administered 3.3 g of a well-characterized oral goldenseal product (550 mg whole root; Solaray, Nutraceutical Corp., Park City, UT) 30 min prior to metformin. Each gram of the goldenseal product contained 28.9 mg of berberine, 18.6 mg of (–)-β-hydrastine, and 0.73 mg of canadine. During Arm 3 (chronic goldenseal exposure), they self-administered 1.1 g of goldenseal thrice daily for 27 consecutive days; compliance was monitored via a video messaging app. On the morning of day 28, participants were administered 1.1 g of goldenseal 30 min prior to metformin; two additional doses of goldenseal (1.1 g) were administered at 4-h intervals. Participants were instructed to administer their daily dose of metformin as prescribed throughout the entire duration of the study without a drug holiday. They were also instructed to take all other medications as prescribed throughout the entirety of

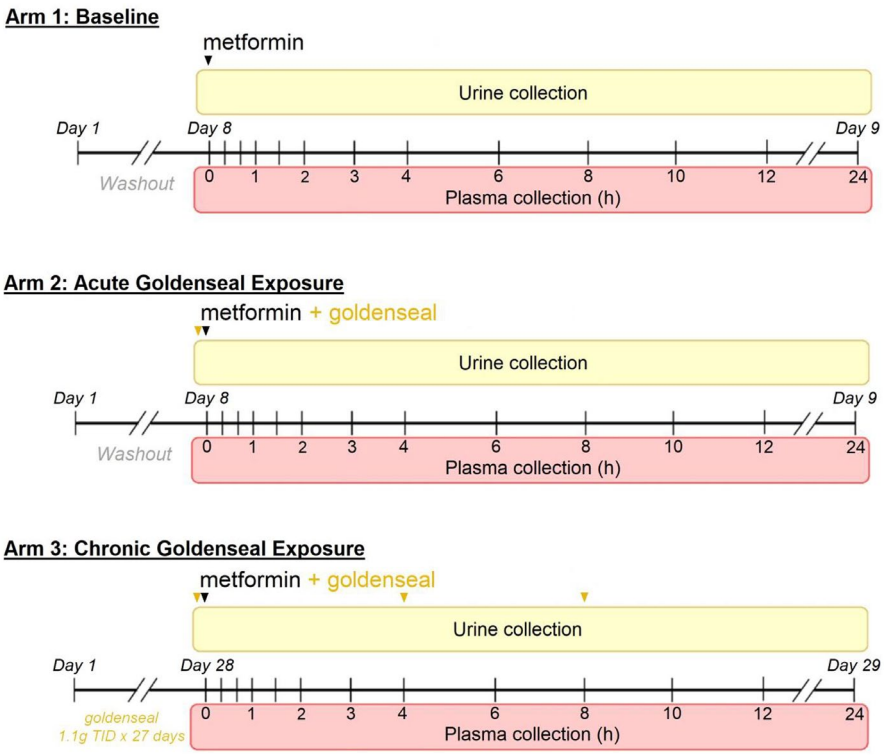

FIGURE 1 | Clinical study design: 3-arm, open-label, crossover. Participants consumed their entire daily dose of metformin (500–2550 mg) at the start of each arm. Plasma and urine were collected up to 24 h after metformin administration. During Arm 2 (acute goldenseal exposure), a single dose of goldenseal (3.3 g) was administered ~30 min prior to metformin. During Arm 3 (chronic goldenseal exposure), participants self-administered goldenseal (1.1 g) thrice daily for 27 days; compliance was monitored via a video messaging app. On Day 28, a single dose of goldenseal (1.1 g) was administered ~30 min prior to metformin; 2 additional doses of goldenseal were administered in 4-h increments (depicted with gold arrows). Each arm was separated by at least a 7-day goldenseal washout period. The washout period was also necessary to ensure participants did not consume food/alcohol known to affect drug metabolizing enzyme and transporters prior to participation in the study.

the study. During all three arms, blood (8 mL) was collected into BD Vacutainer EDTA tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) from an arm vein via an indwelling peripheral venous catheter at designated time points before and up to 12 h post-metformin administration; a single venous blood draw was collected the following morning at the 24-h time point. An additional blood sample (8 mL) was collected into BD Vacutainer SST tubes for clinical biomarker assessment at the beginning of each study arm when participants were fasted overnight. Urine was collected during the 0–12 and 12–24 h intervals after metformin administration. Vital signs (blood pressure, oxygen saturation, pulse) and fasting blood glucose were measured periodically during each visit. Inpatient visits for each arm were separated by a 7-day goldenseal washout period, with Arm 3 being the final arm completed by all participants. A washout period was also necessary to ensure participants did not consume food/liquids known to affect drug metabolizing enzymes and transporters prior to participation in the study.

2.3 | Bioanalytical Method

Plasma was separated from red blood cells via centrifugation (1600 $g \times 10$ min) and subsequently transferred to cryovials. Prior to quantitative analysis, plasma and urine samples were processed by adding 250 μL acetonitrile (containing d_6-metformin as internal standard) to 100 μL of the sample, followed by centrifugation at 20,800 g and 4°C for 10 min to precipitate proteins. Supernatants were collected, diluted 1:50 in acetonitrile, and analyzed using an established UHPLC–MS/MS method [25].

