DYSLIPIDEMIA IS A MAJOR RISK factor for cardiovascular disease known to play a proximal role in the development of obesity and Type 2 diabetes mellitus (T2DM) (39). Abnormal amounts of triglyceride (TG) in the blood and nonadipose tissues result in metabolic disturbances such as impaired glucose uptake, glycolysis synthesis, and glucose oxidation, which can lead to insulin resistance (14, 45, 46, 51, 52) as well as increased risk of developing both microvascular and macrovascular disease-associated complications including nephropathy, neuropathy, retinopathy, and atherosclerosis (48). Two major pathways have been elucidated for the synthesis of TGs: the glycerol phosphate or Kennedy pathway and the monoacylglycerol (MG) pathway. The glycerol phosphate pathway is present in most cells, whereas the MG pathway is utilized in specific cell types such as enterocytes, hepatocytes, and adipocytes. The MG pathway predominates in human small intestine, accounting for ~75% of the formation of TG from the reesterification of hydrolyzed dietary lipids (27). The final reaction in both pathways for synthesizing TG is the formation of an ester bond between a fatty acyl-CoA and diacylglycerol (DG) catalyzed by acyl-CoA:diacylglycerol acyltransferase (DGAT) enzymes. The DGAT enzymes are encoded by two non-homologous genes (DGAT1 and DGAT2) (10, 59). Unlike DGAT2, DGAT1 will accept MG, wax alcohols, and retinol as substrates in addition to DG (10, 58, 59).

Both DGAT1 and DGAT2 are expressed in tissues responsible for the synthesis of TG. In both mice and humans, DGAT1 is expressed most abundantly in the intestines (7) but is also present in other tissues including adipose, liver, and skeletal muscle (58). In humans, DGAT2 is highly expressed in liver, adipose, and mammary glands. In mice, DGAT2 is mostly abundant in adipose followed by expression in liver and many other tissues. DGAT1 knockout mice are phenotypically lean, are resistant to diet-induced obesity and hepatic steatosis, and exhibit increased insulin and leptin sensitivity in addition to having reduced tissue TG concentrations (7–9, 47, 55). Neutral lipid droplet accumulation within the proximal enterocytes is observed in both acute lipid treatment and high-fat diet-fed DGAT1-deficient mice, delaying chylomicron release into circulation (1, 47, 55). Furthermore, when intestinal DGAT1 activity is restored in whole body DGAT1-deficient mice, they are no longer resistant to hepatic steatosis (38). Thus the lack of functional DGAT1 in the intestine could be a major contributor to the improved metabolic profile exhibited in the DGAT1 knockout mice. In contrast, DGAT2 deficiency is not compatible with life as DGAT2 knockout mice die shortly after birth (6). These observations from mice lacking either functional DGAT1 or DGAT2 support the notion that DGAT2 is the critical enzyme for TG homeostasis in mice.

The systemic deletion of DGAT1 as well as its acute pharmacological inhibition has been shown to elicit a blunted postabsorptive lipid profile (4, 5, 19, 32, 38, 60). However, the precise trafficking of dietary lipids without functional DGAT1 has been largely unstudied. Dietary fat absorption involves the hydrolysis of dietary vitamin A and TG to retinol, 2-MG, and fatty acids (FA), respectively, by pancreatic lipases within the lumen of the upper gastrointestinal tract. These hydrolyzed products are readily absorbed by enterocytes for repackaging. Acyl coenzyme A:monoacylglycerol acyltransferase (MGAT) catalyzes the addition of acyl coenzyme A onto 2-MG to form DG. DG is subsequently esterified by DGAT1 to resynthesize TG. It has been reported that in vitro the DGAT1 enzyme has MGAT activities (59). Several enzymes catalyze the esterifi-
cation of vitamin A in the intestine to varying degrees: lecithin: retinol acyltransferase (LRAT), acyl-CoA:retinol acyltransferase (ARAT), and DGAT1 (16, 56). Newly formed TG and retinyl esters are packaged with other lipids to form chylo-
micron lipoprotein particles, which are secreted into the lymph-
Phatic system for entry into the systemic circulation.

In the present study, we evaluated the impact of pharmaco-
logical inhibition of DGAT1 by PF-04620110 on the absorp-
tion of dietary fat and vitamin A in both mice and humans.
PF-04620110, a pyrimidooxazepine, is a competitive inhibi-
tor with a comparable Ki in humans (19 nM), rat (64 nM), and
mice (94 nM) (19, 22). Additionally, we examined the tempo-
ral and spatial patterns of TG absorption in mice following
administration of a single dose of PF-04620110 (19). To
monitor the impact of DGAT1 inhibition on enteroctye lipid
species content, lipid metabolite profiling was performed in
both rat intestine and plasma and human plasma. These studies
using PF-04620110, a pharmacological inhibitor, to probe the
impact of lack of functional DGAT1 on comparable end points
preclinically and clinically provide insight to the translation of
DGAT1 function from mice to humans.

MATERIALS AND METHODS

In vitro assay. The discovery of PF-04620110 has been reported
previously (19) and is a potent and selective small molecule inhibitor
of DGAT1 with 100-fold selectivity vs. human DGAT2, ACAT1,
AWAT1, AWAT2, MGAT2, and MGAT3 and mouse MGAT1.
Briefly, the ability of PF-04620110 to inhibit recombinant human (38
nM), rat (94 nM), and mouse (64 nM) DGAT1 enzymatic activity was
determined by measuring the incorporation of [3H]n-decanoyl coen-
zyme A into DG to form TG. Additionally, in a cell-based assay in
intestinal derived HT-29 cells, PF-04620110 (IC50 < 39 nM) inhibits
the incorporation of 3H-glycerol into TG (19).

