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Article

Pilot Study Assessing Tolerability and Metabolic Effects of Metformin in Patients With Li-Fraumeni Syndrome

Farzana L. Walcott, MD,[1,7] Ping-Yuan Wang, PhD,[2] Christine M. Bryla, MSN,[1] Rebecca D. Huffstutler, CRNP,[2] Neha Singh, MD,[3] Michael N. Pollak, MD,[4] Payal P. Khincha, MD,[5] Sharon A. Savage, MD,[5] Phuong L. Mai, MD,[5,8] Kevin W. Dodd, PhD,[6] Paul M. Hwang, MD, PhD,[2] Antonio T. Fojo, MD, PhD,[1,9,†] Christina M. Annunziata, MD, PhD[1,†]

[1]Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA; [2]Cardiovascular Branch, National Heart Lung Blood Institute, Bethesda, MD, USA; [3]George Washington Medical School, Washington, DC, USA; [4]McGill University, Montreal, QC, Canada; [5]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA; [6]Division of Cancer Prevention, National Cancer Institute, Bethesda, MD, USA; [7]Current affiliation: Oncology R&D,AstraZeneca, Gaithersburg, MD; [8] Current affiliation: Magee-Womens Hospital, University of Pittsburgh, PA and [9] Current affiliation: Columbia University, New York, NY

Correspondence to: Christina M. Annunziata, MD, PhD, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA (e-mail: annunzic@mail.nih.gov).
[†]Authors contributed equally to this work.

Abstract

Background: Li-Fraumeni syndrome (LFS) is a highly penetrant autosomal dominant cancer predisposition disorder caused by germline *TP53* pathogenic variants. Patients with LFS have increased oxidative phosphorylation capacity in skeletal muscle and oxidative stress in blood. Metformin inhibits oxidative phosphorylation, reducing available energy for cancer cell proliferation and decreasing production of reactive oxygen species that cause DNA damage. Thus, metformin may provide pharmacologic risk reduction for cancer in patients with LFS, but its safety in nondiabetic patients with germline *TP53* pathogenic variants has not been documented. **Methods:** This study assessed safety and tolerability of metformin in nondiabetic LFS patients and measured changes in metabolic profiles. Adult patients with LFS and germline *TP53* variant received 14 weeks of metformin. Blood samples were obtained for measurement of serum insulin-like growth factor–1, insulin, and insulin-like growth factor binding protein 3. Hepatic mitochondrial function was assessed with fasting exhaled CO_2 after ingestion of ^{13}C-labeled methionine. Changes in serum metabolome were measured. All statistical tests were 2-sided. **Results:** We enrolled 26 participants: 20 females and 6 males. The most common adverse events were diarrhea (50.0%) and nausea (46.2%). Lactic acidosis did not occur, and there were no changes in fasting glucose. Cumulative mean ^{13}C exhalation was statistically significantly suppressed by metformin ($P = .001$). Mean levels of insulin-like growth factor binding protein 3 and insulin-like growth factor-1 were statistically significantly lowered ($P = .02$). Lipid metabolites and branched-chain amino acids accumulated. **Conclusions:** Metformin was safe and tolerable in patients with LFS. It suppressed hepatic mitochondrial function as expected in these individuals. This study adds to the rationale for development of a pharmacologic risk-reduction clinical trial of metformin in LFS.

Li-Fraumeni syndrome (LFS) is a highly penetrant, autosomal-dominant cancer predisposition syndrome. Sarcomas, breast, brain, and adrenocortical cancers are the most common presenting malignancies, but a wide range of cancers have been associated with LFS. Approximately 80% of families meeting the clinical diagnosis of LFS have pathogenic germline *TP53* pathogenic variants (1). Cumulative cancer incidence is 50% by age 31 years in females, age 46 years in males, and nearly 100% by age 70 years for both sexes (2). Intensive cancer surveillance aimed at early detection, including magnetic resonance imaging–based radiological screening, biochemical surveillance, and colonoscopy, is recommended beginning at diagnosis and statistically significantly reduces cancer-related mortality (3–5). However, no effective primary preventive interventions have been identified other than prophylactic mastectomies for women at risk of breast cancer and colonoscopy that might identify lesions suspicious for colon cancer (6–8).

The role of mitochondrial metabolism in cancer cells is well established (9–11). A growing number of studies have shown that TP53 exerts multiple functions beyond its prototypical

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tumor suppressor activities including its involvement in regulating glycolysis, glutamine metabolism, lipid metabolism, redox homeostasis, and mitochondrial oxidative phosphorylation (12). In addition to regulating genes involved in the cell cycle and maintenance of genomic integrity, TP53 can also transactivate mitochondrial genes and translocate into the mitochondria for maintenance of mitochondrial genomic DNA (13).

TP53 mutations can affect oxidative stress and mitochondrial function in humans. Statistically significant increases in erythrocyte glutathione peroxidase activity, plasma carbonyl content, and plasma malondialdehyde levels (indicating increased lipid peroxidation) occurred in blood samples from carriers of *TP53* mutation (p.R337H) compared with controls (14). Additional evidence suggests increased oxidative metabolism both in humans with LFS and in a mouse model of LFS (15). Specifically, mutant mouse TP53 can retain or enhance the mitochondrial-promoting activity of wild-type TP53 (16). In the setting of human *TP53* mutations with loss of prototypical tumor suppressor activities, the increased mitochondrial activity may promote cancer cell survival and contribute to tumorigenesis. Indeed, in a previous translational study, we showed that metformin in a mouse model can suppress mitochondrial respiration and suppress proliferation (17). Thus, interventions targeting mitochondria may be beneficial for preventing cancer in LFS.