2.4 | Pharmacokinetic Analysis

Metformin pharmacokinetics were determined via noncompartmental analysis using Phoenix WinNonlin (v7.0, Certara, Radnor, PA). Because metformin could not be discontinued in these participants, area under the plasma concentration vs. time curve (AUC) from time zero to the last measured concentration ($AUC_{0\text{-tlast}}$) and maximum plasma concentration (C_{max}) were corrected for residual drug. That is, metformin concentrations in the collected samples were corrected by subtracting the calculated decayed concentrations of residual metformin at each respective timepoint using the terminal elimination rate constant determined for each participant. This correction for residual metformin concentration was necessary to standardize comparisons between study arms due to potential differences in the time during which metformin was administered the day prior. The following additional metrics were recovered from the plasma concentration vs. time data as described: terminal half-life ($t_{1/2}$), time to reach C_{max} (t_{max}), and renal clearance (CL_R) [19].

2.5 | Statistical and Power Analyses

A sample size of 20 evaluable participants was determined to provide > 80% power to detect a 20% change in metformin AUC with a Type I error of 0.05, assuming 29.4% intra-individual variability [26]. The primary endpoints were the geometric mean ratio (GMR; goldenseal exposure-to-baseline) of metformin $AUC_{0\text{-tlast}}$ and C_{max}. The predefined no effect range was 0.80–1.25; that is, if the GMR lay outside this range, a pharmacokinetic interaction was evident. Secondary pharmacokinetic endpoints included $t_{1/2}$, t_{max}, and CL_R. When appropriate, secondary endpoints were compared using the Wilcoxon matched-pairs signed-rank test. Descriptive statistics were recovered using GraphPad Prism 9 (GraphPad Software Inc., La Jolla, CA).

2.6 | Pharmacodynamic Assessments

Clinical biomarkers were measured in serum samples collected at the beginning of study participation and after 28 days of goldenseal administration. Hemoglobin A1c (HbA1c) was evaluated using the Tina-quant assay (Roche Diagnostics International Ltd., Rotkreuz, Switzerland). Glycated albumin % was calculated as the ratio of glycated albumin to albumin, which was measured using the Lucica (EKF Diagonistics, Texas, USA) and ALB Flex (Siemens Healthcare Diagnostics Inc., Delaware, USA) assay, respectively. Insulin and glucose concentrations were measured using recombinant ELISA kits (ab278123; Abcam Inc., Cambridge, UK) and Contour Next glucose monitor kit (Ascensia Diabetes Care, New Jersey, USA), respectively. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated using the following equation: $\frac{\text{glucose}\left(\frac{\text{mg}}{\text{dL}}\right) \times \text{insulin}\left(\frac{\mu\text{IU}}{\text{mL}}\right)}{405}$ [27].

3 | Results

3.1 | Participant Demographics

A total of 22 participants (12 males,10 females) completed the study; none withdrew from the study. Using the NIH demographics form, 20 participants self-identified as White, 1 as American Indian, and 1 as two or more races. Median age [range] was 47 [22–65] years. Mean weight and BMI [90% confidence interval] were, respectively, 106 [96–117] kg and 32 [29–35] kg/m^2 for males and 98 [87–110] kg and 36 [32–40] kg/m^2 for females.

3.2 | Pharmacokinetic Goldenseal-Metformin Interaction Study

All participants tolerated goldenseal well during Arms 2 and 3. No serious adverse effects were reported. Because metformin is known to exhibit nonlinear absorption at therapeutic doses [28–30], the concentration-time profiles (Figure 2) and pharmacokinetics (Table 1) of metformin were categorized according to the total daily dose: ≤ 750, 1000–1500, and ≥ 2000 mg. The changes in metformin AUC and C_{max} did not differ between acute and chronic goldenseal administration; hence, the GMRs for both arms were combined for assessment (Figure 3). At the lower metformin doses (500–750 mg; $n = 8$), AUC_{0-12h} and C_{max} GMRs were 0.80 [0.62–1.04] and 0.79 [0.66–0.94], respectively. At the moderate doses (1000–1500 mg; $n = 15$), AUC_{0-12h} and C_{max} GMRs were 0.85 [0.72–1.02] and 0.86 [0.71–1.02], respectively. At the high doses (2000–2250 mg; $n = 21$), AUC_{0-12h} and C_{max} GMRs were 1.00 [0.91–1.08] and 1.00 [0.90–1.10], respectively. Metformin AUC and C_{max} GMRs were 0.86 [0.76–0.96] and 0.88 [0.78–0.99], respectively, for participants taking immediate release formulations ($n = 14$) and were 1.00 [0.92–1.11] and

