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Metformin Does not Reduce Markers of Cell Proliferation in Esophageal Tissues of Patients with Barrett's Esophagus

Amitabh Chak¹, Navtej S. Buttar², Nathan R. Foster², Drew K. Seisler², Norman E. Marcon³, Robert Schoen⁴, Marcia R. Cruz-Correa⁵, Gary W. Falk⁶, Prateek Sharma⁷, Chin Hur⁸, David A. Katzka², Luz M. Rodriguez⁹, Ellen Richmond⁹, Anamay N. Sharma², Thomas C. Smyrk², Sumithra J. Mandrekar², Paul J. Limburg², and the Cancer Prevention Network

¹University Hospitals Case Medical Center, Cleveland, OH

²Mayo Clinic, Rochester, MN

³St. Michael's Hospital, Toronto, ON, Canada

⁴University of Pittsburgh, Pittsburgh, PA

⁵University of Puerto Rico, San Juan, PR

⁶University of Pennsylvania Perelman School of Medicine, Philadelphia, PA

⁷Kansas City VA, Kansas City, MO

⁸Massachusetts General Hospital, Boston, MA

⁹Division of Cancer Prevention, National Cancer Institute, National Institutes of Health.

Abstract

Background & Aims—Obesity is associated with neoplasia, possibly via insulin-mediated cell pathways that affect cell proliferation. Metformin has been proposed to protect against obesity-associated cancers by decreasing serum insulin. We conducted a randomized, double-blind,

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Address for Correspondence: Amitabh Chak, M.D. University Hospitals/Case Medical Center 11100 Euclid Avenue Cleveland, OH 44106 Telephone: 216-844-5385 amitabh.chak@case.edu.

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Author Involvement:

Amitabh Chak, Navtej S. Buttar, Paul J. Limburg: Concept and design, acquisition of data, analysis and interpretation, drafting of manuscript, critical revision, study supervision

Everyone else: Concept and design, acquisition of data, analysis and interpretation, drafting of manuscript, critical revision.

Luz Rodriguez, Ellen Richmond: Concept and design, analysis and interpretation, drafting of manuscript, critical revision, study supervision

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placebo-controlled, phase 2 study of patients with Barrett's Esophagus (BE) to assess the effect of metformin on phosphorylated S6 kinase (pS6K1) -- a biomarker of insulin pathway activation.

Methods—Seventy-four subjects with BE (mean age 58.7 years; 58 men [78%]; 52 with BE > 2 cm [70%]) were recruited through 8 participating organizations of the Cancer Prevention Network. Participants were randomly assigned to groups given metformin daily (increasing to 2000 mg/day by week 4; n=38) or placebo (n=36) for 12 weeks. Biopsy specimens were collected at baseline and at week 12 via esophagogastroduodenoscopy. We calculated and compared percent changes in median levels of pS6K1 between subjects given metformin vs placebo as the primary endpoint.

Results—The percent change in median level of pS6K1 did not differ significantly between groups (1.4% among subjects given metformin vs -14.7% among subjects given placebo; 1-sided $P=.80$). Metformin was associated with an almost significant reduction in serum levels of insulin (median -4.7% among subjects given metformin vs 23.6% increase among those given placebo, $P=.08$) as well as in homeostatic model assessments of insulin resistance (median -7.2% among subjects given metformin vs 38% increase among those given placebo, $P=.06$). Metformin had no effects on cell proliferation (based on assays for KI67) or apoptosis (based on levels of caspase 3).

Conclusions—In a chemoprevention trial of patients with BE, daily administration of metformin for 12 weeks, compared with placebo, did not cause major reductions in esophageal levels of pS6K1. Although metformin reduced serum levels of insulin and insulin resistance, it did not discernibly alter epithelial proliferation or apoptosis in esophageal tissues. These findings do not support metformin as a chemopreventive agent for BE-associated carcinogenesis.