Metformin is an oral medication approved by the Food and Drug Administration to treat type 2 diabetes. Some reports have associated metformin use with a reduced cancer incidence in diabetics and with improved survival of patients with diabetes and cancer (18,19), but some studies have failed to confirmed these findings (20). One mechanism may be reduction of hyperinsulinemia, which may increase risk of cancer (21–23). Reduction in circulating insulin is achieved by metformin blocking liver gluconeogenesis and stimulating glucose uptake in skeletal muscle. Metformin also decreases circulating insulin-like growth factor-1 (IGF-1) and modulates other IGF axis components such as insulin-like growth factor binding protein 3 (IGFBP3), also associated with increased cancer risk (24).

TP53 biology intersects with metformin through crosstalk with the IGF and insulin signaling pathways, inhibiting mammalian target of rapamaycin (mTOR), inhibiting complex I of the mitochondrial respiratory chain, and increasing cellular levels of adenosine monophosphate by inhibiting oxidative phosphorylation (25). Based on the role of *TP53* in metabolism, increased markers of oxidative stress, and the possibility of increased oxidative metabolism in individuals with LFS (26), we sought to advance the concept that metformin may offer an opportunity for pharmacologic risk reduction of cancer in individuals with LFS. In addition to the previous translational work (17), we now provide a more comprehensive description of its effects in humans with LFS.

Methods

Study Design and Implementation

The institutional review board of the National Cancer Institute approved this single institution phase I study (NCT01981525), and all participants enrolled after providing written informed consent. Patients were volunteers referred from treating physicians in the community, or they could self-refer. The study was advertised on clinicaltrials.gov and cited in presentations at national scientific meetings.

The proposed primary objectives of the trial were to determine 1) the tolerability of standard release, oral metformin up to 2000 mg/day in adult patients with germline *TP53* pathogenic variants and a diagnosis of LFS and 2) the effects of metformin on circulating insulin and insulin-related biomarkers (IGF-1 and IGFBP3), at week 8 compared with baseline. With full measurements on the 3 parameters at 0 and 8 weeks, 22 patients would provide 80% power to detect a change from baseline equal to 0.75 standard deviations of the change (0.75 effect size) using 2-tailed tests at a Bonferroni-adjusted statistical significance level of 0.0167.

Patients returned to the National Institutes of Health Clinical Center (NIHCC) for evaluation at week 8, which was 2 weeks after the last escalation (Figure 1). Patients remained on 2000 mg/day until returning to NIHCC at week 14 for clinical assessment and research testing, at which point metformin was stopped. Participants returned to NIHCC after the 6-week washout period for the final visit at week 20. Patients were asked to keep a log of their missed doses, and adherence to metformin was assessed by pill counts at each visit. Additional details of patient eligibility and metformin administration can be found in Supplementary Methods (available online).

Insulin and Insulin-Related Biomarkers Measurement and Assays

Patients provided a blood sample the morning of their visit after an overnight fast (>8 hours) and 1 hour after ingesting 75 g glucose for measurement of serum IGF-1, insulin, IGFBP3, and connecting C-peptide, which is released upon the conversion of pro-insulin to insulin. Insulin was measured by standard enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden). C-peptide was measured by enzyme-linked immunosorbent assay (ALPCO, Salem, NH). IGF-1 and IGFBP-3 were measured by IDS-iSYS Multi-Discipline Automated System (Boldon Business Park, Tyne & Wear, England) per manufacturer protocols.

^{13}C-Methionine Breath Test (^{13}C-MBT)

At each visit, patients were given carboxyl-^{13}C labeled 200 mg methionine substrate orally after an overnight fast. Exhaled $^{13}CO_2$ was captured and analyzed before and 40 minutes after ingesting substrate. The percentage dose of ^{13}C recovered at 0 and 40 minutes was determined at baseline and weeks 8, 14, and 20 after the start of metformin. Breath test analyses were performed by Metabolic Solutions, Inc (Nashau, NH).

Global Metabolomics Profiling

Metabolomics profiles were determined in human serum samples collected at each of 3 time points (weeks 0, 14, and 20). Global metabolomics was analyzed using the Precision Metabolomics platform per the manufacturer's protocol (Metabolon, Research Triangle, NC).

Statistical Analysis

This study examined the effects of metformin on circulating insulin, IGF-1, IGFBP3, C-peptide, and ^{13}C-MBT using longitudinal analysis of all available measurements at 4 time points (0, 8, 14, and 20 weeks after enrollment) to maximize statistical power.

Table 1. Baseline characteristics of Li-Fraumeni syndrome patients

Characteristics	All patients (n = 26)	Patients completing 20 weeks (n = 21)[a]	Patients not completing 20 weeks (n = 5)
Mean age, y (median) [range]	39.5 (39.3) [19.2-59.3]	39.9 (39.9) [19.2-56.0]	37.8 (37.0) [23.7-59.3]
Male	34.7 (34.2) [19.2-59.3]	34.7 (33.0) [19.2-54.1]	59.3[c]
Female	40.0 (39.7) [23.7-56.0]	41.5 (41.1) [26.3-56.0]	32.4 (33.7) [23.7-38.8]
Mean BMI, kg/m^2 (median) [range]	27.6 (27.4) [19.1-40.5]	27.9 (28.0) [19.1-40.5]	26.3 (25.7) [22.2-28.9]
Male	24.9 (25.6) [19.1-30.8]	24.9 (24.9) [19.1-30.8]	28.9[c]
Female	28.2 (28.0) [21.6-40.5]	28.8 (28.9) [20.7-40.5]	25.2 (24.9) [22.2-28.4]
Baseline fasting glucose, week 0, mg/dL, mean (median) [range]	96.4 (91.5) [82-187]	97.7 (93.0) [85-187]	90.8 (89.0) [82-102]
Baseline HbA1c, week 0, range	4.8-5.8	4.8-5.8	4.9-5.7
Type of germline TP53 variant[b]			
Males			
Missense	3	3	0
Deletion	1	1	0
Nonsense	1	0	1
Other	1	1	0
Females			
Missense	11	9	2
Deletion	5	4	1
Nonsense	3	2	1
Other	1	1	0
Sex			
Male	6	5	1
Female	20	16	4

[a]One patient only reached a maximum dose of 1500 mg but completed study. BMI = body mass index.
[b]Details of the *TP53* pathogenic variant and cancer diagnosis by patient in Supplementary Methods (available online).
[c]Only 1 male dropped out of study (prior to starting metformin).

