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Clinical Research Article

The Use of Ceramides to Predict Metabolic Response to Metformin in Women With PCOS

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Abstract

Context: Polycystic ovarian syndrome (PCOS) is a complex disorder in which metabolic abnormalities are associated with reproductive dysfunction. Women with PCOS have increased ceramide concentrations. Previous studies demonstrated that treating metabolic abnormalities of PCOS with metformin improved glucose effectiveness after 12 weeks.

Objective: We evaluated whether, in women with PCOS, lower baseline ceramide, diacylglycerol (DAG), and triacylglycerol (TAG) concentrations were associated with improved metabolic response to metformin.

Methods: Women (n = 29), aged 29 ± 5 years and diagnosed with PCOS by the NIH criteria underwent an intravenous glucose tolerance test (IVGTT) before and after 12-week treatment with metformin (1500 mg per day). Metabolic responders were defined by improved glucose effectiveness, specifically, the ability of glucose to stimulate uptake and suppress production, after metformin treatment.

Results: Twelve weeks of metformin resulted in weight loss (−1.7 ± 2.6 kg, $P < 0.01$) and a reduction in BMI (−0.6 ± 0.9 kg/m^2, $P < 0.01$) with no change in HbA1c. The concentrations of Cer(d18:1/22:0), Cer(d18:1/24:0), total ceramides, total Cer(d16:0), total Cer(d18:2), DAG, dihydrosphingomyelin (DHSM), and TAG decreased after metformin treatment ($P < 0.05$). Baseline total Cer(d16:0) concentration <204.1 pmol/mL was 82% sensitive (AUC 0.72, $P = 0.03$) and total DHSM concentration <32237 pmol/mL was 100% specific (AUC 0.73, $P = 0.03$) in predicting improved metabolic response to metformin, as measured by IVGTT.

Conclusion: Lower total Cer(16:0) and DHSM concentrations are associated with a beneficial metabolic response to metformin in women with PCOS. Based on the known association between higher ceramide levels and type 2 diabetes, the data suggest that metformin improves metabolic parameters in women with mild metabolic derangements.

Key Words: polycystic ovary syndrome, metformin, ceramides, precision treatment

Abbreviations: AIR_g, acute insulin response to glucose; AMPK, adenosine monophosphate–activated protein kinase; AUC, area under the curve; BMI, body mass index; DAG, diacylglycerol; DHSM, dihydrosphingomyelin; DI, disposition index; G_b, basal glucose; I_b, basal insulin; HOMA-B, homeostatic model assessment for beta-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; IVGTT, intravenous glucose tolerance test; MR, metabolic responder; NR, nonresponder; OR, odds ratio; PCOS, polycystic ovary syndrome; PKC, protein kinase; ROC, receiver operating characteristics curve; S_g, glucose effectiveness; S_i, insulin sensitivity; SM, sphingomyelin; TAG, triacylglycerol.

Polycystic ovarian syndrome (PCOS) is a common [1] yet complex disorder that showcases the intricate interaction of metabolic disruption with reproductive dysfunction. The diagnostic clinical criteria are based on the manifestations of the woman's altered hypothalamic-pituitary-ovarian axis (menstrual irregularities, hyperandrogenism, and/or polycystic ovaries on ultrasound) [2]; however, up to 65% of women are also afflicted with metabolic derangements largely driven by insulin resistance [3, 4]. Metformin has therefore been a drug of choice, targeting insulin resistance in women with PCOS [5–7]. Indeed, metformin has been shown to improve glucose effectiveness, promote weight loss, and restore ovulation in 61% of women with PCOS after 10 to 12 weeks of treatment [8].

Emerging data have revealed the importance of ceramides, diacylglycerol (DAG), and triacylglycerol (TAG) in insulin signaling, insulin sensitivity, fatty acid metabolism, and mitochondrial function [9–13]. Ceramides induce insulin resistance and have been used as a marker for metabolic impairment and cardiovascular disease [14, 15]. Women with PCOS have increased concentrations of ceramides, sphingosine-1-phosphate (S1P), and sphingomyelins, with subtle changes in concentrations based on phenotype (nonobese vs obese; insulin resistant vs non–insulin resistant) [16]. In addition, ceramides have a crucial role in hypothalamic-pituitary signaling for pubertal initiation [17]. Sphingosine-1-phosphate is an essential stimulator of follicular development and ovulation [18]. Taken together, ceramides

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are associated with both the metabolic and reproductive phenotypes in PCOS.

In women with PCOS, 12 weeks of treatment with metformin was associated with a change in ceramide and sphingolipid concentrations [19]. However, not all women with PCOS improve when treated with metformin. We therefore hypothesized that in women with PCOS, metabolic dysfunction characterized by baseline ceramide, DAG, and TAG concentrations will predict metabolic response to metformin.

