Impact of drug distribution into adipose on tissue function: The cholesteryl ester transfer protein (CETP) inhibitor anacetrapib as a test case

Douglas G. Johns1 | Sheng-Ping Wang2 | Raymond Rosa2 | James Hubert2 | Suoyu Xu3 | Ying Chen2 | Thomas Bateman3 | Robert O. Blaustein4

1Department of Translational Pharmacology, Merck & Co., Inc., Kenilworth, NJ, USA
2Department of Cardiometabolic Diseases/Atherosclerosis, Merck & Co., Inc., Kenilworth, NJ, USA
3Department of Pharmacokinetics, Pharmacodynamics and Drug Metabolism, Merck & Co., Inc., Kenilworth, NJ, USA
4Department of Clinical Research, Merck & Co., Inc., Kenilworth, NJ, USA

Correspondence
Douglas G. Johns, Merck & Co., Inc., PO Box 2000, 126 E. Lincoln Ave, Rahway, NJ 07065.
Email: douglas.johns@merck.com

Funding information
Merck, Sharp, & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA

Abstract
Anacetrapib is an inhibitor of cholesteryl ester transfer protein (CETP) previously under development as a lipid-modifying agent that reduces LDL-cholesterol and increases HDL-cholesterol in hypercholesterolemic patients. Anacetrapib demonstrates a long terminal half-life and accumulates in adipose tissue, which contributes to a long residence time of anacetrapib. Given our previous report that anacetrapib distributes into the lipid droplet of adipose tissue, we sought to understand whether anacetrapib affected adipose function, using a diet-induced obese (DIO) mouse model. Following 20 weeks of treatment with anacetrapib (100 mg/kg/day), levels of the drug increased to approximately 0.6 mmol/L in white adipose tissue. This level of anacetrapib was not associated with any impairment in adipose functionality as evidenced by a lack of any reduction in biomarkers of adipose functionality (plasma adiponectin, leptin, insulin; adipose adiponectin, leptin mRNA). In DIO wild-type (WT) mice treated with anacetrapib for 2 weeks and then subjected to 30% food restriction during washout to induce weight loss (18%) and fat mass loss (7%), levels of anacetrapib in adipose and plasma were not different between food restricted and ad lib-fed mice. These data indicate that despite deposition and long-term residence of ~0.6 mmol/L levels of anacetrapib in adipose tissue, adipose tissue function appears to be unaffected in mice. In addition, these data also indicate that even with severe caloric restriction and acute loss of fat mass, anacetrapib does not appear to be mobilized from the fat depot, thereby solidifying the role of adipose as a long-term storage site of anacetrapib.

KEYWORDS
adipose, anacetrapib, cholesteryl ester transfer protein, pharmacokinetics
Anacetrapib, a potent inhibitor of cholesteryl ester transfer protein (CETP), has been shown in several clinical studies to reduce LDL-cholesterol and elevate HDL-cholesterol in both normal healthy volunteers and in hypercholesterolemic patients at high risk of cardiovascular disease on a background of statin therapy.1,2 In a large phase 3 cardiovascular outcomes trial (REVEAL), anacetrapib treatment of patients with atherosclerotic vascular disease for a mean duration of 4 years was associated with a statistically significant relative reduction of ~9% in the primary composite outcome of heart attack, coronary revascularization, or death from coronary heart disease.3 However, despite this reduction in cardiovascular risk, a decision was made based on a comprehensive evaluation of the clinical profile of anacetrapib, not to proceed with regulatory filings (Merck & Co., Inc, Kenilworth, NJ, USA; press release, October 11, 2017).

The development of CETP inhibitors has been challenging and it is difficult to define a benefit-risk profile for this class. Although numerous CETP inhibitors have been tested in preclinical and early clinical studies, none has made it to market. Three CETP inhibitors (torcetrapib, dalcetrapib, and evacetrapib) were evaluated in Phase 3 trials which were terminated prematurely. Torcetrapib was the first of these to be tested clinically but its Phase 3 cardiovascular outcomes trial was stopped early because of an increase in mortality likely due to an off-target effect not seen with dalcetrapib and anacetrapib.4,5 The Phase 3 trial of dalcetrapib was halted early due to the lack of efficacy, which may have been related to its inability to substantially lower LDL-cholesterol.6 Although evacetrapib has robust effects on LDL- and HDL-cholesterol its Phase 3 trial was also terminated early due to the lack of efficacy.7 Given the minimal efficacy seen in REVEAL during the first 2 years of treatment, it is possible that the time point of the interim assessment of the evacetrapib trial was too early to see an effect. Finally, although anacetrapib reduced cardiovascular risk in the REVEAL trial without exhibiting a safety signal of concern, it has two properties that make its benefit-risk profile difficult to assess: (1) it has a long terminal half-life in plasma, and (2) it accumulates in adipose tissue and washes out very slowly.8,9