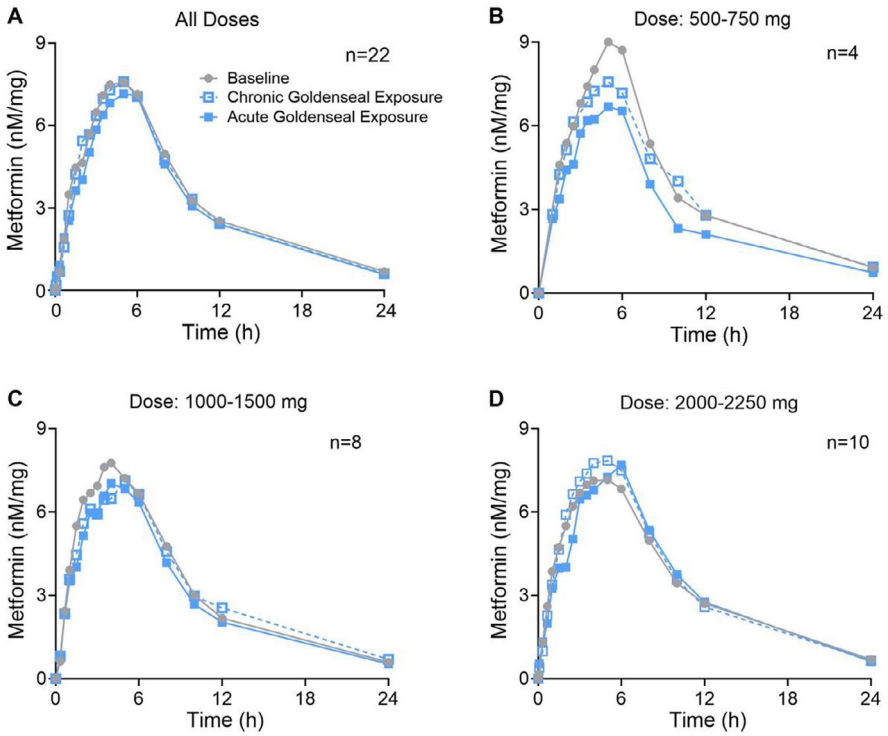

FIGURE 2 | Dose-adjusted metformin plasma concentration versus time profiles for the (A) aggregate data and for data binned into (B) low, (C) moderate, and (D) high metformin dose groups. Symbols denote the geometric mean of metformin concentrations in the absence of goldenseal (gray circles) and after acute (solid blue squares) and chronic (open blue squares) exposure to goldenseal. Error bars are excluded from the graphs for visual clarity; readers are referred to Table 1 for metformin pharmacokinetic variability. One participant reported a change in metformin dose (1000–2000 mg) midway through the study, thus the baseline arm was repeated for this participant at the new dose to provide an appropriate paired analysis.

0.96 [0.86–1.05], respectively, for those taking extended-release dosages ($n = 8$).

3.3 | Clinical Biomarkers of Glucose Control

Clinical biomarker data for three participants were not included in the analysis due to changes to their diabetic pharmacotherapy midway through study participation; an additional four participants were not included in the HbA1c analysis due to the inability to obtain lab results. Of those with evaluable results ($n = 15$), mean [90% confidence interval] HbA1c decreased from 6.8% [6.5%–7.1%] at baseline to 6.5% [6.2%–6.8%] after 28 consecutive days of goldenseal administration (Figure 4A). Mean fasting blood glucose and glycated albumin were 141 mg/dL [131–151 mg/dL] and 14.7% [13.4%–16.0%], respectively, at baseline and were 140 mg/dL [131–150 mg/dL] and 14.5% [13.5%–15.4%], respectively, after goldenseal exposure (Figure 4B,C). Mean HOMA-IR at baseline and after goldenseal exposure were 13.0 [10.2–15.8] and 14.9 [11.6–18.1], respectively (Figure 4D).

4 | Discussion

Natural product–drug interactions are a growing public health concern as more patients seek botanical and other natural alternatives to supplement their pharmacotherapeutic regimens [31, 32]. The botanical product goldenseal was recently shown to precipitate a pharmacokinetic interaction with a subtherapeutic dose of metformin (50 mg) in healthy adults [19]. Specifically, goldenseal decreased metformin systemic exposure by ~25% relative to baseline. The logical question subsequently arose: how do these results translate to people with type 2 diabetes taking metformin? The aims of the current study were to (i) evaluate whether goldenseal precipitates a pharmacokinetic interaction with therapeutic doses of metformin, (ii) determine if duration of exposure to goldenseal affects the magnitude of interaction risk, and (iii) assess if any pharmacokinetic changes affect clinical endpoints in this patient population.

Based on the predefined no-effect range (metformin AUC_{0-12h} GMR: 0.80–1.25), the aggregate data indicated no pharmacokinetic interaction between goldenseal and metformin at therapeutic doses (GMR, 0.93) (Figure 2A). However, when the data were binned into different metformin dose groups, the change in metformin systemic exposure after goldenseal coadministration decreased with increasing metformin dose (Figure 3). Specifically, at the lowest dose range (500–750 mg), goldenseal decreased metformin AUC and C_{max} by ~20% relative to baseline, recapitulating observations from our previous study involving a subtherapeutic dose of metformin [19]. At moderate metformin doses (1000–1500 mg), goldenseal decreased AUC and C_{max} by ~14%. At the highest metformin doses (2000–2550 mg), the risk of an interaction was effectively null (Figure 3). The pharmacokinetic interaction appeared to be dependent on metformin formulation (immediate vs. extended release) but independent of the duration of goldenseal coadministration. These observations,

TABLE 1 | Pharmacokinetics of metformin (500–2550 mg) after oral administration alone and after acute (single 3.3-g dose) and chronic (1.1 g thrice daily for 28 days) exposure to goldenseal. Data represent geometric means [90% confidence intervals] unless noted otherwise.