Mice. C57BL/6J and B6.129S4-Dgat1tm1Kar (DGAT1 knockout
mice) (Jackson Laboratories) male mice (7–12 wk of age) were
allowed ad libitum access to water and normal chow (5001, Purina) on
a 6 AM– 6 PM light-dark cycle. All procedures were approved by the
Institutional Animal Care and Use Committee, and all animals re-
ceived humane treatment according to the criteria stated by the
National Academy of Sciences National Research Council (NRC)

TG/retinol palmitate tolerance test in mice. Mice were randomized
according to body weight on the day of experimentation with eight
mice per group. The mice were fasted for 3 h prior to a single oral
dose of placebo or PF-04620110 at 1, 0.3, 0.1, and 0.01 mg/kg. Thirty
minutes after compound dosing, the animals were administered
with 5 ml/kg corn oil/10,000 IU retinyl palmitate (Sigma) by oral gavage.
Blood was obtained via retroorbital sinus just prior (t = 0) to corn
oil/retinyl palmitate administration and at 1, 2, 3, 6, and 24 h following
corn oil/retinyl palmitate administration. TGs were mea-
sured on a Hitachi c311 clinical analyser (Roche) via a validated
analytical assay (Wako). Retinyl palmitate levels were quantitated by
liquid chromatography-tandem mass spectrometry (LC-MS/MS) us-
ing photospray ionization on an AB Sciex API4000 mass spectrom-
eter (38a).

14C triolein/corn oil tracing in mice. Mice were randomized ac-
cording to body weight on the day of experimentation with four mice
per group. The mice were fasted for 3 h (starting at 6 AM) prior to
administration of a single oral dose of placebo or PF-04620110 at 10
mg/kg. One half-hour after compound dosing, the animals were orally
dosed with corn oil/14C triolein (PerkinElmer) (1 μCi of 14C triolein
per 100 μl corn oil). Blood and tissues were harvested at 0, 1, 2, 4, 6,
8, 10, and 12 h following corn oil/14C triolein bolus. The stomach
was clamped and the small intestine was removed (from base of stomach
to the cecal junction) and sectioned for assessment of 14C counts. The
lumen of the small intestine was rinsed with 0.5 mM of sodium
taurocholate in 1× PBS (Sigma) and then segmented into 2-cm
sections for 14C measurement. Five hundred microliters of 1 N sodium
hydroxide (J. T. Baker) was added to each intestinal sample and
placed at 65°C for 1 h to digest the tissue contents. Plasma was treated
similarly by adding 100 μl of plasma to the sodium hydroxide. To
the stomach and its contents, 1 ml of sodium hydroxide and 10 ml of
scintillation fluid were added to each sample. The samples were
transferred to scintillation glass vials and mixed with 5 ml of Hion-
Fluor liquid scintillation cocktail (PerkinElmer). Samples were then
analyzed for 14C counts (counts per minute (CPM)) by use of a Wallac
1409 DSA scintillation counter (PerkinElmer). In a separate cohort
of mice, feces were collected at 24 and 48 h following 14C triolein/corn
oil bolus. Mice were housed in metabolic cages, five per cage, and
separated as placebo vs. drug treatment (n = 25 per group). Feces
were collected and analyzed for 14C content and thin-layer chroma-
tography (TLC) lipid analysis. In separate cohort of mice, enterocytes
were analyzed for 14C content. Small intestines were obtained at 4
and 12 h following 14C triolein/corn oil administration. The isolated
intestinal sections were then gently scraped and subjected to TLC lipid
analysis. Mean raw CPM were used to quantify the plasma and
stomach + content samples. The small intestine samples are repre-
sented as CPM per weight of intestinal segment per weight of the
entire small intestine per animal. Fecal CPM was normalized to wet
weight of the feces.

TLC lipid analysis. One hundred milligrams of lyophilized fecal
samples or enterocytes were treated by a variation of the Folch
method of lipid extraction (28). In brief, the samples were placed
in glass vials and 2 ml of chloroform-methanol (2:1) was added to each
tube, vortexed, and placed at 65°C for 30 min. Samples were equil-
ibrated to 4 ml of chloroform-methanol and then 1 ml of distilled
water was added to each tube. Samples were then vortexed and
centrifuged for 10 min at 1,000 rpm to separate aqueous and organic
phases. The aqueous phase (bottom layer) was extracted and dried
down by using a Techne nitrogen drier at room temperature. Samples
were then reconstituted in 124 μl of chloroform-methanol and spotted
onto prescored silica plates. Duplicate samples were subject to TLC
using a two-phase extraction method: phase 1 50 ml ethyl acetate, 50
ml of chloroform, 20 ml methanol, 18.05 ml of 0.25% KCl; phase 2 100 ml hexane, 30.7 ml diethyl ether, 2.7 ml acetic acid. Bands for TG, DG, MG, and free fatty acids (FFA) were
scraped from the silica plate into individual scintillation vials, and 5
ml of Aquasol (PerkinElmer) scintillation fluid was added to each
vial and vortexed. Samples were then analyzed for 14C CPM with use of
a Wallac 1409 DSA scintillation counter (PerkinElmer). Fecal lipid
counts are represented as CPM per 100 mg of sample, and enteroctye lipid counts are represented per gram of sample.