We previously reported that anacetrapib levels accumulate in adipose tissue of mice and remain relatively unchanged throughout a 35-week washout period.10 Recently, we reported that anacetrapib distributes into the lipid droplet of adipocyte tissue, via mechanisms independent of active transport or lipase activity.11 Given the prolonged presence of anacetrapib in the lipid droplet of adipocytes, and long elimination phase of anacetrapib from adipose, adipose tissue may represent a depot for anacetrapib. However, the effects of anacetrapib on adipose function have not been characterized. Furthermore, whether rapid loss in body weight or fat content could potentially mobilize anacetrapib from fat depots is unknown. The purpose of the studies described herein is to understand the effects of prolonged adipose accumulation of anacetrapib on adipose tissue function. Furthermore, we sought to understand the effect of weight loss on anacetrapib concentrations in adipose tissue, given the localization and retention of anacetrapib in the lipid droplet of adipocytes.

### 1 INTRODUCTION

### 2 MATERIALS AND METHODS

#### 2.1 Animals

All testing protocols described below were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the US National Institutes of Health, were approved by the Merck Research Laboratories Institutional Animals Care and Use Committee of Merck & Co., Inc., and adhered to the PHS policy on Human Care and Use of Laboratory Animals. Mice were maintained in a 12 hour/12 hour light-dark cycle with free access to food and water, in group housing conditions in a temperature controlled environment (22°C). Male wild-type (WT) C57BL/6J mice were used for these studies. Male mice were used to simplify statistical between-group comparisons, avoid potential error which could be introduced by gender, and to allow nonstatistically significant comparison with previous studies11,12 which also utilized male mice. It is acknowledged that female mice may display different biomarker responses to caloric restriction, and potentially could display differences in the tissue pharmacokinetics of anacetrapib, however, the study of gender differences in the endpoints measured in the current study will require future experimentation. At 6 weeks of age, mice were placed on a high-fat diet to induce obesity (Research Diets, D12492, 60% kcal from fat). Diet-induced obese (DIO) mice were 22-24 weeks of age at the beginning of treatment with anacetrapib. For studies to determine the effect of anacetrapib treatment on adipose functionality, mice were treated with 100 mg/kg anacetrapib, dosed in feed, or control DIO diet for 20 weeks. Epidydimal and retroperitoneal white adipose tissue (eWAT and rWAT, respectively) and blood were collected from N = 4 animals at weeks 1, 4, 8, 12, 14, 18, and 20, following overnight fasting. The tissues were inspected visually for the presence of any adherent connective tissue, which was carefully removed, and briefly washed in PBS before tissues were snap-frozen. Tissues and plasma were frozen in liquid nitrogen for analysis of anacetrapib concentrations and biomarkers of adipose functionality (see below). Anacetrapib concentrations in tissues and plasma were determined as described by Hartmann et al.13 In a separate cohort of DIO mice, following treatment with 100 mg/kg anacetrapib or control DIO diet for 20 weeks, N = 8 animals were treated with CL316243, a selective beta-3 adrenergic receptor agonist14 (5 mg/kg, intraperitoneal injection) to stimulate lipolysis or with vehicle (PBS). Six hours after treatment with CL316243, plasma was collected for the analysis of glycero and free fatty acids, as a measure of lipolysis.

For studies examining the effects of weight loss on anacetrapib levels in adipose and plasma, DIO mice were analyzed via NMR for baseline body composition determination, 3 days before treatment with anacetrapib. Mice were randomized by baseline body weight and fat mass obtained from NMR, such that each treatment group had equal mean starting body weight and fat mass. N = 30 DIO mice were treated with 100 mg/kg anacetrapib (oral gavage in 0.5% methylcellulose) for 2 weeks. Following 2 weeks of treatment with anacetrapib, mice were subjected to food restriction (FR; 30% reduction in normal...
food intake) or ad lib feeding for 28 days (N = 15 per group). Blood was collected on days 1, 7, 10, 14, and 28 following cessation of anacetrapib treatment (blood collected via tail nick on days 1 and 10) for isolation and snap-frozen of plasma. On days 7, 14, and 28 N = 5 animals were analyzed via NMR for total body fat and lean mass determination. Mice were then euthanized and blood was collected for isolation and snap-freezing of plasma for analysis. Epididymal and retroperitoneal white adipose tissue fat pads were also isolated, weighed, and snap-frozen for analysis of anacetrapib concentrations as described in Hartmann et al.11 Anacetrapib mass per fat pad was determined by normalizing the molar concentration of anacetrapib in adipose homogenates by the buffer volume (3 mL per g of tissue) and molecular weight of anacetrapib (637.523g/mol), and the tissue weight for each fat pad.