	Baseline	Acute goldenseal exposure	Chronic goldenseal exposure
Metformin 500–2550 mg (*n*)	23[a]	22	22
AUC_{0-12h} (nM×h/mg)[b]	59.6 [52.0–68.5]	54.5 [48.2–61.7]	57.3 [50.3–65.3]
C_{max} (nM/mg)[b]	8.92 [7.86–10.1]	8.36 [7.55–9.27]	8.26 [7.37–9.25]
$t_{1/2}$ (h)	4.73 [4.18–5.35]	4.70 [4.22–5.23]	4.79 [4.27–5.37]
t_{max} (h)[c]	3.50 [1.00–10.00]	4.50 [1.50–6.00]	4.50 [1.50–6.00]
CL_R (mL/min)	259 [203–331]	249 [200–309]	250 [199–314]
Metformin 500–750 mg (*n*)[d]	4	4	4
AUC_{0-12h} (nM×h/mg)[b]	61.6 [31.8–82.0]	43.0 [29.6–62.9]*	53.6 [23.9–74.2]
C_{max} (nM/mg)[b]	9.87 [7.18–11.4]	6.87 [5.22–9.49]*	7.76 [4.64–10.4]*
$t_{1/2}$ (h)	3.24 [2.12–4.94]	3.83 [2.21–6.63]	3.63 [2.40–5.50]
t_{max} (h)[c]	5.50 [3.00–6.00]	4.5 [3.00–6.00]	4.75 [2.50–6.00]
CL_R (mL/min)	458 [225–712]	282 [186–555]	402 [162–564]
Metformin 1000–1500 mg (*n*)	8	8	7[a]
AUC_{0-12h} (nM×h/mg)[b]	67.2 [50.0–90.2]	58.8 [44.8–77.1]	60.0 [46.2–77.8]
C_{max} (nM/mg)[b]	10.3 [7.94–13.4]	9.15 [7.44–11.2]	8.91 [7.14–11.1]
$t_{1/2}$ (h)	4.43 [3.62–5.42]	4.66 [3.76–5.76]	4.56 [3.38–6.15]
t_{max} (h)[c]	3.50 [1.00–6.00]	4.5 [2.00–6.00]	3.5 [2.00–6.00]
CL_R (mL/min)	279 [189–410]	260 [145–465]	208 [137–316]
Metformin 2000–2550 mg (*n*)	11[a]	10	11[a]
AUC_{0-12h} (nM×h/mg)[b]	56.1 [46.2–68.0]	56.6 [48.7–65.8]	59.7 [50.2–71.1]
C_{max} (nM/mg)[b]	8.00 [6.69–9.57]	8.39 [7.24–9.71]	8.23 [6.94–9.77]
$t_{1/2}$ (h)	5.80 [5.2–6.46]	5.14 [4.70–5.61]	5.46 [5.06–5.89]
t_{max} (h)[c]	3.50 [1.00–10.00]	4.75 [1.50–6.00]	5.00 [1.50–6.00]
CL_R (mL/min)	207 [138–312]	225 [188–270]	249 [200–309]

Note: AUC_{0-12h}, area under the plasma concentration versus time curve from 0 to 12 h. No statistical significance was observed for secondary endpoints ($t_{1/2}$, t_{max}, and CL_R) based on Wilcoxon matched-pairs signed-rank test ($p < 0.05$).
Abbreviations: CL_R, renal clearance; C_{max}, maximum plasma concentration; $t_{½}$, terminal half-life; t_{max}, time to reach C_{max}.
[a]One participant reported a change in metformin dose (1000–2000 mg) midway through the study, thus the baseline arm was repeated to provide an appropriate paired analysis.
[b]Normalized based on metformin hydrochloride dose.
[c]Median [range].
[d]Due to the small sample size, median [range] is reported.
*Significant difference as determined by the pre-defined no effect range of 0.80–1.25 for the goldenseal exposure-to-baseline AUC ratio.

coupled with no significant change in metformin half-life and renal clearance, suggested that goldenseal decreased metformin systemic exposure at low therapeutic doses primarily by attenuating a saturable intestinal process involved in metformin absorption. A limitation with the current observations is that there were a limited number of participants in each of the various dose groups. Hence, a study sufficiently powered to detect differences in the magnitude of the goldenseal-metformin pharmacokinetic interaction at the different metformin dose ranges, as well as differences between acute and chronic goldenseal administration, is necessary to confirm this hypothesis.

The nonlinear absorption kinetics of metformin (less than proportional increase in AUC with increasing dose) are well documented [28–30]. However, the underlying mechanism remains equivocal. Previous in vitro studies identified two mechanisms as potential contributors to this nonlinearity: saturable paracellular diffusion and saturable active transport through a transcellular absorption pathway [22, 33]. If paracellular diffusion is the primary saturable component of metformin absorption, then the relative contribution of active transport will *increase* with increasing metformin dose, which will result in a greater magnitude of change in a transporter-mediated interaction. In contrast,