Human subjects. Healthy male or female (of nonchildbearing
potential) subjects between the ages of 18–55 yr, inclusive [mean
(SD) age was 36.4 (9.5) yr], with a body mass index (BMI) of 27–35
kg/m2 and a total body weight (SD) age was 36.4 (9.5) yr, with a body mass index (BMI) of 27–35
kg/m2 and a total body weight

...
subject received two of three active doses of PF-04620110 plus one dose of placebo over three study periods (periods 1, 2, and 3). Six of the nine cohort B subjects thus received one of two active doses of PF-04620110, plus two doses of placebo, allowing for an assessment of intrasubject variability of pharmacodynamic markers during repeat placebo administration. Subjects in cohort C received two of two active doses of PF-04620110 plus one dose of placebo. In all cohorts, there was a minimum washout period of 2 wk between each dose for any one subject. Subjects were screened for the study within 28 days before dosing in period 1 and admitted to the Pfizer Clinical Research Unit (New Haven, CT) the day before the study. Following an overnight fast (10 h), subjects received a single oral dose of either PF-04620110 (dose range of 0.3–21 mg) or placebo at ~8 AM. At 10 AM, subjects received a standardized high-fat liquid meal consisting of 1,000 kcal (500 ml) of Abbott Nutrition Hi-Cal Liquid (43% carbohydrate, 17% fat, and 40% protein, 2 kcal/ml) supplemented with 100,000 IU vitamin A palmitate (AQUASOL A Parenteral or equivalent) administered orally, which was consumed within 10 min. Subjects were not allowed to eat or drink (except water) until 8 h after the high-fat liquid meal (and after the 10-h postdose biomarker sample had been collected). Blood samples for analysis of postprandial lipid metabolism measures (vitamin A and TG) were collected at 2 h after dosing (immediately before consumption of the high-fat liquid meal) and at 3, 4, 6, 8, and 10 h after dosing. Plasma samples were analyzed for TG and retinyl palmitate concentrations at Pacific Biometrics (Seattle, WA) by using a validated analytical assay in compliance with the sponsor’s standard operating procedures. Plasma samples from the 21-mg dose at the 8-h time point was subjected to lipomic analysis at Tethys Biosciences (Emeryville, CA).

Rats. Sprague-Dawley (Charles River Laboratories) male rats (~300 g) were allowed ad libitum access to water and food (powdered high-fat Western diet, 40% kcal from fat, Research Diets D12079B). All procedures were approved by the Institutional Animal Care and Use Committee and all animals received humane treatment according to the criteria stated by the National Academy of Sciences NRC publication 86-23, 1985.

DGAT1 inhibition in rats. Rats were randomized according to body weight with eight rats per group and dosed orally with placebo or PF-04620110 at 15 mg/kg. A group of pair-fed animals was included to control for lipid biomarker changes due to decreases in daily food intake. Rats were acculturated to the powdered high-fat Western diet for 2 wk prior to being studied. The animals were fasted for 24 h, dosed 30 min prior to ad libitum access to food for 6 h. Blood was taken via cardiac puncture and jejunum was taken for lipomic analysis 6.5 h after compound administration and 6 h after ad libitum access to food.

Lipomic analysis. Lipomic analysis, which comprises quantitative profiling of 450 lipid metabolites in plasma (www.lipomics.com), was performed by Tethys Biosciences on the indicated rat and human samples. The moles of each metabolite are normalized by total moles within the lipid class itself and presented in terms of fold change. The heat maps are colored such that a positive number (in red) represents a positive fold change where the actual fold is the labeled quantity. A negative quantity (in green) represents a negative fold change where the actual fold change is labeled. Furthermore, the inflection point is black. This allows for the distinction between statistically significant nonchange and statistically insignificant change.

Statistical analysis. For the human study, actual sampling times were used to calculate area under the human plasma concentration-time profile [area under the curve (AUC)]. Natural log-transformed AUC values for the TG and retinyl palmitate were analyzed by using a mixed-effects model with sequence, period, and treatment as fixed effects and subject within sequence as a random effect, for each cohort. Estimates of the adjusted mean differences between Test (Dose level of PF-04620110 or Placebo 2) and Reference (Placebo 1) and corresponding 90% confidence intervals (CI) were obtained from the model. The adjusted mean differences and 90% CIs for the differences were exponentiated to provide estimates of the ratio of adjusted geometric means (Test/Reference) and 90% CIs for the ratios. Percent change in mean AUC from Placebo 1 utilized geometric means and was calculated by the following formula: [(Test geometric mean AUC − Reference geometric mean AUC)/Reference geometric mean AUC]*100. Similarly, actual sampling times were used to calculate area under the mouse plasma concentration-time profile (AUC). Natural log-transformed AUC values for the TG and retinyl palmitate were analyzed separately by using a mixed-effects model with treatment as a fixed effect and mouse as a random effect. Estimates of the adjusted mean differences between Test (Dose level of PF-04620110) and Reference (Placebo), and corresponding 90% CIs, were obtained from the model. The adjusted mean differences and 90% CIs for the differences were exponentiated to provide estimates of the ratio of adjusted geometric means (Test/Reference) and 90% CIs for the ratios. Percent change in mean AUC from Placebo utilized geometric means and was calculated by the following formula: [(Test geometric mean AUC - Reference geometric mean AUC)/Reference geometric mean AUC]*100. AUC from time zero (before compound administration) to the last quantifiable compound-plasma concentration (last) was determined by the linear/trapezoidal method. Analyses of 14C trioleoylglycerol content involved using a Student’s t-test to compare treated vs. control groups at each time point. We utilized t-tests with a Satterthwaite approximation to handle unequal group variances where applicable. Group comparisons were conducted at the 5% significance level.