Methods

Participants

Participants recruited were adult women of reproductive age (18-40 years old), diagnosed with PCOS by the National Institutes of Health (NIH) criteria [20]. Participants, who were a subset (based on sample availability) of an open-label single-arm clinical trial (National Clinical Trial no. NCT01389778), underwent an intravenous glucose tolerance test (IVGTT) before and after 12 weeks of treatment with metformin (1500 mg per day) [8]. Participants were excluded if they had abnormalities in thyroid or renal function, had hyperprolactinemia, or were pregnant or planned pregnancy. All individuals were screened for and did not have nonclassic adrenal hyperplasia. Participants were not on oral hypoglycemics, insulin modulators, insulin, lipid-lowering therapy, anti-inflammatory agents, or hormonal medications.

IVGTT

After an overnight fast and baseline lab draw, all participants underwent an IVGTT twice (before and after 12 weeks of treatment with metformin) with an intravenous bolus of glucose 0.3 g/kg at time 0 minutes, followed by regular human insulin 0.03 U/kg intravenously at 20 minutes. Blood samples were drawn for glucose and insulin concentrations over 180 minutes. MinMod Millenium was used to calculate β-cell indices [21]. Calculated indices included the acute insulin response (first phase) to glucose (AIR_g), insulin sensitivity (S_i), glucose effectiveness (S_g), basal glucose (G_b), basal insulin (I_b), disposition index (DI), insulin secretion via the homeostasis model of assessment-B (HOMA-B), and HOMA-insulin resistance (HOMA-IR). Metabolic responders (MR) were defined by improved S_g after metformin treatment whereas nonresponders (NR) had no change or a decrease in S_g.

Assays

Fasting samples were obtained after a 12- to 16-hour overnight fast. Samples were stored at −80 °C. Insulin was measured using an immunochemiluminescent immunoassay (Immulite 2000; Diagnostic Products Corp), with a lower limit of detection of 2.0 μIU/mL (14.4 pmol/L). Sphingolipids were measured on stored serum samples as previously described [8]. Briefly, blood sphingolipids are highly stable and not modified by multiple freeze-thaw cycles, temperature, or long-term storage [22, 23]. Serum samples were thawed at 4 °C for 12 hours then the internal standard (IS) stock mix and protein precipitation solvent were added. The internal standard (IS) stock solution contained sphingomyelin (SM)(d18:1/16:1)-d9 (74 pmol/sample), SM(d18:1/18:1)-d9 (47 pmol/sample), SM(d18:1/20:1)-d9 (23 pmol/sample), SM(d18:1/122:1)-d9 (44 pmol/sample), SM(d18:1/24:1)-d9 (64 pmol/sample), dihydro-cer (d18:0/18:1) (5 pmol/sample), d7-ceramide (d18:1-d7/16:0) (60 pmol/sample), d7-ceramide (d18:1-d7/18:0) (35 pmol/sample), d7-ceramide (d18:1/24:0) (150 pmol/sample), d7-ceramide (d18:1/24:1) (312 pmol/sample), and glucosylceramide (d18:1/17:0) (50 pmol/sample). Tert-Butyl methyl ether (MTBE) was used to extract the lipids in the organic phase. Samples were mixed thoroughly and placed on a shaker at 4 °C for 15 minutes followed by a 10-minute centrifugation at 15 000*g*. The supernatant was then evaporated to dryness using a speedvac. Lipid pellets were reconstituted in methanol/water and then underwent liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis. A total of 12 lipid species were measured, including ceramides with acyl chain lengths of 16 [Cer(d18:1/16:0)], 18 [Cer(d18:1/18:0)], 20 [Cer(d18:1/20:0)], 22 [Cer(d18:1/22:0)], and 24 [Cer(d18:1/24:0)], and a carbon length of 24:1 [Cer(d18:1/24:1)], total ceramides (sum of all ceramides), total dihydroceramides with a backbone of 16 carbons [total Cer(d16:0)], total dihydroceramides with a backbone of 18 carbons and 2 unsaturated bonds [total Cer(d18:2)], total dihydrosphingomyelin (DHSM), total diacylglycerol (DAG), and total triacylglycerol (TAG).

Statistical Analysis

Categorical variables were reported as the number percentage or observations per category and group differences were measured with the Pearson Chi square or Fisher exact test when applicable. Continuous variables were expressed as mean with SD when normally distributed and subjected to the 2-sample *t* test when comparing 2 groups and analysis of variance (ANOVA) when comparing 3 or more groups. Continuous variables with a non-Gaussian distribution were reported as median with interquartile range (25th-75th IQR) and analyzed with nonparametric testing (Wilcoxon rank sum test). Receiver operating characteristic (ROC) curves were calculated for each baseline ceramide/sphingolipid associated with a metabolic response to metformin and the area under the curve (AUC or C-statistic) and sensitivity and specificity for classifying metabolic response status were determined. Multivariable logistic regression analyses, adjusting for age and body mass index (BMI), calculated odds ratios (OR) and 95% CI for the association of each ceramide/sphingolipid with metabolic response to metformin were calculated. We evaluated baseline concentration, and change in concentration over the treatment period, in relation to metabolic response. Calculations were corrected for multiple comparisons. Differences with a significance level $P < 0.05$ were considered to be statistically significant.