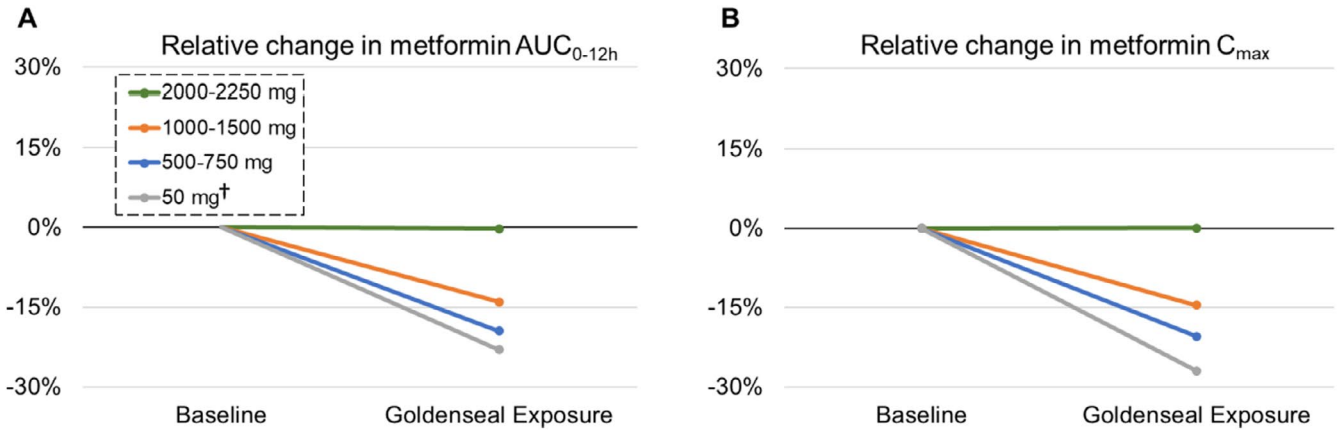

FIGURE 3 | Mean change in metformin systemic exposure after goldenseal co-administration. The relative change in metformin AUC_{0-12h} (A) and C_{max} (B) after goldenseal exposure is grouped by metformin dose. Effects of acute and chronic goldenseal administration are combined. †Effects of goldenseal administration on the pharmacokinetics of metformin (50 mg) in healthy adult participants are reproduced with permission from the American Society for Clinical Pharmacology and Therapeutics (Nguyen et al. 2020).

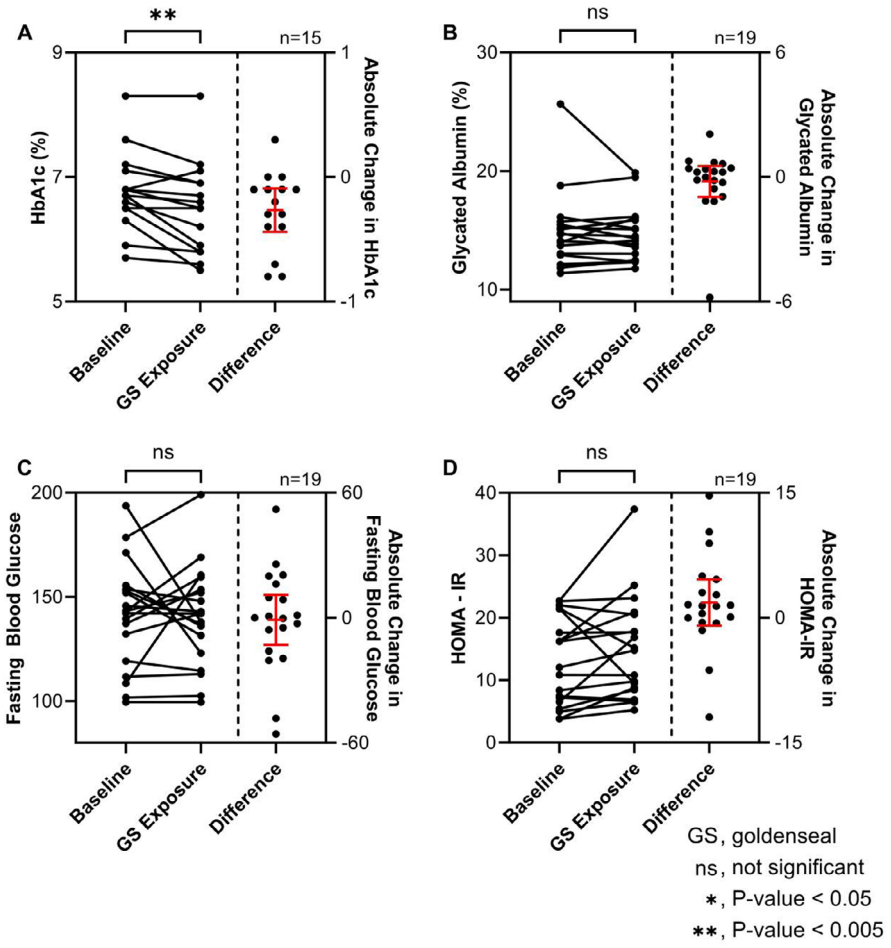

FIGURE 4 | Clinical biomarkers of glucose control measured for each participant at the beginning of the study (Baseline) and after 28 days of goldenseal co-administration (GS Exposure): HbA1c (A), glycated albumin (B), fasting blood glucose (C), and HOMA-IR (D). Intraindividual changes for each participant are shown in the right side of the plots; the red center and extreme horizontal lines denote means and 90% confidence intervals, respectively.

if active transport is the primary saturable component, then the relative contribution of active transport will *decrease* with increasing metformin dose, which will reduce the magnitude of a transporter-mediated drug interaction. Regarding the current study, the diminished interaction at the higher metformin doses indicated that saturation of active transport is the underlying mechanism of the nonlinear absorption of metformin. These clinical results are also consistent with mechanistic studies that

identified goldenseal and berberine, the predominant alkaloid in goldenseal, as inhibitors of select intestinal transporters presumed to be involved in metformin absorption (i.e., OCT1/3, PMAT, ThTr-2) [24, 34–36]. Indeed, the estimated luminal concentration of berberine after administration of a single 1.1 g dose of the goldenseal product (~350 μM) was at least 10-fold higher than the IC_{50} for each transporter, further supporting potential contributions by these transporters to the goldenseal–metformin interaction [19, 24].