Keywords

HOMA-IR; diabetes drug; cancer development; tumorigenesis

BACKGROUND

Obesity has been linked to a variety of malignancies.¹⁻⁴ Recent studies suggest that one explanation for the role of obesity in the development of cancer is activation of the insulin/insulin-like growth factor (IGF) pathway.⁵⁻⁷ A diet high in energy, high in animal fat, and low in fiber in combination with physical inactivity contributes to insulin resistance and resulting hyperinsulinemia. Complex interactions of increased levels of insulin, IGF1, and members of the serum IGF binding protein (IGFBP) family (IGFBP1 thru IGFBP6) determine the levels of insulin and IGF that are available to mediate effects at the cellular level through the insulin receptor (IR) and the IGF-1 receptor (IGF-1R).^{3, 6-8} Activation of the insulin receptor (IR) and IGF-1R stimulates cellular proliferation and inhibits apoptosis via molecular pathways that are mediated by PI3K, AKT, mTOR, S6K1, and other signaling molecules.

Central adiposity is a risk factor that is independently and consistently associated with Barrett's esophagus (BE) and esophageal adenocarcinoma (EAC).⁹ Activation of the insulin/IGF pathway is associated with Barrett's-mediated carcinogenesis.^{10, 11} Metformin is an insulin sensitizer commonly used to treat diabetes mellitus. It lowers serum insulin levels and directly inhibits cell growth. Besides inhibiting gluconeogenesis, this biguanide derivative activates AMP-activated protein kinase (AMPK) in epithelial cells by an LKB-

dependent mechanism. AMPK appears to be a key target for cancers associated with diabetes mellitus and obesity.^{6, 12} Activation of AMPK by metformin increases insulin-stimulated glucose uptake and inhibits mTOR via TSC2/1, resulting in decreased protein synthesis mediated by the down-regulation of ribosomal protein S6 kinase1 (S6K1). This decrease in phosphorylated s6K1 inhibits cell proliferation. Metformin also has AMPK-independent, indirect anti-proliferative effects related to lower systemic levels of insulin. Recent studies have shown its potential as a cancer prevention drug in other common obesity-associated cancers.¹³⁻¹⁸

The prognosis for EAC patients has remained poor with the large majority dying of cancer-related causes within 5 years.¹⁹ Novel interventions, such as chemoprevention in BE, are a high research priority. The goal of this study was to investigate the potential for metformin as a chemoprevention agent by determining its effect on phosphorylated ribosomal s6K in Barrett's epithelium.

METHODS

All aspects of the study protocol were reviewed and approved by the appropriate Institutional Review Board for human research at each participating site. Mayo Clinic in Rochester, MN, served as the coordinating research base. The Data and Safety Monitoring Board of the Mayo Clinic Cancer Center reviewed safety data every 6 months. All authors had access to the study data and reviewed and approved the final manuscript.

Recruiting Sites

Participants were recruited at 8 Cancer Prevention Network (CPN) member organizations: University Hospitals Case Medical Center, Cleveland, OH; Kansas City VA Medical Center, Kansas City, MO; Massachusetts General Hospital, Boston, MA; Mayo Clinic, Rochester, MN; St. Michael's Hospital, Toronto, ON Canada; University of Pittsburgh, Pittsburgh, PA; University of Pennsylvania, Philadelphia, PA; and the University of Puerto Rico, San Juan, PR.

Study Participants

Seventy-five eligible participants were enrolled between February 2012 and January 2013. The target population included participants (≥ 18 years) with histologically-confirmed BE, defined as the presence of specialized columnar epithelium in the tubular esophagus with ≥ 2 cm of involvement and no evidence of high-grade dysplasia or cancer based on both clinical surveillance and additional research biopsies. Participants were required to have documented intestinal metaplasia with goblet cells in ≥ 1 out of 4 research biopsy samples (≥ 50% intestinal metaplasia), use of a proton pump inhibitor (PPI) prior to enrollment, and no history of diabetes mellitus. Women of childbearing potential were required to document a negative pregnancy test prior to enrollment. General exclusion criteria were: history of confirmed esophageal high-grade dysplasia, esophageal carcinoma or any cancer; vitamin B₁₂ deficiency; history of lactic acidosis; medication for weight loss ≥ 2 months prior to enrollment; treatment with other oral hypoglycemia agents or biguanides; receipt of other investigational agents ≥ 3 months prior to enrollment; history of allergic reactions attributed

to compounds of similar composition to the study agent; elective surgery during the study period; genetic disorders such as family history of hereditary gastrointestinal polyp disorder; or comorbidities that might limit adherence to the study protocol.