Results

Clinical Characteristics

Participants with PCOS (age 29 β 5 years) identified as White 34% (n = 10, 34%), African American 14% (n = 4), Asian (n = 3), Latinx (n = 3), mixed ethnicities (n = 8), and Native American (n = 1). 83% (n = 20) reported acne with a median Ferriman-Gallwey Score of 13 [7–19]. Among the participants, 17% (n = 5) were prediabetic (HbA1c 5.7%–6.4%) and 24% (n = 7) had insulin resistance as calculated by their baseline HOMA-IR of ≥3. All participants completed treatment with metformin ER 1500 mg/day for 12 weeks. Treatment with metformin resulted in significant weight loss

Table 1. Anthropometric and laboratory measurements of women with PCOS at baseline and after 12 weeks of metformin

	Baseline (n = 29)	12 weeks (n = 29)	*P* value
Systolic BP, mmHg	113 ± 15	112 ± 10	0.6
Diastolic BP, mmHg	70 ± 10	70 ± 10	0.7
BMI, kg/m^2	30 ± 7	29 ± 7	<0.01
WC, cm	102 ± 17	101 ± 18	0.1
Weight, kg	84 ± 22	82 ± 22	<0.01
Truncal fat mass, g	17095 ± 7822	17840 ± 9291	0.6
Total fat mass, g	34456 ± 13258	35194 ± 13762	0.6
Total lean mass, g	47936 ± 9473	57051 ± 53874	0.7
HbA1c, %	5.5 ± 0.3	5.5 ± 0.3	0.7
HOMA-IR, mM x mU/L^2	2.2 ± 1.7	2.0 ± 1.7	0.3
Total cholesterol, mg/dL	171 ± 37	166 ± 38	0.09
Triglycerides, mg/dL	91 ± 50	80 ± 34	0.08
HDL, mg/dL	49 ± 11	50 ± 12	0.4
LDL, mg/dL	104 ± 33	100 ± 35	0.09
Total testosterone, ng/dL	54 ± 30	45 ± 33	0.1
DHEAS, ng/dL	238 ± 114	246 ± 152	0.4
SHBG, nmol/L	43 ± 26	40 ± 23	0.3

Abbreviations: BP, blood pressure; BMI, body mass index; HOMA-IR, homeostatic model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; DHEAS, dehydroepiandrosterone sulfate; SHBG, sex hormone binding globulin. *P* values computed with the Student *t* test.

($P < 0.01$) with no difference in fat mass or lean body mass distribution (Table 1).

Calculated β-cell Indices

Treatment with metformin resulted in a decrease in fasting glucose concentrations (−3.3% change from baseline, $P = 0.01$), with improved glucose effectiveness (+39% change from baseline, $P = 0.01$) and an increase in the first phase insulin secretion (+60% change from baseline, $P = 0.01$). There was no change in DI, likely due to the absence of differences in either insulin sensitivity (S_i) or insulin secretion parameters (I_b) (Table 2).

Metabolic Responders vs Nonresponders

Participants were then compared based on an increase in S_g after metformin treatment. Metabolic responders (MR, n = 18) were similar in age, race/ethnicity, BMI, cholesterol, androgens, and baseline HOMA-IR compared with nonresponders (NR, n = 11) (Table 3). MRs had significantly lower S_g at baseline compared with NR with no difference in AIR_g, S_i, or DI (Fig. 1). Compared with NR, baseline total Cer(d16:0) concentrations were lower in MR [total Cer(d16:0) MR 174 ± 30 pmol/mL vs NR 215 ± 66 pmol/mL, $P = 0.03$] (Fig. 2). Similarly, baseline total DHSM concentrations were lower in MR compared with NR (MR 36662 ± 8421 pmol/ml vs 45900 ± 13897 pmol/ml, $P = 0.03$) (Fig. 2). In MR, there were significant reductions in Cer(d18:1/16:0), Cer(d18:1/20:0), Cer(d18:1/24:0), total ceramides, and total Cer(d18:2) concentrations after 12 weeks of metformin treatment. In NR, however, only total Cer(d16:0) concentrations were reduced after 12 weeks of metformin treatment, achieving concentrations comparable to those found at baseline in

Table 2. IVGTT-derived glucose homeostasis parameters of women with PCOS at baseline and after 12 weeks of metformin