The effects of goldenseal on clinical biomarkers of glucose control were discordant with those expected from the changes in metformin systemic exposure. Based on the decrease in metformin AUC and C_{max} (dose range: 500–1500 mg) after goldenseal coadministration, a decrease in drug efficacy (increased blood glucose concentrations) was anticipated. However, efficacy was apparently enhanced, as mean HbA1c decreased from 6.8% to 6.5% after coadministration of goldenseal with metformin for 28 days (Figure 4A). These observations are consistent with those from previous clinical studies where daily administration of 1500 and 1000 mg berberine to adults with type 2 diabetes reduced HbA1c from 9.5% to 7.5% and from 7.5% to 6.6%, respectively [37, 38]. The amount of berberine administered in the current study was ~95 mg daily. Collectively, these observations suggest that the magnitude of HbA1c reduction may depend on berberine dose. Regarding the current study, the presumed antihyperglycemic effects of goldenseal could have offset the projected adverse impact from the pharmacokinetic interaction (if any) with metformin.

Because HbA1c is a measure of average blood glucose for the preceding 3–4 months, this clinical biomarker may have underestimated the presumed antihyperglycemic effects of goldenseal, which was administered for only ~1 month. Glycated albumin, an emerging but less established biomarker, reflects average blood glucose for the preceding 6–8 weeks, representing a potential tool for assessing therapeutic effects over a shorter duration [39, 40]. Results from this secondary assessment, as well as fasting blood glucose and HOMA-IR, did not support the antihyperglycemic effects of goldenseal (Figure 4B–D). A potential confounding factor is a sequence effect stemming from the study design (chronic goldenseal exposure was the final arm completed by all participants), which was implemented due to logistical constraints. That is, participants may have altered their behavior (e.g., improved dietary habits and increased physical activity) because they were monitored throughout the study. This type of participation bias may have contributed to the apparent decrease in HbA1c. Another confounder is that the study was not powered to detect clinically meaningful changes in these biomarkers. Collectively, results support the need for an expanded study with a larger sample size and extended administration duration (> 90 days) to confirm the effects of goldenseal on blood glucose.

Pharmacokinetic results from this clinical study are the first to demonstrate that the nonlinear absorption of metformin is driven primarily by saturable intestinal transporter(s). This concept can be applied to other orally administered drugs that are absorbed predominantly via this mechanism (e.g., uptake of fexofenadine by OATP2B1). That is, at higher therapeutic doses of the object drug, the risk of transporter-mediated drug interactions in the intestine diminishes because of the high luminal concentrations saturating active transport processes, assuming transporter inhibition occurs in a competitive manner. A range of goldenseal doses would provide further insights into the mechanism of inhibition.

The current study demonstrated the utility of including clinical biomarkers in the evaluation of pharmacokinetic natural product–drug interactions. In general, a change in object drug systemic exposure should correlate with a change in pharmacological effects. However, because natural products contain multiple constituents (many of which could be pharmacologically active), the change in drug systemic exposure resulting from a pharmacokinetic natural product–drug interaction may not necessarily correlate with the anticipated effects on clinical endpoints. Regarding the current study, had the clinical biomarkers not been measured, the potential glucose lowering effects of goldenseal would not have been detected. These observations highlight the need to measure changes in clinical biomarkers to comprehensively assess the impact of natural product–drug interactions.

In summary, results from this comprehensive clinical study provided mechanistic insight into a pharmacokinetic natural product–drug interaction and identified potential pharmacologic effects of the natural product that are independent of the interaction. The aggregate pharmacokinetic data suggested that there was no interaction between goldenseal and metformin. However, at metformin doses < 1000 mg, goldenseal coadministration appeared to decrease exposure to the antidiabetic drug. Finally, the combination of metformin and goldenseal could have additive/synergistic antihyperglycemic effects as observed by the decrease in HbA1c, potentially necessitating closer monitoring of blood glucose. Additional studies are needed to determine conclusively whether goldenseal has therapeutic benefits, which are essential to guide patients and healthcare providers about supplementing pharmacotherapeutic regimens with this natural product.

Author Contributions

J.T.N. and M.F.P. wrote the manuscript; J.T.N., K.E.T., J.R.W., and M.F.P. designed the research; J.T.N., C.M.A., R.S.T., M.G.C., M.E.L., and J.R.W. performed the research; J.T.N. and C.M.A. analyzed the data; C.M.A. and K.E.T. contributed the analytical tools.

Acknowledgments

The authors thank Judy Griffin and Kate Newbill for their expert nursing skills and Deena Hadi for her assistance with clinical study logistics. M.F.P. dedicates this article to Dr. David P. Paine.

Conflicts of Interest

The authors declare no conflicts of interest.