Baseline Evaluation

Following informed consent, participants completed a focused interview, physical exam, peripheral blood draw, anthropometric measurements (height, weight, calculated BMI, and waist-hip ratio), and esophagogastroduodenoscopy (EGD) with biopsies for eligibility testing.

Baseline Endoscopy

Endoscopic landmarks including the diaphragmatic hiatus, end of the tubular esophagus as marked by the proximal margin of gastric folds, and squamocolumnar junction were recorded. Extent of the circumferentially involved BE segment was determined using the Prague classification system.²⁰ Hiatal hernia size was measured by linear distance between the end of the tubular esophagus and the diaphragmatic hiatus. Short- and long-segment BE were defined as specialized columnar epithelium lining < 3 cm and ≥ 3 cm of the distal esophagus, respectively.

Four quadrant endoscopic surveillance biopsies were obtained at 2-cm intervals along the length of the Barrett's epithelium. Additional specimens were obtained from any irregularities. Endoscopic research biopsies (up to 8) were obtained with the same forceps from a 1 cm zone of Barrett's epithelium 1-2 cm above the proximal margin of the gastric folds. Research biopsies were washed for 5 seconds in PBS. Half of the biopsies were snap frozen in liquid nitrogen. Half were placed in 10% neutral buffered formalin at room temperature.

Findings of irregularities within the Barrett's segment; erosive esophagitis >LA class A; high-grade dysplasia or cancer; or inadequate Barrett's mucosa to satisfy the study endpoints (defined as < 1 of 4 research samples with ≥ 50% intestinal metaplasia by central pathology review) excluded further study participation.

Study Design

Willing, eligible participants were randomly assigned in double-blind fashion to receive a 12-week intervention with one of two study agent combinations (metformin vs. placebo) using a 1:1 randomization scheme based on the Pocock-Simon dynamic allocation procedure,²¹ which stratified by regular NSAID use (regular use vs. no regular use), obesity (BMI ≥ 30 vs. < 30), gender, length of Barrett's segment (< 5 cm versus ≥ 5 cm circumferential involvement), and participating site. Extended release metformin/placebo was self-administered according to a defined escalation schema: Week 1: 500 mg/day; one 500mg tablet qd po; Week 2: 1000 mg/day; one 500mg tablet bid po; Week 3: 1500 mg/day; 2 tablets po qam and 1 tablet po qpm; Weeks 4-12 (+/- 7 days): 2000 mg/day; two 500mg tablets bid po. The primary objective of this trial was to compare the percent change in mean phosphorylated S6K1 (pS6K1) value, based on esophageal biopsies obtained at the baseline and post-intervention EGD procedures, between participants randomly assigned to the

metformin and placebo arms. Phosphorylation of S6K1 was selected as the primary effect biomarker because this primary substrate of mTOR is a common downstream mediator of both the AMPK and insulin pathways. Thus, pS6K1 assays both direct and indirect effects of metformin.

Adverse events were classified and graded using NCI Common Terminology Criteria for Adverse Events, version 4 (available at www.ctep.cancer.gov). Attribution of agent-related adverse events was performed by the site investigator who was blinded to the intervention assignments.

Post-Intervention Evaluation

Participants returned at Week 12 (+/-7 days) post-randomization to assess adherence, concomitant medication use, and adverse events. Physical exam and peripheral blood draw were performed. The post-intervention blood draw was not mandated. Post-intervention endoscopy, research biopsies, and tissue handling were performed according to the standardized protocol applied at baseline.