Pharmacokinetic parameters in mice are represented as geometric means. Pharmacokinetic parameters in humans are represented as geometric means except for Fig. 2E and peak concentrations achieved at 30 min (Tmax) in Fig. 2F, which is represented as median to ensure the relationship is not obscured by outliers.

Pharmacokinetic analysis of PF-04620110 quantitation in mouse and human plasma. Mouse and human concentration PF-04620110 was determined by validated LC-MS/MS methods. In brief, aliquots of plasma were precipitated by use of acetoniitrile containing a structurally similar internal standard (IS; 200 ng/ml). The effluent was analyzed by a mass spectrometer detector (AB Sciex API-4000). PF-04620110 and IS were monitored by multiple reaction monitoring at transitions of 409.3/238.0 and 395.4/237.9, respectively. The dynamic range of the assay was 0.001–10 μg/ml. Pharmacokinetic parameters of PF-04620110 in mouse and human were calculated by using Watson version 7.2.

RESULTS

Administration of a single dose of a DGAT1 inhibitor, PF-04620110, reduces postprandial plasma TG and retinyl palmitate excursions in mice and humans. To determine the impact of DGAT1 inhibition on dietary lipid absorption, fasted C57/BL6J mice were challenged with a corn oil/retinyl palmitate bolus following administration of PF-04620110, a potent and selective DGAT1 inhibitor (19). Maximal plasma TG concentrations were achieved in placebo-treated animals at 2 h post-challenge whereas TG levels were significantly attenuated in mice receiving 0.1, 0.3, and 1 mg/kg PF-04620110 (Fig. 1A). At the 1 mg/kg dose of PF-04620110, a 47.5% decrease in TG mean AUC compared with placebo was seen (Fig. 1B). Similarly, retinyl palmitate levels in plasma were also reduced in a dose-responsive manner upon DGAT1 inhibition compared with placebo-treated animals (Fig. 1C). At the 1 mg/kg dose of PF-04620110, a 72.9% decrease in retinyl palmitate mean AUC relative to placebo was achieved (Fig. 1D). Compound exposures were monitored during the course of the tolerance test (Fig. 1E). PF-04620110 exhibited a dose-proportional increase in exposure with Tmax following oral administration.
Fig. 1. Acute administration of a DGAT1 inhibitor, PF-04620110, reduces postprandial plasma triglyceride (TG) and retinyl palmitate excursions in wild-type (WT) mice and has no effect in DGAT1 knockout (KO) mice. Fasted male C57BL/6J mice \( (n/8/100) \) were administered either placebo or indicated doses of PF-04620110 by oral gavage. Thirty minutes following compound administration, mice received a bolus of corn oil/retinyl palmitate (challenge). Blood was collected just prior to bolus (time 0) and at 1, 2, 3, 6, and 24 h afterward for determination of PF-04620110, TG, and retinyl palmitate concentrations.

A: TG (mg/dl) excursion following challenge plotted as average \( \pm \) SE.

B: retinyl palmitate (ng/ml) excursion following challenge plotted as average \( \pm \) SE.

B and D: area under the curve (AUC) was calculated for TG and retinyl palmitate excursion from 0–6 h and represented as geometric mean AUC percent change from placebo, ratio of geometric means, and lower and upper 90% confidence intervals (CI) for TG \( (B) \) and retinyl palmitate \( (D) \) concentrations \( (*) P < 0.05% \).

E: pharmacokinetic analysis of PF-04620110 in plasma.

F: mean PF-04620110 pharmacokinetic parameters in mice \( (N = \text{number of patients per treatment; NC, not calculated}) \).

G: DGAT KO mice \( (-/-) \) and their WT littermates \( (+/+>) \) were administered either placebo or 10 mg/kg PF-04620110 by oral gavage. Thirty minutes later a corn oil/retinyl palmitate bolus was given. TG levels were significantly reduced in drug-treated WT \( (+/+) \), placebo-treated DGAT1 KO \( (-/-) \), and drug-treated DGAT1 KO mice compared with placebo-treated WT mice.

H: fasted male C57BL/6J mice \( (n/8/100) \) were administered either placebo or indicated doses of PF-04620110 by oral gavage for 5 consecutive days. Thirty minutes following compound administration on the fifth day, mice received a bolus of corn oil/retinyl palmitate (challenge). Blood was collected just prior to bolus \( (time 0) \) and at 1, 2, 3, 6, and 24 h afterward for determination of TG levels.
PF-04620110 plasma concentrations during the tolerance test (AUC [0–6 h]) at 0.1, 0.3, and 1.0 mg/kg were ~0.5–1.8–, and 9.7-fold over the \( K_i \) values (Fig. 1F). To demonstrate that functional DGAT1 is required for PF-04620110 to elicit its effects on postprandial dietary lipid excursions, we administered PF-04620110 at 10 mg/kg to DGAT1 knockout and their wild-type littermates. PF-04620110 did not alter postprandial TG or retinol excursions in the DGAT1 knockout mice (7, 8) using tracer studies performed up to 48 h. Additionally, the response of pharmacological inhibition in wild-type mice is comparable to DGAT1 knockout mice with respect to dietary lipid absorption (Fig. 1G). PF-04620110 was also chronically administered to C57BL6J mice for 5 consecutive days and postprandial lipid absorption was measured (Fig. 1H). Although basal TG levels were elevated compared with acute dosing studies, significant blunting of the TG excursion was observed at all doses of PF-04620110 compared with placebo treatment.