Prior Presentation

Parts of this work were presented as a poster at the American Society for Clinical Pharmacology and Therapeutics annual meeting held in Atlanta, GA, 22–24 March 2023.

References

1. B. Guthrie, B. Makubate, V. Hernandez-Santiago, and T. Dreischulte, "The Rising Tide of Polypharmacy and Drug-Drug Interactions: Population Database Analysis 1995–2010," *BMC Medicine* 13 (2015): 74.

2. B. Percha and R. B. Altman, "Informatics Confronts Drug–Drug Interactions," *Trends in Pharmacological Sciences* 34 (2013): 178–184.

3. N. P. Tatonetti, G. H. Fernald, and R. B. Altman, "A Novel Signal Detection Algorithm for Identifying Hidden Drug–Drug Interactions in Adverse Event Reports," *Journal of the American Medical Informatics Association* 19 (2012): 79–85.

4. R. Kiguba, S. Olsson, and C. Waitt, "Pharmacovigilance in Low- and Middle-Income Countries: A Review With Particular Focus on Africa," *British Journal of Clinical Pharmacology* 89 (2023): 491–509.

5. R. Hussain, M. A. Hassali, A. Ur Rehman, J. Muneswarao, and F. Hashmi, "Physicians' Understanding and Practices of Pharmacovigilance: Qualitative Experience From A Lower Middle-Income Country," *International Journal of Environmental Research and Public Health* 17 (2020): 2209.

6. M. F. Paine, D. D. Shen, and J. S. McCune, "Recommended Approaches for Pharmacokinetic Natural Product-Drug Interaction Research: A NaPDI Center Commentary," *Drug Metabolism and Disposition* 46 (2018): 1041–1045.

7. D. G. Bailey, G. K. Dresser, B. F. Leake, and R. B. Kim, "Naringin Is a Major and Selective Clinical Inhibitor of Organic Anion-Transporting Polypeptide 1A2 (OATP1A2) in Grapefruit Juice," *Clinical Pharmacology and Therapeutics* 81 (2007): 495–502.

8. V. Vuksan and J. L. Sievenpiper, "Herbal Remedies in the Management of Diabetes: Lessons Learned From the Study of Ginseng," *Nutrition, Metabolism, and Cardiovascular Diseases* 15 (2005): 149–160.

9. J. Kesavadev, B. Saboo, S. Sadikot, et al., "Unproven Therapies for Diabetes and Their Implications," *Advances in Therapy* 34 (2017): 60–77.

10. T. Smith, H. Resetar, and C. Morton, "US Sales of Herbal Supplements Increase by 9.7% in 2021," *HerbalGram* 136 (2022): 42–69.

11. "National Center for Complementary and Integrative Health (NCCIH) Goldenseal," *National Center for Complementary and Integrative Health* (2021), https://nccih.nih.gov/health/goldenseal.

12. L. Shane-McWhorter, "Goldenseal," *Merck Manual* (2023), https://www.merckmanuals.com/professional/special-subjects/dietary-supplements/goldenseal.

13. E. D. Wallace, N. H. Oberlies, N. B. Cech, and J. J. Kellogg, "Detection of Adulteration in *Hydrastis canadensis* (Goldenseal) Dietary Supplements via Untargeted Mass Spectrometry-Based Metabolomics," *Food and Chemical Toxicology* 120 (2018): 439–447.

14. W. Xie, F. Su, G. Wang, et al., "Glucose-Lowering Effect of Berberine on Type 2 Diabetes: A Systematic Review and Meta-Analysis," *Frontiers in Pharmacology* 13 (2022): 1015045.

15. Y. Liang, X. Xu, M. Yin, et al., "Effects of Berberine on Blood Glucose in Patients With Type 2 Diabetes Mellitus: A Systematic Literature Review and a Meta-Analysis," *Endocrine Journal* 66 (2019): 51–63.

16. X. Xia, J. Yan, Y. Shen, et al., "Berberine Improves Glucose Metabolism in Diabetic Rats by Inhibition of Hepatic Gluconeogenesis," *PLoS One* 6 (2011): e16556.

17. B. J. Gurley, S. F. Gardner, M. A. Hubbard, et al., "In Vivo Effects of Goldenseal, Kava Kava, Black Cohosh, and Valerian on Human Cytochrome P450 1A2, 2D6, 2E1, and 3A4/5 Phenotypes," *Clinical Pharmacology and Therapeutics* 77 (2005): 415–426.

18. B. J. Gurley, A. Swain, M. A. Hubbard, et al., "Supplementation With Goldenseal (*Hydrastis canadensis*), but Not Kava Kava (*Piper methysticum*), Inhibits Human CYP3A Activity In Vivo," *Clinical Pharmacology and Therapeutics* 83 (2008): 61–69.

19. J. T. Nguyen, D. D. Tian, R. S. Tanna, et al., "Assessing Transporter-Mediated Natural Product–Drug Interactions via In Vitro–In Vivo Extrapolation: Clinical Evaluation With a Probe Cocktail," *Clinical Pharmacology and Therapeutics* 109 (2021): 1342–1352.

20. K. Kanto, H. Ito, S. Noso, et al., "Effects of Dosage and Dosing Frequency on the Efficacy and Safety of High-Dose Metformin in Japanese Patients With Type 2 Diabetes Mellitus," *Journal of Diabetes Investigation* 9 (2018): 587–593.