Statistical Analyses

The primary objective was to compare the percent change in mean pS6K1 level in BE mucosal biopsies between the active versus placebo intervention arms. The null hypothesis for this study was that the percent change (%) in mean pS6K1 values (from baseline to post-intervention) would be the same or increased for the metformin arm as compared to the placebo arm. The alternative hypothesis was that the percent change in mean pS6K1 values would be decreased in the metformin arm as compared to placebo. We estimated that the standard deviation for the distribution of % in pS6K1 would be approximately the range of possible values (i.e. -1 to 1) divided by 4 (i.e. 0.50 or 50%) given a lack of prior data in this disease setting. Assuming equal standard deviations (i.e. 50%) across the metformin and placebo groups, 30 participants per arm yielded 84% power to detect at least a 35% decrease in the metformin arm as compared to placebo, using a 1-sided t-test with a significance level of 0.05. If the primary endpoint data were not normally distributed, the Wilcoxon Rank-Sum test would be used for this analysis to compare the medians instead. Study participants were considered evaluable for the primary endpoint if pS6K1 data were available from both the baseline and post-intervention evaluations based on the modified intent-to-treat principle.

RESULTS

Cohort Description

A CONSORT overview of participant recruitment is shown in Figure 1. Ninety-three unique participants provided informed consent and were pre-registered for the baseline evaluation. A total of 18 participants were deemed screen failures, and one participant withdrew prior to receiving any study drug, for a total of 74 participants in the intervention cohort (38 metformin; 36 placebo). Reasons for screen failure included: out of range lab values (n=8), high grade dysplasia, esophagitis, or esophageal stricture (n=4), intestinal metaplasia on < 25% of biopsies (n=2), and other (n=4). Pre-registered screen failures (n=18) and the one participant who withdrew prior to receiving study drug were similar to intervention cohort

participants with respect to age, sex, smoking history, BMI, and length of Barrett's Segment ($p = 0.15$). Within the intervention cohort ($n=74$), 5 participants were not evaluable for the pS6K1 analyses since they dropped out of the study due to adverse events prior to the post-EGD evaluation, leaving 69 evaluable participants for the primary endpoint.

Intervention Arms, Adverse Events, and Agent Adherence

Intervention arms were evenly balanced at baseline with respect to all factors (Table 1) including sex ($p=1.00$), length of BE segment ($p=1.00$), age ($p=0.43$), BMI ($p=0.58$), smoking history ($p=0.73$), NSAID use ($p=1.00$), and dysplasia status ($p=0.57$) in the 74 randomized participants who also received study drug (38 metformin; 36 placebo).

Adverse events were reported by 49/74 (66%) trial participants after starting the assigned study intervention. The observed overall adverse event (AE) rates were higher for the metformin participants, but none of the differences reached statistical significance (Table 1). Specifically, 19 of 38 metformin participants (50%) had a treatment related AE (possibly, probably, or definitely related) compared to 36% for the placebo participants ($p=0.25$). The incidence of grade 1 events were similar between arms ($p=0.82$), while grade 2 events were reported at a higher rate for metformin vs. placebo participants (34% vs. 22%, $p=0.31$). Only 1 subject had a grade 3 adverse event (dyspepsia), which occurred in the placebo arm.

Commonly occurring maximum grade adverse events (3 or more events) were also compared between arms. Metformin treated subjects had a higher rate of abdominal pain (Grade 1: 8% vs. 0%; Grade 2: 8% vs. 0%), diarrhea (Grade 1: 21% vs. 11%; Grade 2: 5% vs. 3%), fatigue (Grade 1: 8% vs. 3%), headache (Grade 1: 8% vs. 3%), and nausea (Grade 1: 13% vs. 6%), but none of these observed differences reached statistical significance for each AE grade. When pooling across grades due to the small numbers, metformin treated subjects had a significantly higher rate of grade 1/2 abdominal pain compared to placebo (16% vs. 0%; $p=0.025$). None of the other pooled comparisons were significant between arms. The most common AEs overall were diarrhea and abdominal pain for metformin treated subjects, where 10/38 (26%) subjects had a grade 1 or 2 diarrhea and 6/38 (16%) had a grade 1 or 2 abdominal pain. There was an SAE for a subject treated with metformin. This SAE was a grade 2 amnesia that started on 8/24/2012 and resolved the next day. The number of subjects that went off study early due to an AE was similar for metformin (5/38, 13%) and placebo (3/36, 8%).