To translate the pharmacology of DGAT1 on dietary lipid absorption in humans using PF-04620110, healthy overweight and obese human subjects were given a high-fat liquid meal supplemented with pharmacological doses of vitamin A. Following the administration of a single oral dose of PF-04620110 ranging from 0.3 to 21 mg, plasma TGs were decreased with significance achieved in the 7-, 14-, and 21-mg doses (Fig. 2A). At the highest dose (21 mg) tested, a 15.2% TG decrease in mean AUC compared with placebo was demonstrated (Fig. 2B). To demonstrate whether DGAT1 in humans impacts retinoid absorption, we also monitored the absorption of pharmacological doses of retinyl palmitate in plasma following DGAT1 inhibition. Plasma retinyl palmitate levels were significantly decreased following a single dose at 7, 14, and 21 mg of PF-04620110 (Fig. 2C). Strikingly, a 50.3% decrease in retinyl palmitate mean AUC compared with placebo at the 21-mg dose was achieved (Fig. 2D). Compound exposures were monitored during the course of the tolerance test (Fig. 2E). PF-04620110 exhibited a dose-proportional increase in exposure up to 14 mg with a plateau between 14 and 21 mg and \( T_{\text{max}} \) at 2 h (Fig. 2F). Although the magnitude of maximal inhibition of vitamin A absorption differs between mice and humans, pharmacokinetic/pharmacodynamic (PK/PD) modeling indicates the \( ED_{50} \) is comparable (Fig. 2G).

**DGAT1 inhibition delays gastric emptying and elicits a malabsorption in mice.** We then explored whether pharmacological inhibition of DGAT1 is truly inhibiting or merely delaying the absorption as previously reported in whole body knockout mice (7, 8) using tracer studies performed up to 48 h. We monitored radioactive counts over time following oral gavage of \(^{14}\)C triolein/corn oil in the plasma, stomach, and feces in C57/BL6J mice in the absence and presence of PF-04620110. Based on the exposure profile (Fig. 1E), a dose of 10 mg/kg was chosen to maintain PF-04620110 concentration over the \( K_i \) for ~20 h. Initially, \(^{14}\)C levels in plasma were significantly lower during the first 6 h in the DGAT1 inhibitor-treated mice compared with placebo, consistent with the TG tolerance test (Fig. 3A). However, levels of \(^{14}\)C detected in plasma started to rise in PF-04620110-treated mice at 8 h following lipid challenge (Fig. 3A). Concomitantly, with the appearance in plasma, \(^{14}\)C counts in the stomach were rapidly eliminated in the placebo-treated mice, whereas in mice treated with PF-04620110 radioactive counts remained in the stomach for 4 h following corn oil administration before decreasing over time (Fig. 3B). PF-04620110 delays the emptying of radioactive triolein in mice and recapitulates the delayed lipid absorption seen in DGAT1 knockout mice. This delayed gastric emptying is part of the mechanism by which DGAT1 inhibition results in a blunted absorption of dietary lipids.

To determine the impact of DGAT1 inhibition on the efficiency of dietary lipid absorption, feces were collected 24 and 48 h after \(^{14}\)C triolein/corn oil administration. The 24 h fecal collection indicated a significant 52% increase in \(^{14}\)C excreted from PF-04620110-treated mice relative to placebo (Fig. 3C). At 48 h, \(^{14}\)C fecal collection revealed a 45% increase, which was not significant. We performed identical studies in DGAT1 knockout mice and did not detect an increase in lipid in the feces (data not shown). Additionally, we evaluated TG, DG, and FFA content in placebo- and DGAT1 inhibitor-treated 24-h feces by TLC. There was a uniform trend toward increase in all lipid species assessed following DGAT1 inhibition (Fig. 3D). Therefore, acute pharmacological inhibition of DGAT1 in mice results in an increase in lipid in the feces. In summary, the temporal absorption of dietary lipids as well as the efficiency of lipid absorption is altered in DGAT1 inhibitor-treated mice.

**DGAT1 inhibition spatially alters lipid absorption in mice.** Next, we sought to monitor whether DGAT1 inhibition alters the spatial absorption of dietary lipids throughout the small intestine. A time course from 0–12 h following \(^{14}\)C triolein/corn oil administration examining radioactive counts and lipid species by TLC throughout the small intestine was performed. In placebo-treated mice, maximal levels of \(^{14}\)C occur during the first 4 h following lipid challenge in the proximal intestine (duodenum and upper jejunum) (Fig. 4, A–C). At 6 h and beyond, the levels of \(^{14}\)C throughout the small intestine were negligible in placebo-treated mice (Fig. 4, D–G). In contrast, mice treated with PF-04620110 maximal levels of \(^{14}\)C occur at two distinct time points, 1 h (Fig. 4A) and 12 h (Fig. 4G) following dose in the jejunum. Additionally, the levels of \(^{14}\)C in small intestine exposed to PF-04620110 were lower than placebo for the duration of the experiment (Fig. 4, A–G).

Intestinal lipid content for the small intestine was determined by using TLC at the 4- and 12-h time points, which represent the peak lipid absorption for placebo and PF-04620110-treated mice, respectively. At 4 h following \(^{14}\)C triolein/corn oil administration, all lipid classes, TG, DG, MG, and FFA, were significantly decreased by 79.3, 72.1, 60.4, and 55.9%, respectively, in DGAT1 inhibitor-treated enterocytes relative to placebo (Fig. 4H). In contrast, at 12 h following corn oil, all lipid classes, TG, DG, MG, and FFA were significantly increased by 76.1, 74.2, 61.4, and 60.1% in DGAT1 inhibitor-treated enterocyte lipids relative to placebo (Fig. 4H).