2.2 | Ex vivo assays of adipose functionality

Plasma adipokines adiponectin and leptin were measured using immunoassay kits (Meso Scale Discovery) per manufacturer’s instructions. Plasma insulin was measured using an immunoassay kit (AlphaLISA, Perkin Elmer) per manufacturer’s instructions. Plasma glycerol and free fatty acids were measured using assay kits for glycerol (Randox) and nonesterified fatty acid (NEFA-HR assay kit, Wako Diagnostics) per manufacturer’s instructions. Tissue mRNA expression in adipose tissue was performed using TaqMan RT-PCR. mRNA was isolated from adipose tissue using RNeasy mini kit (Qiagen, Germantown) per manufacturer’s instructions. 2 μg RNA was reverse transcribed using ABI high-capacity reaction kit and TaqMan PCR was performed using the primer/probe sequences for adiponectin, leptin, serum amyloid A3 (a tissue marker of adipose inflammation),14,15 and beta-actin as a control (Applied Biosystems/ThermoFisher primer/probe ID numbers: Mm00456425_m1, Mm00440181_m1, Mm00441203_m1, Mm00607939_s1, respectively).

2.3 | Statistics

For two group comparisons, Student’s t-test (two-tailed) was performed with P < .05 being considered significant.

FIGURE 1 (A) Concentration of anacetrapib in white adipose tissue and plasma during 20 weeks of dosing (eWAT: epididymal white adipose tissue; rWAT: retroperitoneal white adipose tissue), N = 4 per time point, with the exception of week 20, where N = 8; (B) plasma cholesterol and triglyceride levels following 20 weeks of dosing with anacetrapib (100 mg/kg dosed in feed), N = 8 per time point. Data points in both panels represent means ± SEM.
2.4 | Chemicals and compounds

Reagents were obtained from commercial sources as indicated. Anacetrapib was synthesized and prepared in the Medicinal Chemistry Department at Merck & Co., Inc. (Rahway, NJ, USA).

3 | RESULTS

3.1 | Anacetrapib accumulates in adipose tissues following 20 weeks of dosing

In a previous study, we reported that anacetrapib levels increase in white adipose tissue following 6 weeks of dosing.11 In this study, we increased the dosing duration to determine whether extended exposure of adipose tissue to anacetrapib would have any impact on adipose function. As shown in Figure 1, anacetrapib levels increased in adipose tissue up to approximately 600 μmol/L in epididymal and retroperitoneal white adipose (eWAT and rWAT, respectively) after 20 weeks of dosing. Plasma levels of anacetrapib appeared to plateau between weeks 4 and 8 of dosing (Figure 1A). Plasma total cholesterol, LDL-C, HDL-C, and triglycerides were also not affected by anacetrapib treatment (Figure 1B), however, it should be noted that these WT mice do not express CETP, and changes in lipoprotein-associated cholesterol or plasma triglycerides were not expected. Body weight was not different between anacetrapib- and vehicle-treated animals at baseline or at week 20 (data not shown).

3.2 | Anacetrapib treatment has no negative effect on plasma and tissue markers of adipose functionality

Plasma levels of fasting adiponectin, leptin, and insulin were not different between vehicle-treated controls at 20 weeks and baseline/
Baseline lipolysis, as well as beta-3 adrenergic-stimulated lipolysis. Glycerol levels, suggesting that anacetrapib treatment had no effect on NEFA and glycerol levels in the PBS groups between anacetrapib-treated animals and controls (Figure 3). The vehicle for CL316243 was PBS, and comparison of NEFA and glycerol levels were not different between anacetrapib-treated animals and controls (Figure 3B). CL316243-stimulated plasma NEFA and glycerol levels were not different between anacetrapib-treated and vehicle-treated controls (Figure 2A). Expression of mRNA for adiponectin, leptin, and the tissue biomarker for adipose inflammation SAA3 were not adversely affected by anacetrapib treatment in white adipose tissue (retroperitoneal and epididymal). As shown in Figure 2B, adiponectin mRNA was not different between anacetrapib- and vehicle-treated mice in inguinal and epididymal adipose, but was increased by approximately 50% in the retroperitoneal fat pad. Leptin and SAA3 mRNA were not significantly different between treatment groups in any fat pad examined.