Agent adherence was excellent (median of 97% and 98% for metformin and placebo arms, respectively), with nearly all participants receiving the majority of the assigned study doses, and was similar across the randomization arms ($p=0.18$) (Table 1). The placebo participants did take more pills on the average (median of 280 vs. 257, $p=0.09$), but this was due to being on treatment longer on the average.

Primary Endpoint: Tissue pS6K1 Concentration

Of the 74 participants in the intervention cohort, 69 were evaluable for the primary endpoint based on the modified intent-to-treat principle ($n=36$ and 33 for metformin vs. placebo, respectively). There was no statistically significant difference between the baseline or post-intervention pS6K1 values across the 2 intervention arms (Table 2). There was no significant

change (percent and absolute) in the pS6K1 values from pre-intervention to post-intervention within each arm (Wilcoxon signed-rank $p = 0.61$). The metformin and placebo arms were similar with respect to median percent change from baseline for pS6K1 values (1-sided $p=0.80$; Table 2). Specifically, the median percent change from baseline in pS6K1 values was 1.4% for metformin compared to a decrease of 14.7% in placebo treated participants (Table 2). These primary endpoint results were also similar when excluding the 8 outliers (4/arm), where the percent change from baseline exceeded 200% (1-sided $p=0.87$, Figure 2). Finally, the two arms were also similar with respect to absolute change from baseline (1-sided $p=0.72$; Table 2), even after excluding the two major outliers (1/arm), where the absolute change from baseline was -500 or less (1-sided $p=0.72$).

DISCUSSION

We report the first double-blind, randomized, controlled prospective chemoprevention trial of metformin in BE patients. Mean fasting serum insulin levels and insulin resistance assessed by HOMA-IR in the BE patients were similar to those reported in a previous study¹⁰ and 2000 mg a day metformin for 3 months was associated with a borderline reduction in insulin as well as insulin resistance. However, metformin had no discernible effects on levels of phosphorylation of S6Kinase1, the intracellular mediator of insulin and IGF activation in Barrett's epithelium, compared to placebo. Metformin 2000 mg a day also did not alter proliferation or apoptosis in Barrett's epithelium significantly as assayed by Ki-67 and caspase-3 indicating no effect on insulin/AMP Kinase independent proliferation pathways. This study suggests that metformin at tolerable doses will not be effective as a single agent in preventing the progression of BE in the general population to esophageal adenocarcinoma.

The current strategy of screening for BE in older patients with chronic GERD followed by periodic endoscopic surveillance is based on the premise that BE is the primary precursor of EAC and EAC can be most effectively cured when detected early.^{22, 23} However, given that the five-year survival for EAC remains below 20%,²⁴ it is clear that we need to develop improved, less invasive approaches. Chemoprevention in the early metaplastic stage is an attractive strategy and discovery of an effective and tolerable chemopreventive agent would avoid the need for repeated endoscopic surveillance. Unfortunately, data from our study do not support a chemopreventive role for metformin in BE-associated carcinogenesis.

To date, most BE chemoprevention trials have focused on reducing inflammation with PPIs and/or cyclooxygenase inhibitors.²⁵⁻²⁷ The role of central obesity in esophageal carcinogenesis is of growing interest.⁹ Molecular mechanisms by which central obesity could lead to cancer include inflammatory mediators, changes in immune function, oxidative stress/DNA damage, hormones/growth factors, and metabolic detoxification factors. One mechanism that has been implicated in obesity associated cancers is the insulin pathway.^{3, 28} Epidemiological studies suggest that obesity mediated insulin resistance and diabetes contribute to BE and its progression to EAC.^{29, 30} Some cross sectional studies have found an association between hyperinsulinemia and BE whereas others have not.^{10, 31, 32} Furthermore, epidemiological studies suggest that metformin might be protective against certain cancers.¹³⁻¹⁸ Given the null findings from the currently reported trial, future

intervention trials may be more appropriately focused on modulating adipokines such as leptin and adiponectin, alterations of which have also been postulated to be involved in esophageal carcinogenesis.³²⁻³⁸ Recent observational studies propose a chemopreventive role for statins in patients with BE that should also be pursued prospectively.³⁹ It is also possible that metformin could be chemopreventive in the subset of BE subjects who are centrally obese or those with elevated insulin levels, although trials much larger than ours would be required to address this possibility.