**DGAT1 inhibition induces a differential FA composition in rats and humans following a mixed meal.** To examine how DGAT1 inhibition affects the FA composition in TG during a mixed meal, the plasma lipid metabolome was examined at a single time point in both rats (6 h following access to food and water and 6.5 h following compound administration) and humans (6 h following meal administration and 8 h following PF-04620110 dosing) (Fig. 5). The plasma TG profile in humans and rats portrays an overall decrease in TG levels with an enrichment in polyunsaturated fatty acids (PUFA) within the TG class. Additionally, we evaluated the FA composition of TG and FFA in the rat jejunum. Rat jejunum TG profile follows the same trend as the plasma, but interestingly the
Fig. 2. Acute DGAT1 inhibition reduces postprandial TG and retinoid excursions in healthy overweight and obese volunteers. Healthy overweight or obese volunteers (n = 27) were admitted to the Pfizer Clinical Research Unit the day before the study. Following an overnight (10 h) fast, subjects received a single oral dose of either PF-04620110 at dose indicated or placebo at 8 AM. At 10 AM, subjects received a liquid high-fat meal supplemented with vitamin A. Blood samples were collected at 2 h after PF-04620110 dosing (immediately before liquid meal) and at 3, 4, 6, 8, and 10 h after dosing for determination of PF-04620110, TG, and retinyl palmitate concentrations.

A: triglyceride levels were significantly reduced in a dose-dependent manner. C: retinyl palmitate levels were also significantly reduced in a dose-dependent manner. B and D: geometric mean AUC % change from placebo for TG (B) and retinyl palmitate (D) concentrations (*P < 0.05). E: median pharmacokinetic exposure of PF-04620110 in plasma. F: geometric mean AUC and Cmax (concentration); median Tmax (time) PF-04620110 pharmacokinetic parameters in humans. G: mouse-human comparison of retinyl palmitate dose response.
jejenum FFA profiling most closely resembles the plasma TG data. Because treatment with DGAT1 inhibition produces an unusual pattern of changes in PUFA metabolism, we investigated whether the diet contained long-chain PUFA as a possible partial explanation for their enrichment. The diet contained no PUFA longer than 18:2n6 and 18:2n3, implying that the changes are a result of changes in endogenous FA metabolism.

**DISCUSSION**

This is the first demonstration in humans that pharmacological inhibition of DGAT1 following administration of a single dose of PF-04620110 decreases both TG and vitamin A postprandial absorption. Strikingly, the EC50 for inhibition of vitamin A was similar between mice and humans, suggestive that the role of DGAT1 in reesterification of vitamin A is comparable. Thus, by using a functional assessment of DGAT1 enzymatic activity, PF-04620110 allows us to probe the role of DGAT1 in humans via loss of enzymatic activity. These data also suggest that DGAT1 reesterifies pharmacological doses of vitamin A in humans, consistent with its role in retinoid metabolism elucidated in mice (16, 56). To understand the metabolic fate of dietary lipids upon DGAT1 inhibition, radio-

**Fig. 3.** Mice treated with PF-04620110 have delayed gastric emptying and increased fecal lipid content following a corn oil14C-triolein load. C57BL/6J mice (fed a normal chow diet, n = 4/group) were fasted for 3 h prior to receiving an oral gavage of placebo or PF-04620110 (10 mg/kg). Thirty minutes later a bolus of corn oil14C-triolein was administered, and radioactive counts were monitored at indicated time points. A: 14C plasma levels graphed as 14C counts per minute (CPM) per 100 μl of plasma at each indicated time point. B: the entire stomach was removed and total radioactive counts are shown at the indicated time points. C: feces were collected at 24 and 48 h following 14C triolein/corn oil bolus. Radioactive counts per gram of feces per mouse are shown. D: lipids were extracted from feces and analyzed by thin-layer chromatography (TLC). Fecal lipids [TG, diacylglycerol (DG), and free fatty acids (FFA)] shown by counts associated with respective lipid species band on TLC plate per 100 mg of feces. DGAT1 inhibitor treatment compared with placebo (*P < 0.05, **P < 0.01, ***P < 0.001 Student’s t-test). Values are means ± SE.

**Fig. 4.** Acute administration of PF-04620110 alters the spatial absorption of dietary lipids in the small intestine. C57BL/6J mice (fed a normal chow diet, n = 4 per group) were fasted for 3 h prior to receiving an oral gavage of placebo or PF-04620110 (10 mg/kg). Thirty minutes later a bolus of corn oil14C-triolein was administered, and radioactive counts were monitored at indicated time points in the small intestine. A–G: radioactive counts are shown as a function of counts per intestinal segment weight divided by the total weight of the small intestine in placebo vs. DGAT1 inhibitor-treated mice at the indicated time point. H: lipids were extracted from the small intestine at 4 and 12 h following corn oil14C-triolein bolus and quantitated by TLC. Enterocyte lipid content [TG, DG, monoacylglycerol (MG), and FFA] shown as counts per gram of small intestine. (*P < 0.05, **P < 0.01, ***P < 0.001; Student’s t-test). Values are means ± SE.
active tracer studies in mice were conducted. Acute pharmacological inhibition of DGAT1 in mice causes a delay in gastric emptying, increases fecal fat content, and induces a temporal and spatial shift of TG absorption within the small intestine. Furthermore, lipomic profiling indicated a unique enrichment in PUFA moieties associated with TG in enterocytes in rats treated with PF-04620110. Collectively, these data provide a further understanding of the integral role of DGAT1 in intestinal fat absorption and lipid metabolism in mice and rats and the translation of DGAT1 function in humans.