### 3.3 Baseline and beta-adrenergic agonist-induced lipolysis is not affected by anacetrapib treatment

To determine whether the presence of anacetrapib in adipose tissue impacts the ability of adipose to undergo lipolysis, a group of mice was treated with anacetrapib or vehicle for 20 weeks, after which time, mice were treated with CL316243, a beta-3 adrenergic agonist, to stimulate lipolysis. Following 20 weeks of treatment with anacetrapib or vehicle, and after acute treatment with a beta-adrenergic agonist (CL316243, 5 mg/kg for 6 hours) plasma NEFA and glycerol levels were significantly increased (Figure 3B). CL316243-stimulated plasma NEFA and glycerol levels were not different between anacetrapib-treated animals and controls (Figure 3). The vehicle for CL316243 was PBS, and comparison of the NEFA and glycerol levels in the PBS groups between anacetrapib- and control diet-treated animals showed no difference in NEFA and glycerol levels, suggesting that anacetrapib treatment had no effect on baseline lipolysis, as well as beta-3 adrenergic-stimulated lipolysis.

### 3.4 Caloric restriction results in significant reduction in body weight and total fat mass, but has no effect on plasma and adipose levels of anacetrapib

Because anacetrapib resides in the lipid droplet of adipose tissue, a separate group of mice was treated with anacetrapib for 2 weeks, then subjected to caloric/food restriction (FR; placed on 70% of normal food intake) during the washout phase following dosing, to determine if rapid reduction in fat mass would promote egress of anacetrapib from adipose tissue. During the off-drug phase, body weight, total fat mass (NMR), and the weight of epididymal and retroperitoneal white adipose tissue fat pads were measured. Food restriction resulted in a body weight reduction of approximately 18%, and a change in fat mass (% of body weight) from 45% to 41%, with a reciprocal increase in lean mass (Figure 4). The difference in total fat mass between food restricted and ad lib-fed mice at day 28 was approximately 7% (FR 41.3 ± 1.2%, Ad Lib 48.5 ± 1.2%, P < .001).

Plasma levels of anacetrapib were not different between food restricted and ad lib-fed mice throughout the 28 day monitoring period (Figure 5). During food restriction, the weight of the epididymal fat pad was not different from that of ad lib-fed mice, however, retroperitoneal fat pad weight was significantly lower in food restricted mice compared to ad lib-fed mice (Figure 6). The difference in retroperitoneal fat pad weight between food restricted and ad lib-fed mice at day 28 was approximately 37% (retroperitoneal fat pad weight Ad lib 3.2 ± 0.1 g, FR 2.0 ± 0.2 g, P < .001). No difference was observed in the mass of anacetrapib present in either epididymal or retroperitoneal adipose between food restricted and ad lib-fed mice (Figure 6).

### 4 DISCUSSION

Anacetrapib displays a long terminal half-life, with low levels detected in the plasma of humans, years after dosing is complete. Anacetrapib distributes into and accumulates in adipose tissue of human subjects, and studies in mice showed that anacetrapib levels persist in white adipose tissue relatively unchanged for up to 35 weeks following the cessation of dosing. These observations could be explained by retention and very slow elimination of anacetrapib from adipose tissue.

Recently, we reported that anacetrapib distributes into the lipid droplet of adipocytes in vivo via mechanisms independent of active