Metformin 2000 mg for 12 weeks was found to be generally well tolerated by our study participants, none of whom had clinically diagnosed diabetes mellitus. A few subjects were unable to tolerate metformin because of gastrointestinal side effects but these side effects ceased once metformin was discontinued. These findings suggest that metformin may be reasonably considered for chemoprevention trials involving subjects with other premalignant conditions, regardless of diabetes mellitus status.

Strengths of the present trial include the rigorous definition of BE and the double-blind, randomized, placebo controlled, multicenter study design. To maximize the efficiency of accrual, participants were allowed to take aspirin or NSAIDs during the intervention period. Trial limitations include the fact that the sample size was powered to detect a fairly large, 35% relative decrease in phosphorylated S6K1. Thus, we could have missed a smaller but clinically significant chemopreventive effect of metformin. Although, the pS6K1 assay has previously been utilized to determine the effects of bile acids on signaling pathways in BE and EAC cell lines,⁴⁰ the variation of this assay in assessing BE tissue has not been assessed. A large variance in the assay would also make it difficult to detect a metformin effect. Also, metformin was given for a period of only 12 weeks to improve adherence to the study protocol. It remains conceivable that longer exposure may have yielded more pronounced effects on the endpoints of interest. Phosphorylated S6K1 was selected as our primary effect biomarker because it is the common downstream molecular mediator of PI3K as well as AMPK pathways. There are other carcinogenic pathways that were not assayed in this study. It is possible that metformin could affect BE without affecting S6K1. Our study did not mandate a blood draw at the post- intervention endoscopy. Thus, secondary endpoints such as change in serum insulin and HOMA-IR were only measured in half the study subjects. Finally, markers of proliferation and apoptosis such as Ki-67 and caspase-3 are only indirect markers of carcinogenesis. Long-term studies would be required to determine whether an agent truly does or does not prevent cancer.

In summary, metformin 2000 mg a day in BE patients on PPI, although tolerable and safe, was not effective in altering pS6K1, proliferation, or apoptosis pathways in non- dysplastic BE. Although this study could have missed moderate effects of metformin on pS6K1 or effects of metformin on alternate carcinogenic pathways, we suggest that future chemoprevention trials consider targeting alternate carcinogenic pathways, perhaps involving adiponectin and/or leptin, in BE-associated carcinogenesis.

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Figure 1. CONSORT Diagram that shows the number of participants that were pre-registered, the number of screen failures, along with the number of participants that were randomized and evaluable for the primary endpoint.

Figure 2. Boxplots for Percent Change in pS6K1 values by each treatment arm (metformin and placebo) after excluding the 8 outliers (4/arm), where the percent change from baseline exceeded 200%.