The blunting of TG and retinyl palmitate absorption with pharmacological inhibition of DGAT1 in humans is in agreement with previously reported rodent (1, 4, 19, 32, 60) and human (1) studies. Dietary lipids and retinoids are hydrolyzed in the lumen of the gastrointestinal tract into MG, FFA, and free retinol, which are consequently reesterified through enzymatic activity of DGAT1 and other acyltransferases (1, 19, 42, 56, 58). Although we have not directly measured DGAT1 enzymatic activity in the intestine in these studies, we are inferring the actions of PF-04620110 on dietary lipid excursions as a consequence of modulating DGAT1 activity in the enterocyte; however, other mechanisms cannot be conclusively excluded. A steep dose-response relationship is evident on the postprandial retinoid excursions in humans. There is a discrepancy between the maximal inhibition achieved on TG and retinyl palmitate AUC in humans. It is difficult to ascertain whether this is merely due to biological variability in TG measures or whether another acyltransferase contributes sig-

Fig. 5. DGAT1 inhibitor induced differential fatty acid composition in rats and humans following a mixed meal. Rats (n = 8 per group) were fasted overnight and given a single oral dose of PF-04620110 (15 mg/kg) and then allowed ad libitum access to food (Western diet) and water for 6 h. Subsequently blood and jejunum were removed for lipomics analysis (Tethys Biosciences). Healthy overweight/obese human subjects (n = 6 per group) were fasted overnight and received a single oral dose of PF-04620110 (21 mg) and then received a high-fat liquid meal. Blood was collected 8 h following dose for lipomics analysis (Tethys Biosciences). Data are represented in a heat map as fold change of DGAT1 inhibitor treatment relative to placebo. Within each sample (i.e., human TG plasma), the left-hand column (nmol) is the molar concentration of the respective fatty acid and the right-hand column is the molar concentration of each fatty acid within the lipid class (%). Samples ordered as follows: human plasma TG, rat plasma TG, rat jejunum TG, and rat jejunum FFA. The heat maps are colored such that a positive number (in red) represents a positive fold change where the actual fold is the labeled quantity. A negative quantity (in green) represents a negative fold change where the actual fold change is labeled. Furthermore, the inflection point is black. This allows for the distinction between statistically significant nonchange and statistically insignificant change.

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G966 TRANSLATIONAL PHYSIOLOGY

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nificantly in human enterocytes to reesterification of dietary TG. A clear PK/PD relationship for both TG and retinoid postprandial excursions exists in mice with a near-complete reduction seen over the 6-h testing period. We next sought to understand the fate of this exogenous dietary lipid bolus with DGAT1 inhibition.

To this end, we monitored the fate of radioactive triolein in mice. DGAT1 inhibition exhibited a 6- to 8-h delay in the appearance of radioactive counts in the plasma. The radioactive counts remained in the stomach during this delay, suggesting that delayed gastric emptying is part of the mechanism by which DGAT1 inhibition impacts postprandial lipid absorption. On the basis of the dose of PF-04620110 chosen, we are still achieving concentrations of PF-04620110 in excess of our in vitro settings when dietary lipids begin to emerge from the stomach. Thus the delayed gastric emptying is a biological consequence of DGAT1 inhibition and not due to compound clearance and diminished inhibition of DGAT1, consistent with observations in DGAT1-deficient mice (11, 41). Similar findings were also observed in MGAT2 and SRC-2 knockout mice, where lipids enter circulation at a decreased but more sustained rate (12, 57). Delayed gastric emptying can occur through increased incretin hormone levels (41). Indeed, DGAT1-deficient mice exhibited an enhanced incretin response to dietary lipids (41). Pharmacological inhibition of DGAT1 also recapitulates the increase in incretin levels in rats postprandially treated with PF-04620110 (22, 42a) and with other DGAT1 inhibitors in mice (1).

Increased fecal fat in the feces collected for 24 h following DGAT1 inhibition suggest an acute malabsorption. Because we are monitoring counts in the feces, we cannot discern whether these radioactive counts are associated triolein that never entered the enterocytes or contents of sloughed off enterocytes. Perhaps the consequence of altering temporal and spatial dietary lipid absorption or a signal generated by the DGAT1 inhibition within the enterocytes accounts for this apparent malabsorption. Alternatively, the enterocytes are evoking a feedback mechanism by which they “sense” DGAT1 activity is abolished and downregulate lipid absorption to avoid potential lipotoxicity. Interestingly, we also performed metabolic experiments on DGAT1 knockout and wild-type mice and no differences in fecal fat content were detected (data not shown) (47). These data suggest that upon chronic dosing with a DGAT1 inhibitor adaptations may occur that would allow for efficient lipid absorption and reversal of the malabsorption observed with a single dose. Additionally, PF-04620110 was administered to DGAT1 knockout mice, and fecal fat was not increased relative to placebo, implying that functional DGAT1 is required for PF-04620110 to exert its effects on dietary lipid malabsorption (data not shown). Contrary to rodents, humans that are homozygous for a splice variant on chromosome 8, which results in a deletion of exon 8 and consequentially a nonfunctional DGAT1 enzyme, exhibit hyperlipidemia and a diarrhea disorder (23). The most commonly reported adverse events in our human single-dose study were diarrhea, nausea, and vomiting at the highest two doses of PF-04620110. These gastrointestinal side effects have also been reported for other DGAT1 inhibitors in humans (17, 40). These contrasting data suggest the human intestine is more sensitive to a lack of functional DGAT1.