	Baseline (n = 29)	16 weeks (n = 29)	*P* value
AIR_g, mIU/L × min	481 ± 328	770 ± 463	0.01
DI	1564 ± 921	2463 ± 2440	0.3
S_i, mIU/L-1 × min^{-1}	4.0 ± 2.5	3.9 ± 2.2	0.3
S_g, min^{-1}	0.021 ± 0.0079	0.026 ± 0.011	0.01
G_b, mg/dL	81 ± 6	79 ± 6	0.01
I_b, mIU/L	10 ± 8	10 ± 7	0.5
HOMA-B, mIU/mM	218 ± 181	255 ± 203	0.1
HOMA-IR, mM × mU/L^2	2.0 ± 1.5	1.9 ± 1.5	0.3

Abbreviations: AIR_g, acute insulin response to glucose; DI, disposition index; G_b, basal glucose; HOMA-B, homeostatic model assessment of β-cell function; HOMA-IR, homeostatic model assessment of insulin resistance; I_b, basal insulin; S_g, glucose effectiveness; S_i, insulin sensitivity.

Table 3. Demographic and metabolic parameters of women with PCOS by metabolic response to metformin

	Metabolic responders (n = 18)	Nonresponders (n = 11)	*P* value
Age, yrs	29 ± 6	29 ± 4	0.8
Ethnicity, n (%)			0.4
Caucasian	5 (28)	5 (45)	
African	3 (17)	1 (10)	
Other	10 (55)	5 (45)	
Acne, n (%)	14 (78)	6 (55)	0.09
FGS, median (IQR)	14 (8-19)	12 (6-19)	0.9
Systolic BP, mmHg	114 ± 16	111 ± 14	0.9
Diastolic BP, mmHg	71 ± 11	69 ± 10	0.5
BMI, kg/m^2	30 ± 7	30 ± 7	0.8
WC, cm	102 ± 17	102 ± 19	0.7
Weight, kg	87 ± 23	79 ± 21	0.4
Total fat mass, g	36635 ± 13502	30891 ± 12639	0.3
Total lean mass, g	48706 ± 9998	46676 ± 8861	0.6
HbA1c, %	5.5 ± 0.3	5.6 ± 0.2	0.3
HOMA-IR, mM x mU/L^2	2.1 ± 1.6	2.3 ± 1.9	0.8
Total cholesterol, mg/dL	171 ± 36	172 ± 38	0.9
Triglycerides, mg/dL	93 ± 50	87 ± 52	0.7
HDL, mg/dL	48 ± 10	51 ± 13	0.5
LDL, mg/dL	105 ± 33	103 ± 34	0.8
Total testosterone, ng/dL	52 ± 19	57 ± 44	0.7
DHEAS, ng/dL	242 ± 118	231 ± 113	0.8
SHBG, nmol/L	44 ± 25	41 ± 30	0.8

Metabolic responders had improved glucose effectiveness after 12 weeks of metformin treatment. *P* values computed with the Student *t* test.
Abbreviations: BMI, body mass index; BP, blood pressure; DHEAS, dehydroepiandrosterone sulfate; FGS, Ferriman-Gallwey Score; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; LDL, low-density lipoprotein; SHBG, sex hormone binding globulin; WC, waist circumference.