Based on Bland-Altman plots and histograms of within- and between-individual measurements, analyses were carried out on the log scale, where laboratory errors looked homoscedastic and approximately normal, and the distributions of biomarker measurements were not skewed. Linear mixed models were used to analyze each biomarker separately for the fasting and nonfasting observations. An indicator variable with value 1 for "on-agent" time periods (8 and 14 weeks) and 0 otherwise was the only fixed-effect covariate—that is, weeks 8 and 14 were treated as measures of a patient's single on-agent value, whereas weeks 0 and 20 were treated as measures of their single off-agent value. A compound symmetry covariance structure was assumed to allow a fixed correlation for repeated measures on the same patient; this is analogous to the assumption made when using a paired t test to analyze only 2 time points. Significance testing was performed on the parameters estimated from the mixed models fit to log-scale data, but for ease of clinical interpretation, we present geometric means and associated confidence intervals (CI) in the original scale. Note that because the log of a ratio is the difference in the log of the numerator and the log of the denominator, it follows that the mean difference in the log scale is equivalent to the log of the mean ratio in the original scale. Thus, the coefficient in the mixed models for on vs off represents the log of the ratio of the mean on-agent to the mean off-agent. Accordingly, we also present the exponentiated parameter estimate and its associated 95% confidence interval, which can be interpreted as the modeled ratio in the original scale. In all, 9 models were fit to data from this study (breath test + 4 biomarkers x fasting/nonfasting). Even though the longitudinal models have more power to detect differences than paired t tests performed with only 2 time points, we still adjusted for multiple comparisons, although we used the Benjamin-Hochberg step-up procedure to control the false discovery rate rather than an extremely conservative Bonferroni adjustment. Because the data set is very small, and the study exploratory, we set the false discovery rate to 10%.

Results

Patient Characteristics

Accrual occurred from January 2014 to February 2016. The last patient visit occurred in June 2016. We enrolled 26 individuals with LFS: 20 females and 6 males (Table 1). Patients had a diverse cancer history (Supplementary Table 1, available online). The higher percentage of females reflects the known incidence of early onset breast cancers (2).

Of the 26 patients enrolled, 21 had fasting blood glucose less than 100 mg/dL, and 4 had fasting glucose 100-110 mg/dL; 1 patient had a fasting blood glucose measured at 187 mg/dL, but a subsequent glucose tolerance test decreased to 161 mg/dL, suggesting error in the fasting measurement. Body mass index ranged from 19.1 to 40.5 kg/m^2 (Table 1). Body mass index and glucose were not correlated.

Four female patients and 1 male patient ended study participation prior to 20 weeks (Figure 1). Of these, 2 participants (1 female, 1 male) ended participation prior to starting metformin because of personal or family issues, and 1 participant withdrew at 6 weeks for personal reasons. One participant withdrew at 2 weeks because of adverse events (grade 2 dyspepsia and reflux, noncardiac chest pain relieved with antacids) while taking 500 mg metformin daily. One patient withdrew at 13 weeks because of a new cancer diagnosis. One female patient did not tolerate 2000 mg dose, which

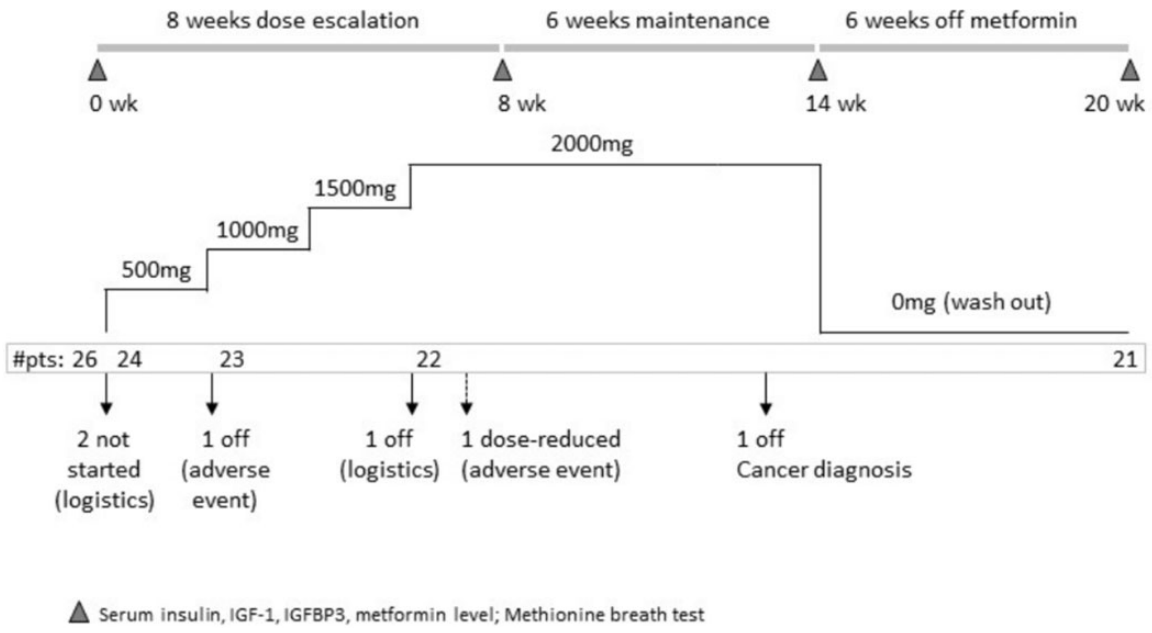


Figure 1. Study design and accrual. participants enrolled and escalated dose of metformin per protocol. Five participants withdrew from study for reasons indicated.