Table 1

Baseline Characteristics, Adherence and Adverse Events by Randomized Arm

	Placebo (N=36)	Metformin (N=38)	p value
Age (years)			0.43 ¹
Mean (SD)	58.1 (8.4)	59.2 (11.0)	
Median	60.5	60.5	
Q1, Q3	52.0, 63.0	53.0, 66.0	
Range	(39.0-79.0)	(20.0-81.0)	
Sex, n (%)			1.00 ²
Female	8 (22.2%)	8 (21.1%)	
Male	28 (77.8%)	30 (78.9%)	
Performance Score, n (%)			1.00 ²
0	35 (97.2%)	37 (97.4%)	
1	1 (2.8%)	1 (2.6%)	
BMI			0.58 ¹
Mean (SD)	30.4 (6.4)	30.9 (5.1)	
Median	29.9	30.1	
Q1, Q3	26.6, 32.9	28.0, 33.8	
Range	(20.2-52.0)	(22.3-44.5)	
Waist to hip ratio			0.61 ¹
N	36	37	
Mean (SD)	1.0 (0.1)	1.0 (0.1)	
Median	1.0	1.0	
Q1, Q3	1.0, 1.0	0.9, 1.0	
Range	(0.7-1.1)	(0.8-1.1)	
Length of Barrett's segment, n (%)			1.00 ²
<5 cm of circumferential involvement	11 (30.6%)	11 (28.9%)	
>=5 cm of circumferential involvement	25 (69.4%)	27 (71.1%)	
Dysplasia status at pre-intervention, n (%)			0.57 ²
No dysplasia	30 (83.3%)	29 (76.3%)	
Low grade or indefinite for dysplasia	6 (16.7%)	9 (23.7%)	
Smoking History, n(%)			0.73 ²
Current Smoker	2 (5.6%)	4 (10.5%)	
Never Smoked	20 (55.6%)	18 (47.4%)	
Quit/Former Smoker	14 (38.9%)	16 (42.1%)	
NSAID use, n (%)			1.00 ²
Regular Use	8 (22.2%)	9 (23.7%)	
No Regular Use	28 (77.8%)	29 (76.3%)	
No. of pills taken, median (range)			0.09 ¹

	Placebo (N=36)	Metformin (N=38)	p value
N	35	38	
Mean (SD)	253.9 (73.4)	235.8 (73.6)	
Median	280.0	256.5	
Q1, Q3	249.0, 300.0	232.0, 288.0	
Range	(1.0-300.0)	(68.0-320.0)	
Adherence (%), median (range)			0.18 ¹
N	35	38	
Mean (SD)	93.7 (11.8)	91.3 (13.8)	
Median	98.0	96.7	
Q1, Q3	92.0, 99.7	89.6, 99.2	
Range	(54.5-104.9)	(32.9-100.7)	
Any adverse event regardless of treatment or grade			0.81 ²
No	13 (36.1%)	12 (31.6%)	
Yes	23 (63.9%)	26 (68.4%)	
Adverse events related to study treatment			0.25 ²
No	23 (63.9%)	19 (50.0%)	
Yes	13 (36.1%)	19 (50.0%)	
Grade 1 Adverse Events			0.82 ²
No	18 (50.0%)	17 (44.7%)	
Yes	18 (50.0%)	21 (55.3%)	
Grade 2 Adverse Events			0.31 ²
No	28 (77.8%)	25 (65.8%)	
Yes	8 (22.2%)	13 (34.2%)	
Grade 3 Adverse Events			0.49 ²
No	35 (97.2%)	38 (100.0%)	
Yes	1 (2.8%)	0 (0.0%)	

¹ Wilcoxon Rank-Sum test

² Fisher's Exact test

Table 2

pS6K1 Results by Treatment Arm

	Placebo (N=33)	Metformin (N=36)	p-value
pS6K1 (Pre-Intervention)			0.58 ¹
Mean (SD)	163.0 (221.6)	188.5 (306.9)	
Median	99.7	73.9	
Range	(2.5-1218.6)	(4.2-1566.5)	
pS6K1 (Post-Intervention)			0.38 ¹
Mean (SD)	122.9 (133.8)	123.6 (114.0)	
Median	71.4	102.8	
Range	(5.9-530.8)	(13.0-627.9)	
pS6K1 (Absolute Change)			0.72 ²
Mean (SD)	-40.1 (166.5)	-64.9 (252.4)	
Median	-9.9	0.9	
Range	(-687.7-283.2)	(-1379.8-132.6)	
pS6K1 (Percent Change)			0.80 ²
Mean (SD)	60.6 (301.0)	42.5 (157.1)	
Median	-14.7	1.4	
Range	(-97.7-1646.1)	(-88.1-694.0)	
pS6K1 (Percent Change (no outliers[*]))			0.87 ²
N	29	32	
Mean (SD)	-14.1 (62.5)	-3.3 (51.1)	
Median	-24.4	-4.0	
Range	(-97.7-134.2)	(-88.1-143.6)	

¹ Wilcoxon Rank-Sum Test² Wilcoxon Rank-Sum Test (1-sided per protocol)

* excludes percent change values > 200%.