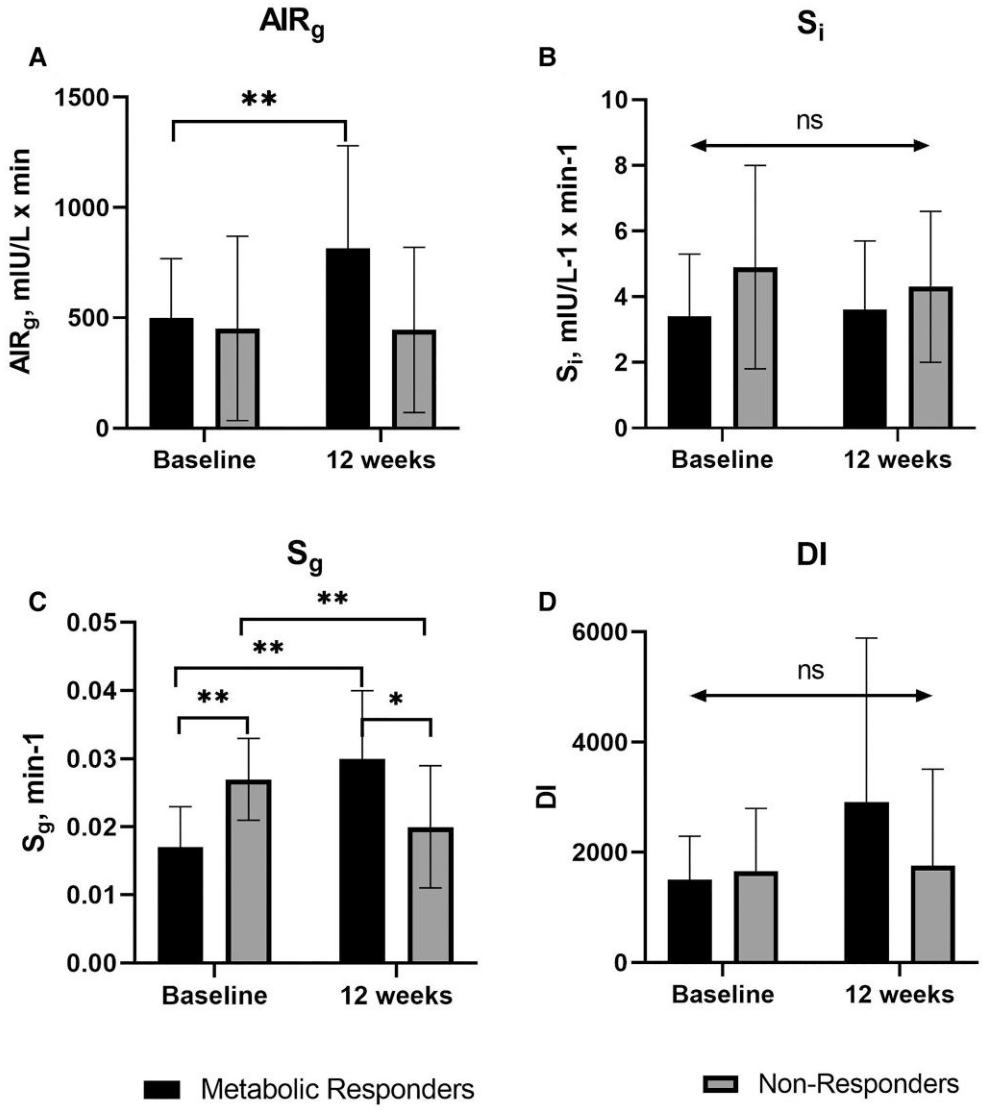

Figure 1. IVGTT-derived glucose homeostasis parameters (A, acute insulin response to glucose or AIR_g; B, insulin sensitivity or S_i; C, glucose effectiveness or Sg; D, disposition index or DI) in metabolic responders (black bars) and nonresponders (gray bars) at baseline and after 12 weeks of metformin.

MR (Table 4). Total Cer(d16:0) concentrations < 204 pmol/mL were 82% sensitive (AUC 0.72, $P = 0.02$) and total DHSM concentrations < 32237 pmol/mL were 100% specific (AUC 0.73, $P = 0.03$) in predicting improved metabolic response to metformin, as measured by IVGTT (Table 5). Utilizing the ROC curve's optimal threshold to predict a metabolic response, only a total Cer(d16:0) concentration < 204.1 pmol/mL was significantly associated with metabolic response to metformin (OR 6.1; 95% CI, 1.2–32.1) (Fig. 3). The relationship was no longer statistically significant after multivariable adjustment.

Ceramide Concentrations and Calculated β-cell Indices

After adjusting for multiple comparisons using a False Discovery Rate < 0.05, baseline DAG and TAG concentrations were associated with baseline HOMA-B (DAG $r^2 = 0.47$, $P < 0.01$; TAG $r^2 = 0.32$, $P < 0.01$), I_b (DAG $r^2 = 0.48$, $P < 0.01$; TAG $r^2 = 0.33$, $P < 0.01$) and HOMA-IR (DAG $r^2 = 0.45$, $P < 0.01$; TAG $r^2 = 0.3$, $P < 0.01$). Baseline ceramide concentrations were not associated with S_g or improved ovulation.

Discussion

In women with PCOS, total Cer(16:0) concentrations were sensitive in predicting a metabolic response to metformin, as defined by improved glucose effectiveness, after 12 weeks of treatment. In addition, total DHSM concentrations were specific in predicting a metabolic response to metformin. Women with an improved metabolic response to metformin after 12 weeks of treatment had lower baseline total Cer(16:0) and total DHSM concentrations compared with women who did not respond (Fig. 4). While baseline DAG and TAG were associated with baseline β-cell functional indices (HOMA-B, I_b, and HOMA-IR), they were not associated with a response to metformin. Even though many baseline ceramide concentrations were similar in responders and nonresponders, metformin was effective in reducing multiple ceramide concentrations in metabolic responders [Cer(d18:1/16:0), Cer(d18:1/22:0), Cer(d18:1/