Table 2. Adverse events attributed to metformin

Adverse event	Grade 1, No. (%)	Grade 2, No. (%)
Gastrointestinal		
Diarrhea	13 (50.0)	3 (11.5)
Nausea	12 (46.2)	3 (11.5)
Abdominal pain	10 (38.5)	1 (3.8)
Anorexia	2 (7.7)	0 (0.0)
Dyspepsia	2 (7.7)	3 (11.5)
Flatulence	2 (7.7)	0 (0.0)
Bloating	1 (3.8)	0 (0.0)
Constipation	1 (3.8)	0 (0.0)
Dry mouth	1 (3.8)	0 (0.0)
Belching	1 (3.8)	0 (0.0)
Sore throat	1 (3.8)	0 (0.0)
Vomiting	1 (3.8)	0 (0.0)
General		
Malaise	11 (42.3)	0 (0.0)
Fatigue	2 (7.7)	0 (0.0)
Nervous system		
Headache	6 (23.1)	2 (7.7)
General muscle weakness	1 (3.8)	0 (0.0)
Dizziness	1 (3.8)	0 (0.0)
Noncardiac chest pain	0 (0.0)	1 (3.8)

caused grade 2 diarrhea, and completed the study taking 1500 mg metformin daily. The remaining 21 patients tolerated 2000 mg of metformin and completed the study. Very few patients missed doses of metformin (Supplementary Table 2, available online); 14 patients did not miss any doses. Most missed doses were due to the patient forgetting to take the drug.

Adverse Events

The most common side effects of this study were grade 1 diarrhea (50.0%) and nausea (46.2%) (Table 2). Grade 2 adverse events occurred in 3 participants; there were no grade 3-5 events. Two participants experienced no side effects while taking metformin. There were no episodes of lactic acidosis. Fasting glucose levels did not change per patient throughout the study. The majority of study participants reported improvement or complete resolution of side effects by week 14 on the maximum dose of metformin at 2000 mg/day.

Insulin, IGF-1, IGFBP3, and C-Peptide Biomarkers

Insulin-related markers were measured at 4 time points in both the fasting (Table 3) and nonfasting (Table 4) state. Time points reflect pretreatment (0 weeks), initial steady state after reaching maximal dose (8 weeks), longer-term steady state (14 weeks), and after washout (20 weeks). Time points 0 and 2 weeks are combined as off-agent, and time points 8 and 14 weeks are on-agent. Metformin levels were considered to be at steady state once a patient had been taking the same dose for more than 2 weeks, although metformin blood levels were not measured. Statistically significant changes occurred in the mean levels of fasting IGFBP3, measured at 4752.4 ng/mL off metformin and 4548.7 ng/mL on metformin ($P = .02$). Similarly, both fasting and nonfasting IGF-1 were lower in participants while taking metformin (Table 3). Mean fasting IGF-1 levels were 159.8 and 149.9 ng/mL off and on metformin, respectively ($P = .02$), and nonfasting IGF-1 levels were 159.5 and 148.2 ng/mL off and on metformin, respectively ($P = .01$). Ratios less than 1 (on and off) indicate that biomarker levels in patients on metformin are decreased compared with those off metformin but were not statistically significant in most cases. Two individuals were missing fasting insulin levels at baseline, 1 individual was missing fasting insulin level at 8 weeks, 3 individuals were missing fasting insulin levels at 14 weeks, and 2 more were missing fasting insulin levels at 20 weeks. Fasting insulin results did not vary on or off metformin.

Table 3. Insulin-related markers in participants on and off metformin

Insulin-related markers	Off-agent geometric mean (95% CI) weeks 0 and 20	On-agent geometric mean (95% CI) weeks 8 and 14	Ratio (on/off)[a]	P[b]
Fasting				
Insulin, mIU/L	8.9 (6.9 to 11.4)	7.8 (6.1 to 10.0)	0.879 (0.73 to 1.05)	.16
IGF-1, ng/mL	159.8 (139.9 to 182.7)	149.9 (131.2 to 171.3)	0.938 (0.89 to 0.99)	.02
IGFBP3, ng/mL	4752.4 (4461.3 to 5061.9)	4548.7 (4270.1 to 4845.0)	0.957 (0.92 to 0.99)	.02
C-peptide, pmol/L	562.8 (449.5 to 704.6)	502.7 (401.6 to 629.4)	0.893 (0.79 to 1.01)	.07
Nonfasting				
Insulin, mIU/L	54.0 (40.6 to 71.8)	50.1 (37.6 to 66.6)	0.927 (0.77 to 1.12)	.42
IGF-1, ng/mL	159.5 (138.8 to 183.3)	148.2 (129.0 to 170.3)	0.929 (0.88 to 0.98)	.01
IGFBP3, mg/mL	4727.3 (4434.2 to 5040.2)	4617.9 (4331.2 to 4923.1)	0.977 (0.94 to 1.02)	.24
C-peptide, pmol/L	1989.0 (1712.1 to 2310.7)	1965.3 (1691.7 to 2283.1)	0.988 (0.89 to 1.10)	.82

[a]exp(mean[on]—mean[off] in log scale).
[b]Nominal P value used in Benjamini-Hochberg false discovery rate procedure. CI = confidence interval; IGF-1 = insulin-like growth factor; IGFBP3 = insulin-like growth factor binding protein 3.