Mice treated with placebo during a lipid challenge containing radioactive triolein exhibited high radioactive counts in the upper third of the small intestine. However, in mice treated with PF-04620110, radioactive counts were detected more distal in the small intestine. When DGAT1 knockout mice are fed a high-fat diet, their enterocytes accumulate cytoplasmic lipid droplets that are TG rich (5), which are reduced by the introduction of functional DGAT1 in the intestine (38). In contrast following acute DGAT1 inhibition, we find decreased TG content in enterocytes of mice. It is also noteworthy that all lipid species are decreased with acute DGAT1 inhibition.

On the basis of the enzymatic activity of DGAT1, one would not anticipate all lipid species to be impacted within the enterocyte. One might anticipate that if we monitored earlier time points, we would detect DG and MG elevated with DGAT1 inhibition, whereas when we assessed lipid species at the 4 h time point the enterocyte shunted these accumulating species to other pathways to avoid lipotoxicity. Reduced fat uptake in the duodenum with altered kinetics of fat absorption has been observed in MGAT2 knockout mice (57), with speculation that delayed fat reaching the distal small intestine triggers gut-derived neuroendocrine responses promoting fat expenditure over storage (57). Causal reasoning engine analysis of transcriptomic changes in the jejunum from rats treated with PF-04620110 postulates that DGAT1 inhibition increases FA oxidation along with decreasing FA synthesis generating a satiety signal and reversal of high-fat diet induced insulin resistance (22, 36). It is likely that continued delay in lipid absorption and jejunum absorptive shift with DGAT1 inhibition could reduce fat storage and improve fat oxidation and metabolic inefficiency (57) combined with sustained incretin levels postprandially, resulting in increased insulin sensitivity.

The notion of a signal emanating from the impact of DGAT1 inhibition to improve whole body insulin sensitivity is supported by the compositional analysis of circulating TG in both our rats and humans with an enrichment in long chain-PUFA in the context of lower circulating TG. The exact molecular mechanism and target tissue driving altered composition of the TG pool with DGAT1 inhibition are unclear. Because the enrichments in long-chain FAs are not derived from the diet, we can potentially infer that enterocyte lipid metabolism is altered such that enzymatic activity associated with desaturation and elongation is upregulated, a mechanism downregulated with insulin resistance (53). Alternatively, the enrichment might be the result of substrate preference of enzymes by which the DGAT1 substrates are fluxing through in the face of inhibition of DGAT1 or preferential retention of the enriched FAs with DGAT1 inhibition. There is also clear evidence that low PUFA and high saturated fatty acid (SFA) in circulation can lead to increased risk of coronary artery disease, insulin resistance, dyslipidemia, and hypertension (15, 29, 33). In all the lipid classes analyzed postprandially, SFAs were decreased in both human and rat plasma. Also of note, n6 and n3 PUFA pathways metabolism are differentially altered downstream of DGAT1 inhibition. Typically, these two pathways are regulated similarly (24). Long-chain PUFAs reduce hepatic VLDL lipogenesis, enhance FA oxidation (possibly through PPAR, CD36, or GPR40, GPR119, and GPR120) and facilitate TG lipoprotein removal by increasing lipoprotein lipase activity (13, 20, 21, 25, 26, 29, 31, 52). This systematic lipidomic analysis in human plasma demonstrates that DGAT1 inhibition...
alters FA composition to increase circulating PUFA in the context of decreased postprandial TG levels. This acute reduction of newly synthesized TG at maximum postprandial levels has been associated with decreased cardiovascular risk in humans (3) and decreased diabetic risk in ob/ob mice (32).

The current therapies available for treatment of dyslipidemia, including statins, fibrates, niacin, bile acid sequestrants, and cholesterol absorption inhibitors, only modestly improve the lipid profile (39, 52, 54) while bariatric surgery remains the most effective and permanent intervention (49). Surgical reorganization of the gastrointestinal tract not only results in sustained weight loss but also changes the metabolic physiology beyond diet restriction and malabsorption, leading toward improved glucose homeostasis and insulin sensitivity (2, 43, 49). It is also well known that increased energy expenditure and fat utilization through neuroendocrine and nutrient signaling are enhanced with gastric bypass surgery (30, 34, 37, 43, 44). Thus the improvements in metabolic parameters following gastric bypass are attributed to a combination of physiological and physiological events that are initiated within the gastrointestinal tract. Pharmacological inhibition of DGAT1 in the intestine generates many signals that resemble the physiological sequelae of events following gastric bypass, exemplified by decreased food intake (22, 42a), increased incretin secretion (22, 42a), malabsorption, and alterations in temporal and spatial absorption of lipids. These collective results suggest that using DGAT1 inhibition could have the potential to be a pharmacological alternative to gastric bypass surgery. Our findings provide significant and new insights for the impact of pharmacological inhibition of DGAT1 and future longer term studies in patients with T2DM will inform the potential of this therapeutic intervention.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


REFERENCES


10. Cheng D, Iqbal J, Devenny J, Chu CH, Chen L, Dong J, Seethala R, Ahn SM, Pomp A, Rubino F. Impaired glucose secretion (22, 42a), malabsorption, and alterations in temporal and spatial absorption of lipids. These collective results suggest that using DGAT1 inhibition could have the potential to be a pharmacological alternative to gastric bypass surgery. Our findings provide significant and new insights for the impact of pharmacological inhibition of DGAT1 and future longer term studies in patients with T2DM will inform the potential of this therapeutic intervention.