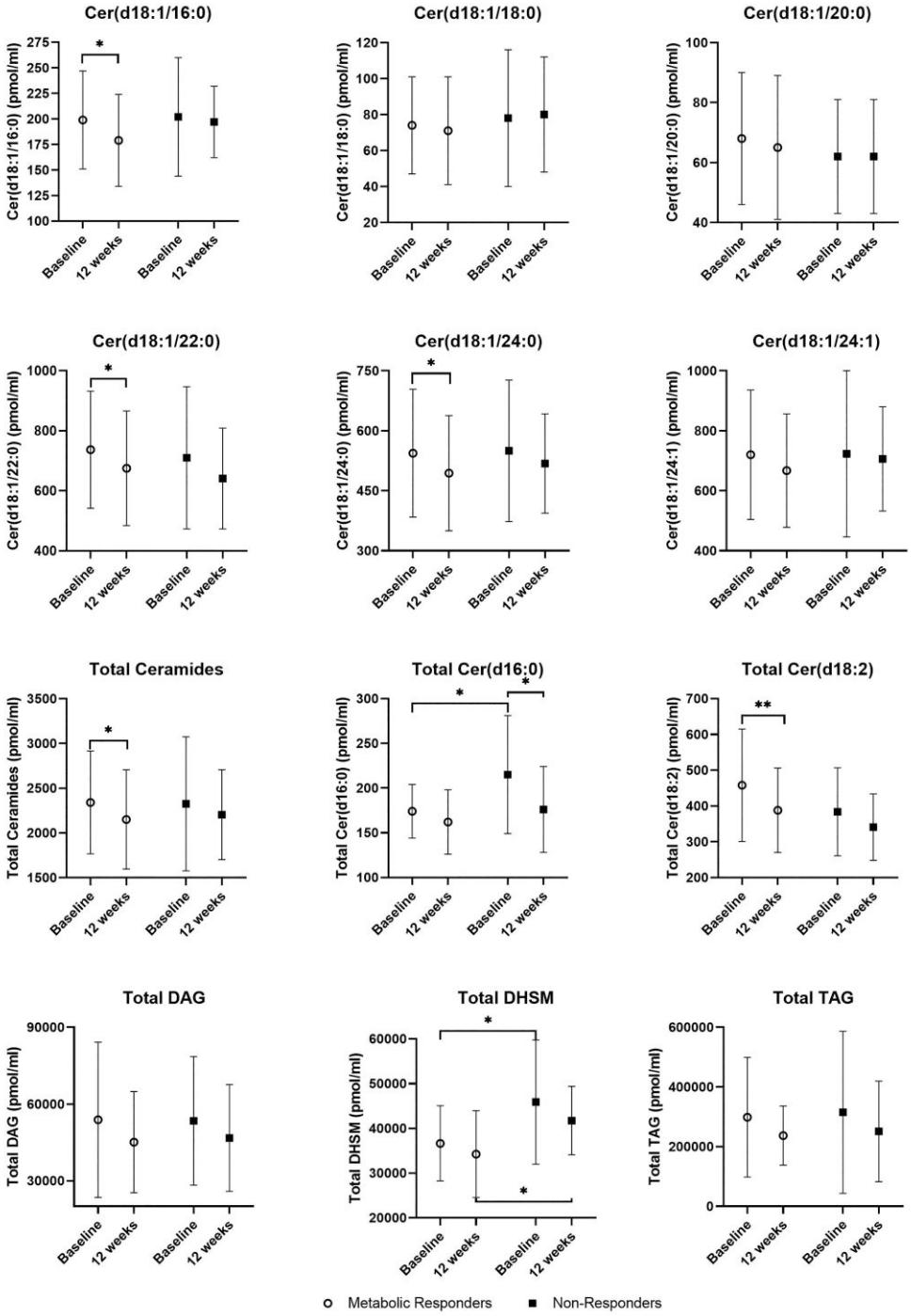

Figure 2. Sphingolipid concentrations in metabolic responders (white circles) and nonresponders (black squares) at baseline and at 12 weeks of metformin treatment. Abbreviations: DAG, diacylglycerol, DHSM, dihydrosphingomyelin; TAG, triacylglycerol.

24:0), total ceramides, total Cer(d16:0), total Cer(d18:2)] but only one in nonresponders [total Cer(d16:0)]. This highlights the potential to apply precision therapy to the treatment of PCOS by utilizing ceramides to identify women with PCOS who are more likely to respond to metformin.

Ceramides, in particular total Cer(d16:0), independently predict insulin resistance, diabetes mellitus type 2, and cardiovascular mortality [24–26]. Women with PCOS have elevated ceramide concentrations compared with controls [27, 28], and exhibit insulin resistance with an increased risk of diabetes mellitus type 2 and cardiovascular disease [3, 29, 30]. In this study, metformin effectively reduced multiple ceramide concentrations only in metabolic responders. Metabolic responders also had significantly lower total Cer(d16:0) and DHSM concentrations at baseline. Our data expands results from a previous study demonstrating that a reduction in ceramide concentrations after metformin treatment depends on a more favorable metabolic profile at baseline [19]. Women with PCOS have lower plasma adiponectin concentrations and increased intramuscular ceramide and lipid contents, resulting in impaired adenosine monophosphate–activated protein kinase (AMPK) expression and thus skeletal