Table 4. ^{13}C-MBT in participants on and off metformin

Off-agent[a]	On-agent[b]	Ratio (on/off)[c]	P-value[d]
2.41 (2.15, 2.71)	2.013 (1.79, 2.26)	0.834 (0.743, .935)	.001*

[a]Geometric means (95% CI) of values at week 0 and week 20; CI = confidence interval
[b]Geometric means (95% CI) of values at week 8 and week 14;
[c]exp(mean(on) – mean(off) in log scale)
[d]Nominal P-value used in Benjamini-Hochberg FDR procedure; FDR = false discovery rate.
*Statistically significant result after FDR adjustment

^{13}C-MBT

The ^{13}C-MBT measurements assess hepatic mitochondrial function and have been shown to be reproducible (24). We analyzed ^{13}C-MBT at baseline and weeks 8, 14, and 20 after the start of metformin. Cumulative ^{13}C exhalation 40 minutes after ingestion of ^{13}C-labeled methionine in participants indicated hepatic mitochondrial function was statistically significantly suppressed by metformin in participants at weeks 8 and 14 of metformin treatment compared with baseline, and recovery of hepatic mitochondrial function was measured at week 20 (after 6 week washout) (Figure 2). Geometric mean ^{13}C-MBT levels were statistically significantly lower on metformin (mean-= 2.013, 95% CI = 1.79 to 2.26), suggesting a decrease in hepatic mitochondrial function compared with off metformin (mean-= 2.41, 95% CI = 2.15 to 2.71; P = .001), with a ratio of .834 (95% CI = .743 to .935) on to off metformin. Measurements at week 20 were not statistically significantly different than week 0 baseline. Using the last-observation-carried-forward method to impute missing breath test data, we saw no evidence that missing data affected the results.

Global Untargeted Metabolomics Profiling

Our primary goals with metabolomics profiling were to confirm direct effects of metformin in vivo on oxidative metabolism and to determine if differences in circulating levels of insulin, IGF-1, or IGFBP3 correspond to metabolite differences at each time point (baseline, week 14, and postwashout week 20).

Consistent with evidence showing that metformin can increase fatty acid β-oxidation (27), many acylcarnitines, long-chain fatty acids (FA), and 3-hydroxy FA were increased in participants on metformin at week 14 compared with baseline (Figure 3). Short-, medium-, and long-chain acylcarnitines (3-hydroxybutyrylcarnitine, suberoylcarnitine, and palmitoylcarnitine, respectively) were statistically significantly elevated following metformin treatment, suggesting increased FA β-oxidation (Figure 3,A). We also observed increased levels of 3-hydroxybutyrate (BHBA), which can elevate with robust β-oxidation. However, inhibition of mitochondria and tricarboxylic acid cycle by metformin may have caused accumulation of the intermediates aconitate, malate, and fumarate. By week 20, after clearance of metformin, these levels returned to baseline values. Metformin administration also perturbed branched-chain amino acid (BCAA) levels (Figure 3,B). We observed increases in BCAA intermediates leucine and isoleucine, as well as immediate catabolites, α-hydroxyisovalerate, 3-methyl-2-oxovalerate, and 2-hydroxy-2-methylvalerate, with metformin treatment.

Because IGF-1 levels statistically significantly changed metformin exposure, we examined whether participants with higher IGF-1 levels experienced a larger change in any serum metabolite. participants with the IGF-1 levels above the median showed increased measurements of several short- and medium-chain acylcarnitines (eg, acetylcarnitine, 3-hydroxybutyrylcarnitine [1 and 2; enantiomers], hexanoylcarnitine, octanoylcarnitine, and laurylcarnitine) while at steady-state dosing of metformin (week 14) (Figure 3,C). Similarly, these participants also exhibited increased levels of the ketone body BHBA, which becomes elevated with increased β-oxidation. Again, clearance of metformin resulted in normalization of BHBA to levels comparable with those at baseline. These data suggest that plasma IGF-1 levels could serve as a response marker of metformin effect on FA β-oxidation in this patient population.

Discussion

We report a pilot study evaluating the safety, tolerability, and metabolic effects of metformin in nondiabetic adults with LFS due to pathogenic germline *TP53* variants (NCT01981525). Metformin is known to be safe and tolerable in nondiabetic individuals, including those with cancer, but its safety has never been demonstrated in nondiabetic individuals harboring a germline *TP53* pathogenic variant. We postulated that

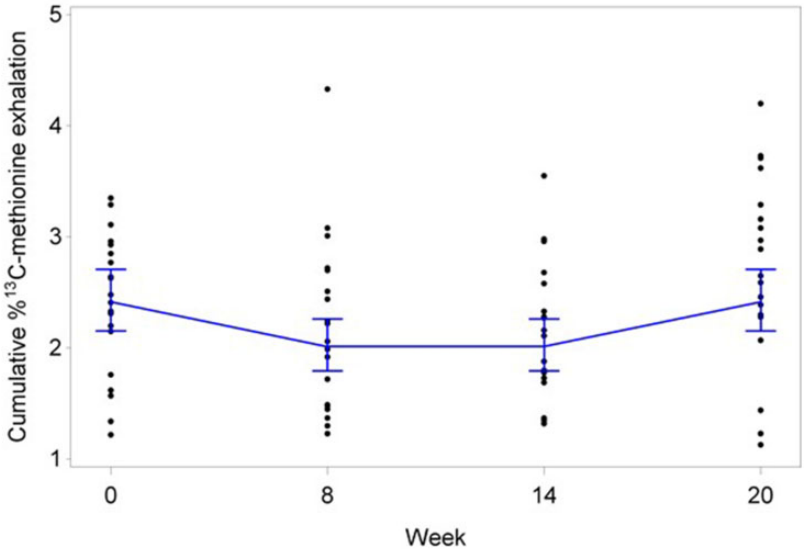


Figure 2. 13C-MBT: metformin treatment inhibits hepatic mitochondrial function. Cumulative ^{13}C exhalation was measured 40 minutes after ingestion of ^{13}C-labeled methionine on week 0 (baseline), weeks 8 and 14 (on metformin), and week 20 (6 week washout off metformin).

metformin would inhibit mitochondria function in individuals with germline *TP53* pathogenic variants, given prior evidence of increased oxidative metabolism capacity in these individuals (14). This pilot study of metformin in participants with LFS demonstrated that it was well tolerated at standard dosing and therefore could be safely administered as a cancer prevention agent if shown to be effective in this patient population through larger randomized clinical trials.