21. K. M. Huttunen, A. Mannila, K. Laine, et al., "The First Bioreversible Prodrug of Metformin With Improved Lipophilicity and Enhanced Intestinal Absorption," *Journal of Medicinal Chemistry* 52 (2009): 4142–4148.

22. W. R. Proctor, D. L. Bourdet, and D. R. Thakker, "Mechanisms Underlying Saturable Intestinal Absorption of Metformin," *Drug Metabolism and Disposition* 36 (2008): 1650–1658.

23. X. Liang and K. M. Giacomini, "Transporters Involved in Metformin Pharmacokinetics and Treatment Response," *Journal of Pharmaceutical Sciences* 106 (2017): 2245–2250.

24. V. O. Oyanna, K. Y. Garcia-Torres, B. J. Bechtold, et al., "Goldenseal-Mediated Inhibition of Intestinal Uptake Transporters Decreases Metformin Systemic Exposure in Mice," *Drug Metabolism and Disposition* 51 (2023): 1483–1489, https://doi.org/10.1124/dmd.123.001360.

25. O. Scherf-Clavel, M. Kinzig, M. S. Stoffel, U. Fuhr, and F. Sörgel, "A HILIC-MS/MS Assay for the Quantification of Metformin and Sitagliptin in Human Plasma and Urine: A Tool for Studying Drug Transporter Perturbation," *Journal of Pharmaceutical and Biomedical Analysis* 175 (2019): 112754.

26. A. Frid, G. N. Sterner, M. Löndahl, et al., "Novel Assay of Metformin Levels in Patients With Type 2 Diabetes and Varying Levels of Renal Function: Clinical Recommendations," *Diabetes Care* 33 (2010): 1291–1293.

27. T. M. Wallace, J. C. Levy, and D. R. Matthews, "Use and Abuse of HOMA Modeling," *Diabetes Care* 27 (2004): 1487–1495.

28. N. C. Sambol, J. Chiang, M. O'Conner, et al., "Pharmacokinetics and Pharmacodynamics of Metformin in Healthy Subjects and Patients With Noninsulin-Dependent Diabetes Mellitus," *Journal of Clinical Pharmacology* 36 (1996): 1012–1021.

29. G. T. Tucker, C. Casey, P. J. Phillips, H. Connor, J. D. Ward, and H. F. Woods, "Metformin Kinetics in Healthy Subjects and in Patients With Diabetes Mellitus," *British Journal of Clinical Pharmacology* 12 (1981): 235–246.

30. A. J. Scheen, "Clinical Pharmacokinetics of Metformin," *Clinical Pharmacokinetics* 30 (1996): 359–371.

31. E. J. Johnson, V. González-Peréz, D. D. Tian, et al., "Selection of Priority Natural Products for Evaluation as Potential Precipitants of Natural Product–Drug Interactions: A NaPDI Center Recommended Approach," *Drug Metabolism and Disposition* 46 (2018): 1046–1052.

32. D. Safari, E. C. DeMarco, L. Scanlon, and G. T. Grossberg, "Over-The-Counter Remedies in Older Adults: Patterns of Use, Potential Pitfalls, and Proposed Solutions," *Clinics in Geriatric Medicine* 38 (2022): 99–118.

33. Y. Shirasaka, M. Seki, M. Hatakeyama, et al., "Multiple Transport Mechanisms Involved in the Intestinal Absorption of Metformin: Impact on the Nonlinear Absorption Kinetics," *Journal of Pharmaceutical Sciences* 111 (2022): 1531–1541.

34. T. Han, W. R. Proctor, C. L. Costales, H. Cai, R. S. Everett, and D. R. Thakker, "Four Cation-Selective Transporters Contribute to Apical Uptake and Accumulation of Metformin in Caco-2 Cell Monolayerss," *Journal of Pharmacology and Experimental Therapeutics* 352 (2015): 519–528.

35. R. Shi, Z. Xu, X. Xu, et al., "Organic Cation Transporter and Multidrug and Toxin Extrusion 1 Co-Mediated Interaction Between

Metformin and Berberine," *European Journal of Pharmaceutical Sciences* 127 (2019): 282–290.

36. M. Kwon, Y. A. Choi, M.-K. Choi, and I.-S. Song, "Organic Cation Transporter-Mediated Drug–Drug Interaction Potential Between Berberine and Metformin," *Archives of Pharmacal Research* 38 (2015): 849–856.

37. J. Yin, H. Xing, and J. Ye, "Efficacy of Berberine in Patients With Type 2 Diabetes Mellitus," *Metabolism* 57 (2008): 712–717.

38. H. Zhang, J. Wei, R. Xue, et al., "Berberine Lowers Blood Glucose in Type 2 Diabetes Mellitus Patients Through Increasing Insulin Receptor Expression," *Metabolism* 59 (2010): 285–292.

39. N. Furusyo and J. Hayashi, "Glycated Albumin and Diabetes Mellitus," *Biochimica et Biophysica Acta* 1830 (2013): 5509–5514.

40. F. C. Chume, M. H. Kieling, P. A. Correa Freitas, G. Cavagnolli, and J. L. Camargo, "Glycated Albumin as a Diagnostic Tool in Diabetes: An Alternative or an Additional Test?," *PLoS One* 14 (2019): e0227065.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.