Table 4. Ceramide and sphingolipid concentrations in metabolic responders and nonresponders at baseline and at 12 weeks of metformin treatment

Ceramide/sphingolipid	Metabolic responder (n = 18)		*P* values	Nonresponder (n = 11)		*P* values	Baseline *P* values
	Baseline	12 weeks		Baseline	12 weeks		
Cer(d18:1/16:0)	199 ± 48	179 ± 45	0.02	202 ± 58	197 ± 35	0.7	0.9
Cer(d18:1/18:0)	74 ± 27	71 ± 30	0.3	78 ± 38	80 ± 32	0.8	0.8
Cer(d18:1/20:0)	68 ± 22	65 ± 24	0.2	62 ± 19	62 ± 19	1.0	0.5
Cer(d18:1/22:0)	737 ± 195	675 ± 191	0.02	710 ± 237	641 ± 168	0.1	0.7
Cer(d18:1/24:0)	544 ± 160	494 ± 144	0.04	550 ± 177	518 ± 124	0.4	0.9
Cer(d18:1/24:1)	720 ± 216	667 ± 189	0.1	723 ± 283	706 ± 174	0.8	0.9
Total Ceramides	2341 ± 574	2151 ± 554	0.02	2326 ± 748	2204 ± 501	0.4	0.9
Total Cer(d16:0)	174 ± 30	162 ± 36	0.2	215 ± 66	176 ± 48	0.01	0.03
Total Cer(d18:2)[a]	458 ± 157	388 ± 118	<0.01	384 ± 123	341 ± 93	0.09	0.2
Total DAG	53850 ± 30363	45102 ± 19757	0.08	53427 ± 25108	46736 ± 20896	0.2	0.9
Total DHSM[a]	36662 ± 8421	34250 ± 9713[b]	0.07	45900 ± 13897	41754 ± 7628[b]	0.1	0.03
Total TAG	298593 ± 200485	237193 ± 99043	0.2	315189 ± 271608	251351 ± 168461	0.2	0.8

Abbreviations: DAG, diacylglycerol; DHSM, dihydrosphingomyelin; TAG, triacylglycerol.
[a]Two-way ANOVA difference overall in responders vs nonresponders.
[b]$P = 0.04$ metabolic responders vs nonresponders at 12 weeks.

Table 5. ROC-AUC analysis of serum ceramide concentrations in classification of metabolic response to metformin among women with PCOS

Ceramide/ sphingolipid	Threshold (pmol/mL)	C-statistic	Sensitivity (%)	Specificity (%)
Cer(d18:1/16:0)	<198.4	0.53	53	64
Cer(d18:1/18:0)	<79	0.51	71	55
Cer(d18:1/20:0)	<85.2	0.53	29	91
Cer(d18:1/22:0)	>507	0.49	100	18
Cer(d18:1/24:0)	<562	0.53	71	45
Cer(d18:1/24:1)	<812	0.50	76	45
Total Ceramide	>1644	0.50	100	19
Total Cer(d16:0)	<204.1	0.72[a]	82	64
Total Cer(d18:2)	>340	0.6	83	45
Total DAG	>108267	0.47	12	100
Total DHSM	<32237	0.73[a]	41	100
Total TAG	<140239	0.52	24	91

Abbreviations: AUC, area under the curve; DAG, diacylglycerol, DHSM, dihydrosphingomyelin; ROC, receiver operating characteristics curve; TAG, triacylglycerol.
[a]$P < 0.05$.

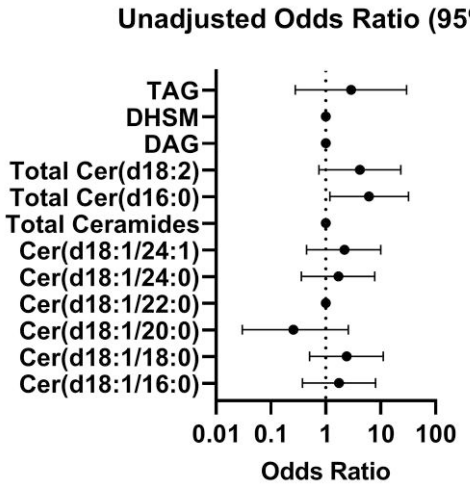

Figure 3. Forrest plot of odds ratios (95% CI) for metabolic response to metformin of sphingolipid concentrations. Abbreviations: DAG, diacylglycerol; DHSM, dihydrosphingomyelin; TAG, triacylglycerol.

muscle insulin resistance [31]. Metformin activates AMPK and PKC-dependent glucose uptake in muscle [32], and may, in part, counteract the detrimental effects of ceramides. Intramyocellular ceramides also potentiate insulin resistance via mitochondrial dysfunction [14] and inhibition of insulin mediated GLUT4 translocation through protein phosphatase 2 (PP2)- and PKC-dependent pathways [33]. Metformin improves mitochondrial function via inhibition of mitochondrial respiratory-chain complex 1 which reduces NADH oxidation but does not act through ceramide inhibition of mitochondrial function [34]. Given metformin's variable response in PCOS [8, 35], women with severe metabolic disease as evidenced by high ceramide concentrations may be less likely to respond due to competitive and parallel pathways.