Metformin inhibits hepatic gluconeogenesis, and hepatocytes express the organic cation transporter-1 needed for metformin uptake into the hepatic cell (28). It is unclear at this time whether metformin would enter potential cancer cells the same way it enters hepatocytes at an effective concentration. We therefore assessed hepatic mitochondrial function using the noninvasive ^{13}C-MBT to quantify exhaled nonradioactive isotope–labeled CO_2. We performed global untargeted metabolomics profiling on serum samples. Unbiased profiling and pathway analysis at each time point allowed inquiry into metabolic pathways that change with metformin treatment, further enhancing knowledge of the pleiotropic effects of metformin in these patients.

We show that metformin statistically significantly decreased serum levels of insulin-related proteins in nondiabetic LFS patients, including reduction in circulating fasting IGF-1 and IGFBP3, which could contribute to the various observed metabolic changes in addition to inhibition of respiration (Figure 4). This may be particularly relevant in participants with LFS, who carry germline *TP53* pathogenic variants. Wild-type p53 induces IGFBP3, which binds to IGF-1 and prevents its binding to the IGF receptor, resulting in the down regulation of the PI3K-AKT-mTOR–signaling pathways (29–31). The aberrant activation of these 2 pathways plays an important role in stimulating glucose consumption and promoting growth and proliferation of tumor cells. In particular, metformin may suppress the glucose metabolism regulated by *TP53* (17,32). It is important to note, however, that although the changes were statistically significant, the numerical changes amounted to less than 10% difference in the levels of IGF-1 and IGFBP3, consistent with the assessment that metformin was clinically well tolerated with few adverse events in this patient population.

There is growing evidence that mitochondria play an important role in tumorigenesis, and metformin's effects on mitochondria may be a means of preventing tumorigenesis. One intriguing hypothesis is that of synthetic lethality, whereby metformin may be cytotoxic only in the context of loss of a tumor suppressor, implying the possibility of an inhibitory effect of metformin on cancer development in the setting of *TP53* dysfunction (33). Wild-type TP53 promotes growth arrest during cellular stress, whereas its mutation may abrogate this inhibitory function, allowing proliferation to progress unchecked. Inhibition of mitochondrial function by metformin here could induce an energy crisis causing cell cycle arrest or death. We show statistically significantly lower hepatic metabolism of ^{13}C-MBT in patients on metformin compared with when they were not taking the drug, providing in vivo evidence of mitochondrial function suppression by metformin in individuals with germline *TP53* pathogenic variants.

Individuals harboring pathogenic germline *TP53* variants can display altered oxidative metabolism and have a high propensity to develop cancer (14). The role of mitochondria and metabolism in cancer cells has been postulated, most specifically in the context of the Warburg effect, whereby cancer cells showed increases of both glycolysis and mitochondrial function (11). In this study, we demonstrated changes in the metabolomics profile that may indicate changes in mitochondrial function. Treatment of this patient population with metformin statistically significantly altered metabolism of branched-chain amino acids, which may affect glucose utilization because BCAAs, particularly leucine, are potent inducers of insulin secretion (34).

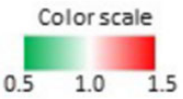
Although our study did not include non-LFS patients, the metabolic effects of metformin have been widely studied. Consistent with our observation of altered lipid metabolism in LFS participants, metformin treatment of non-LFS diabetic patients statistically significantly decreases hepatic glucose output, adipose tissue, and plasma triglyceride concentration (35,36). Similarly, metformin interacts with the IGF pathway in the presence of either wild-type or mutant *TP53* (37). Consistent with the effect of metformin on BCAA metabolism in our LFS participants, treatment of mice with metformin can normalize changes in BCAA metabolism caused by a high-fat diet (38).

A

Lipid metabolism		Fold change	
Pathway	**Biochemical Name**	**Wk 14:Wk 0**	**Wk 20:Wk 0**
Acyl Carnitine Metabolism	3-hydroxybutyrylcarnitine (2)	2.43	0.97
	3-hydroxybutyrylcarnitine (1)	1.66	0.96
	acylcarnitine (C2)	1.30	1.02
	margaroylcarnitine	1.22	1.02
	suberoylcarnitine (C6-OC)	1.35	0.91
	hexanoylcarnitine (C6)	1.24	0.93
	palmitoylcarnitine (C16)	1.10	1.04
	nervonoylcarnintine (C24:1)	0.75	0.92
Keytone Bodies	3-hydroxybutyrate (BHBA)	1.65	0.99
Fatty Acid, Monohydroxy	3-hydroxysebacate	4.23	0.93
	3-hydroxyoctanoate	2.49	0.91
	3-hydroxydecanoate	2.14	0.94
	3-hydroxyhexanoate	1.53	0.93
	3-hydroxystearate	1.13	1.08
	3-hydroxylaurate	1.44	0.93
	3-hydroxymyristate	1.19	0.95
	2-hydroxyoctanoate	0.78	0.81
TCA cycle	aconitate (cis or trans)	1.31	1.07
	malate	1.35	1.15
	fumarate	1.17	1.15
	succinate	0.93	1.06

B

Branched chain amino acid		Fold change	
Pathway	**Biochemical name**	**Wk 14:Wk 0**	**Wk 20:Wk 0**
Leucine, isoleucine, Valine metabolism	Leucine	1.12	0.96
	Isoleucine	1.12	0.98
	2-hydroxy-3-methylvalerate	1.40	1.02
	α-hydroxyisovalerate	1.34	1.09
	3-hydroxy-2-ethylproprionate	1.27	0.97
	α-hydroxyisocaproate	1.22	1.02
	3-methyl-2-oxovalerate	1.14	0.99
	methylsuccinoylcarnitine (1)	0.80	1.12
	3-methylglutarylcarnintine (2)	0.81	0.76



C

IGF-1 stratification		High IGF-1		Low IGF-1	
Pathway	**Biochemical name**	**Wk 14:Wk 0**	**Wk 20:Wk 0**	**Wk 14:Wk 0**	**Wk 20:Wk 0**
Acyl Carnitine Metabolism	acylcarnitine (C2)	1.43	0.98	1.18	1.08
	3-hydroxybutyrylcarnitine (1)	1.94	0.91	1.41	1.03
	3-hydroxybutyrylcarnitine (2)	2.83	0.93	2.24	1.01
	hexanoylcarnitine (C6)	1.34	0.90	1.14	0.95
	octanoylcarnitine (C8)	1.24	0.86	1.04	0.90
	laurylcarnitine (C12)	1.29	0.89	1.05	0.93
Ketone bodies	3-hydroxybutyrate (BHBA)	2.64	1.14	0.99	0.84

Figure 3. Metformin results in changes in serum metabolites in Li-Fraumeni syndrome participants after 14 weeks of metformin treatment. Global metabolomic profiling was performed in serum samples from participants at baseline (premetformin), 14 weeks (on metformin), and 20 weeks (6 weeks off metformin). **A)** Metabolites of lipid metabolism increased while participants were taking metformin and returned to baseline levels after washout period. **B)** Branched-chain amino acid metabolites showed a similar pattern. **C)** Products of acyl carnitine metabolism were differentially changed between patients with IGF-1 level above the median at baseline, and less effect was seen in patients whose IGF-1 level was below median prior to starting metformin.

Importantly, we asked whether metformin reversed the increased oxidative metabolism in LFS patients, a biomarker associated with delayed cancer in a mouse model of LFS (17).

This study was not powered to detect differences between individual variants. Given the small number of patients with each type of *TP53* alteration, we are unable to identify associations between mutation type and molecular changes. Additionally, our study was not designed to show whether these metabolic responses are specific to individuals with LFS by any molecular change. Taken together, however, our results highlight potential mechanisms by which metformin may protect them from developing cancer.

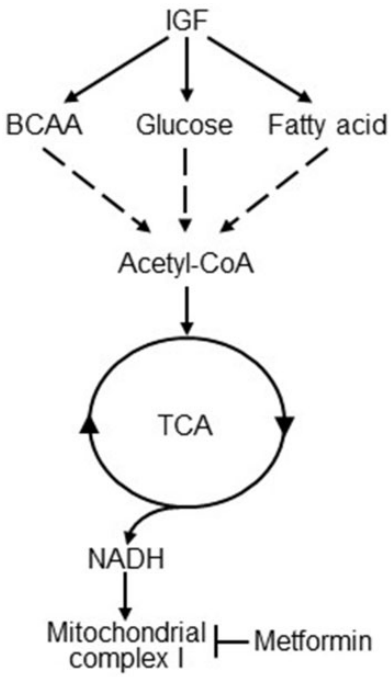


Figure 4. Summary of the pathways. Pathways by which metformin could cause the various integrated metabolic changes observed in the current study are shown, including inhibition of mitochondrial respiration. BCAA = branched-chain amino acid; IGF = insulin-like growth factor; NADH = nicotinamide adenine dinucleotide; TCA = tricarboxylic acid cycle.

In summary, metformin up to 2000 mg/day is safe and tolerable in nondiabetic individuals with germline *TP53* pathogenic variants. Adverse events were low grade and infrequent, dose reductions were rare, compliance was high, and blood glucose levels remained stable in all patients. Correlative markers tested in this study resulted in the expected biological effect of metformin in this patient population. Our findings with metabolomic profiling have yielded new insights and provide support for testing the pharmacologic risk-reducing effects metformin in a prospectively designed clinical trial for patients with LFS.

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Data availability

Raw data are available upon request.