The higher ceramide concentrations of total Cer(d16:0) and DHSM in nonresponders are likely contributing to their lack of response to metformin. Metformin decreases hepatic glucose production resulting in improved glucose-mediated glucose disposal (S_g) and first phase insulin response (AIR_g). In rats with elevated hepatic ceramide and DAG concentrations, metformin improved glucose tolerance but did not reverse it [36]. Cer(d16:0) is associated with obesity, hepatic steatosis, and insulin resistance [14]. In fact, reduction in Cer(d16:0) concentrations via ablation of the *Cers6* gene, which encodes the enzyme that makes Cer(d16:0), prevented high-fat diet–induced obesity and glucose intolerance in a *Cers6*-deficient animal model [9]. In addition, diacylation of ceramides via ceramidase reduces PKC activation, prevented hepatic

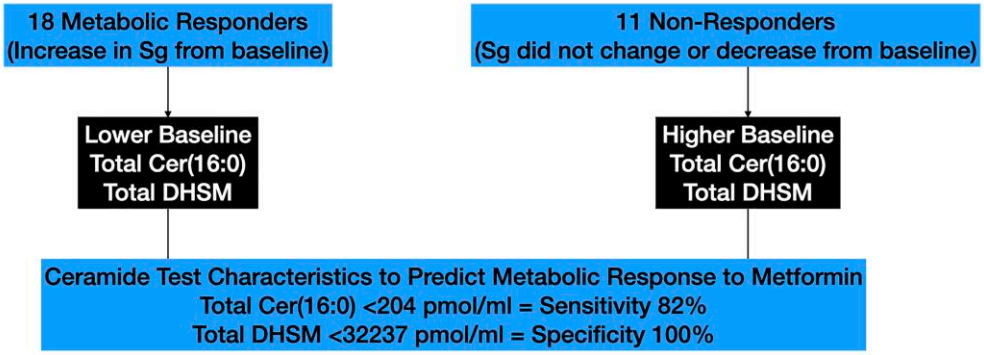

Figure 4. Summary of study results. Sg, glucose effectiveness; Total Cer(16:0), total dihydroceramides with a backbone of 16 carbons; DHSM, dihydrosphingomyelin.

steatosis, and improved insulin action [37]. Preventing the formation of dihydrosphingolipids mitigates the risk of hepatic steatosis, insulin resistance, and prediabetes [38]. While metformin reduces ceramide and dihydroceramide concentrations, it does not decrease them to normal concentrations. This again supports the greater effectiveness we observed of metformin in women with PCOS who have mild metabolic abnormalities. The significance of total Cer(16:0) in predicting a metabolic response was lost after adjusting for BMI in our cohort, since higher ceramide concentrations are associated with higher BMI; thus, total Cer(16:0) is not independent of BMI.

Ceramide measurement in clinical practice has not been widespread. Nevertheless, recognition of their utility has been demonstrated in predicting cardiovascular disease [15, 39, 40] and laboratory testing is available commercially [41]. Ceramides have been studied in precision medicine initiatives in the fields of oncology, cardiovascular disease, Alzheimer's disease, multiple sclerosis, and type 2 diabetes mellitus [42–45]. While data on the genetic and environmental susceptibility to PCOS continue to be explored [46, 47], there are limited data on precision therapeutics for PCOS. This is the first study, to our knowledge, to establish a potential role of ceramides, in particular Cer(d16:0), to inform treatment decisions based on patient risk profile.

One of the major strengths of this study is its novelty in exploring circulating ceramides as a tool to determine the likelihood of a woman with PCOS to experience improved metabolic health with a pharmacologic treatment (metformin). Metabolic parameters were measured via an IVGTT, providing a validated measurement of glucose metabolism. Limitations include the study's sample size, lack of a randomized controlled study design, and its short, although adequate duration [19, 48].

In conclusion, our study highlights the potential use of ceramides to aid treatment decisions regarding the use of metformin in women with PCOS. While metformin improved glucose effectiveness, the response was limited to a subset of participants who had milder metabolic dysfunction. Women with higher ceramide concentrations, which are associated with a more severe metabolic profile and future risk of cardiometabolic diseases, did not demonstrate improved glucose metabolism with metformin. Future directions involve larger-scale studies utilizing ceramides for precision diagnostics and treatment. While the data are not strong enough to recommend the measurement of ceramides in all women with PCOS, we strongly recommend more studies using ceramides for prediction and additional medications that focus on improving the individualized care of women with PCOS.

Disclosures

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Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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