References

1. Malkin D. Li-Fraumeni syndrome. *Genes Cancer*. 2011;2(4): 475–484.
2. Mai PL, Best AF, Peters JA, et al Risks of first and subsequent cancers among TP53 mutation carriers in the National Cancer Institute Li-Fraumeni syndrome cohort. *Cancer*. 2016;122(23):3673–3681.
3. Ballinger ML, Best A, Mai PL, et al Baseline surveillance in Li-Fraumeni syndrome using whole-body magnetic resonance imaging: a meta-analysis. *JAMA Oncol*. 2017;3(12):1634–1639.
4. Villani A, Shore A, Wasserman JD, et al Biochemical and imaging surveillance in germline TP53 mutation carriers with Li-Fraumeni syndrome: 11 year follow-up of a prospective observational study. *Lancet Oncol*. 2016;17(9): 1295–1305.
5. Villani A, Tabori U, Schiffman J, et al Biochemical and imaging surveillance in germline TP53 mutation carriers with Li-Fraumeni syndrome: a prospective observational study. *Lancet Oncol*. 2011;12(6):559–567.
6. National Comprehensive Cancer Network. Clinical practice guidelines in oncology: genetic/familial high-risk assessment: breast and ovarian, v1. 2020. https://www.nccn.org/professionals/physician_gls/pdf/genetics_bop.pdf. Accessed July 27, 2020.
7. Macfarland SP, Zelley K, Long JM, Mckenna D, et al Earlier colorectal cancer screening may be necessary in patients with Li-Fraumeni syndrome. *Gastroenterology*. 2019;156(1):273–274.
8. Nirvana Da Cruz Formiga M, Ashton-Prolla P, Alves de Souza Waddington Achatz MI. Early-onset colorectal cancer in patients with Li Fraumeni syndrome: is it really enough to justify early colon cancer screening? *Gastroenterology*. 2019;157(1):264–264.
9. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science*. 2009; 324(5930): 1029–1033.
10. Ward PS, Thompson CB. Metabolic reprogramming: a cancer hallmark even Warburg did not anticipate. *Cancer Cell*. 2012;21(3): 297–308.
11. Koppenol WH, Bounds PL, Dang CV. Otto Warburg's contributions to current concepts of cancer metabolism. *Nat Rev Cancer*. 2011;11(5):325–337.
12. Vousden KH, Ryan KM. p53 and metabolism. *Nat Rev Cancer*. 2009;9(10): 691–700.
13. Lago CU, Sung HJ, Ma W, et al p53, aerobic metabolism, and cancer. *Antioxid Redox Signal*. 2011;15(6): 1739–1748.
14. Macedo GS, Lisbôa da Motta L, Giacomazzi J, et al Increased oxidative damage in carriers of the germline TP53 p. R337H mutation. *PLoS One*. 2012;7(10): e47010.
15. Wang P-Y, Ma W, Park J-Y, et al Increased oxidative metabolism in the Li-Fraumeni syndrome. *N Engl J Med*. 2013;368(11): 1027–1032.
16. Kamp WM, Wang PY, Hwang PM. TP53 mutation, mitochondria and cancer. *Curr Opin Genet Dev*. 2016;38:16–22.
17. Wang P-y, Li J, Walcott FL, et al Inhibiting mitochondrial respiration prevents cancer in a mouse model of Li-Fraumeni syndrome. *J Clin Invest*. 2016;127(1): 132–136.
18. Campbell JM, Bellman SM, Stephenson MD, Lisy K. Metformin reduces all-cause mortality and diseases of ageing independent of its effect on diabetes control: a systematic review and meta-analysis. *Ageing Res Rev*. 2017;40: 31–44.
19. Heckman-Stoddard BM, DeCensi A, Sahasrabuddhe VV, Ford LG. Repurposing metformin for the prevention of cancer and cancer recurrence. *Diabetologia*. 2017;60(9):1639–1647.
20. Suissa S, Azoulay L. Metformin and cancer: mounting evidence against an association. *Diabetes Care*. 2014;37(7):1786–1788.
21. Pollak M. Potential applications for biguanides in oncology. *J Clin Invest*. 2013; 123(9):3693–3700.

22. Wang Y, Nasiri AR, Damsky WE, et al Uncoupling hepatic oxidative phosphorylation reduces tumor growth in two murine models of colon cancer. *Cell Rep*. 2018;24(1):47–55.
23. Pollak M. The insulin and insulin-like growth factor receptor family in neoplasia: an update. *Nat Rev Cancer*. 2012;12(3):159–169.
24. Yu H, Rohan T. Role of the insulin-like growth factor family in cancer development and progression. *J Natl Cancer Inst*. 2000;92(18): 1472–1489.
25. Algire C, Moiseeva O, Deschenes-Simard X, et al Metformin reduces endogenous reactive oxygen species and associated DNA damage. *Cancer Prev Res (Phila)*. 2012;5(4): 536–543.
26. Labuschagne CF, Zani F, Vousden KH. Control of metabolism by p53 - cancer and beyond. *Biochim Biophys Acta Rev Cancer*. 2018;1870(1):32–42.
27. Collier CA, Bruce CR, Smith AC, Lopaschuk G, Dyck DJ. Metformin counters the insulin-induced suppression of fatty acid oxidation and stimulation of triacylglycerol storage in rodent skeletal muscle. *Am J Physiol Endocrinol Metab*. 2006;291(1):E182–E189.
28. Pollak M. Overcoming drug development bottlenecks with repurposing: repurposing biguanides to target energy metabolism for cancer treatment. *Nat Med*. 2014;20(6): 591–593.
29. Feng Z, Levine AJ. The regulation of energy metabolism and the IGF-1/mTOR pathways by the p53 protein. *Trends Cell Biol*. 2010;20(7): 427–434.
30. Feng Z. p53 regulation of the IGF-1/AKT/mTOR pathways and the endosomal compartment. *Cold Spring Harb Perspect Biol*. 2010;2(2): a001057.
31. Levine AJ. Coordination and communication between the p53 and IGF-1-AKT-TOR signal transduction pathways. *Genes Dev*. 2006;20(3): 267–275.
32. Matoba S. p53 regulates mitochondrial respiration. *Science*. 2006;312(5780): 1650–1653.
33. Buzzai M, Jones RG, Amaravadi RK, et al Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. *Cancer Res*. 2007;67(14):6745–6752.
34. Yang J, Chi Y, Burkhardt BR, Guan Y, Wolf BA. Leucine metabolism in regulation of insulin secretion from pancreatic beta cells. *Nutr Rev*. 2010;68(5): 270–279.
35. Stumvoll M, Nurjhan N, Perriello G, Dailey G, Gerich JE. Metabolic effects of metformin in non-insulin-dependent diabetes mellitus. *N Engl J Med*. 1995; 333(9):550–554.
36. Wu MS, Johnston P, Sheu WH, et al Effect of metformin on carbohydrate and lipoprotein metabolism in NIDDM patients. *Diabetes Care*. 1990;13(1):1–8.
37. Sarfstein R, Friedman Y, Attias-Geva Z, Fishman A, Bruchim I, Werner H. Metformin downregulates the insulin/IGF-I signaling pathway and inhibits different uterine serous carcinoma (USC) cells proliferation and migration in p53-dependent or -independent manners. *PLoS One*. 2013;8(4):e61537.
38. Zemdegs J, Martin H, Pintana H, et al Metformin promotes anxiolytic and antidepressant-like responses in insulin-resistant mice by decreasing circulating branched-chain amino acids. *J Neurosci*. 2019;39(30): 5935–5948.