![FIGURE 3](Image) Effect of anacetrapib on lipolysis. Following 20 weeks of dosing, levels of NEFA (left panel) and glycerol (right panel) were not different between anacetrapib-treated mice (solid bars) and vehicle-treated mice (open bars). CL316243 treatment resulted in a significant increase in plasma NEFA and glycerol in both anacetrapib-treated (vertical hatched bars) and control/vehicle-treated mice (diagonal hatched bars), but the increased levels of NEFA and glycerol were not significantly different (P > .05). Data points represent means ± SEM. ***P < .001 vs PBS vehicle
Because anacetrapib appears to reside in the lipid droplet at concentrations which are nearly 100-fold higher than is observed in plasma, and resides in the lipid droplet for an extremely long time with very slow egress, we sought to understand whether this phenomenon is associated with any alteration in adipose function. In the current study, the concentration of anacetrapib observed in white adipose tissue by 20 weeks of dosing was approximately 600 μmol/L. Previously, we reported the levels of anacetrapib in DIO WT mouse white adipose tissue (epididymal) in a similar range, approximately 400 μmol/L after 6 weeks of dosing. In this study, white adipose tissue concentrations of anacetrapib at 4 and 8 weeks were 131 μmol/L and 306 μmol/L, respectively, which are in a similar range as observed in Hartmann et al at similar time points. Furthermore, in Hartmann et al., in lean WT mice, anacetrapib levels were ~30-40 μmol/L in liver and <0.1 μmol/L in the brain, following 6 weeks of dosing. While plasma levels of anacetrapib appeared to plateau during the 20 week dosing period, levels of anacetrapib in adipose tissue appeared to continue to increase, so a true tissue steady state might not be reached even after 20 weeks of dosing in mice. Adipose tissue performs myriad functions and key biomarkers were selected to assess fundamental pathways. These included adiponectin and leptin (plasma levels and adipose tissue mRNA levels) as adipokines which regulate metabolic homeostasis, serum amyloid A3, which is a biomarker of murine adipose inflammation, and plasma glycerol and free fatty acids as markers of lipolysis. While numerous other biomarkers representing multiple pathways within adipose tissue can potentially be explored, the abovementioned biomarkers were selected to represent key functions of adipose. Despite anacetrapib being present at ~0.5 mmol/L concentrations by week 20, no adverse difference in the levels of adiponectin or leptin in plasma or tissue mRNA was observed, suggesting that there was no alteration in the ability of adipose tissue to produce these adipokines in DIO mice treated with anacetrapib. Adiponectin mRNA in adipose tissue was increased in retroperitoneal fat, but was not accompanied by a change in circulating plasma adiponectin levels. Whether this change in adiponectin mRNA translates to a change in transcribed/stored adiponectin protein within retroperitoneal tissue requires additional study. Fasting insulin levels were also no different, indicating in the DIO state, high concentrations of anacetrapib in adipose tissue were not impacting the ability of adipose tissue to regulate plasma insulin levels. SAA3 is a marker of adipose inflammation in mice and we saw no increase in this biomarker with anacetrapib treatment, compared to vehicle. Indeed, we observed lower
SAA3 mRNA levels in anacetrapib-treated animals compared to vehicle, raising the possibility that this may represent an anti-inflammatory effect. Because adipose tissue also regulates mobilization of fatty acids through lipolysis, we tested whether the markers of lipolysis (NEFA and glycerol) were affected by anacetrapib treatment. The lack of any significant difference in the markers of baseline or CL316243-stimulated lipolysis after 20 weeks of treatment suggests that the prolonged presence of high concentrations of anacetrapib in the lipid droplet of adipose does not affect the ability of adipocytes to mobilize fatty acids. Taken together, these data suggest that major functions of adipose tissue are not affected by the presence of 600 μmol/L anacetrapib.

An important caveat to the observations that the prolonged presence of anacetrapib in adipose tissue is not associated with any changes in the biomarkers of adipose function, is that these studies were conducted in WT mice, which do not express CETP. The purpose of these studies was to examine whether the high concentrations of anacetrapib impacted adipose function; however, the role of CETP inhibition, either in the plasma compartment or in adipose tissue itself, was not tested. The role of CETP expressed in adipose tissue is unclear. However, studies by Zhou et al, where human CETP is overexpressed in mouse adipose tissue via an adipose tissue-specific promoter, suggest that it could play a role in cholesterol homeostasis. Furthermore, changes in the plasma lipoprotein profile in response to CETP inhibition could theoretically impact the delivery of lipid to the adipose tissue and, ultimately, the fatty acid composition of the lipid droplet. The examination of the role of CETP biology in adipose function (ie, via inhibition with anacetrapib) was beyond the scope of the current set of experiments, but remains an important question. However, the results from the current study support the notion that the presence of ~0.5 mmol/L concentrations of anacetrapib in adipose tissue has no off-target impact on adipose function.

The observation that anacetrapib resides in the lipid droplet of adipose tissue at high concentrations gave rise to the question of whether adipose tissue represents a "depot" for anacetrapib. The data collected to date suggest that very slow egress of anacetrapib from adipose tissue might explain the very long terminal half-life in plasma years observed after discontinuation of treatment in humans. We sought to understand whether a rapid reduction in fat mass could accelerate the removal of anacetrapib from adipose tissue during a washout period in mice following treatment with anacetrapib for 2 weeks. This dosing/washout regimen was chosen so that the adipose tissue would not be overloaded with anacetrapib, and the reduction in fat mass was occurring during the washout period. It was believed that this might increase the chance of detecting anacetrapib egress from adipose tissue. The 30% reduction in food/calorie intake was associated with a ~18% reduction in body weight and a ~7% lower total fat mass in food restricted mice vs ad lib-fed DIO mice. The epididymal fat pads were selected since previous studies examining anacetrapib deposition into adipose tissue utilized this fat pad to assess anacetrapib concentrations in white adipose tissue, however, this fat pad weight did not change in response to food restriction. The retroperitoneal fat pad was studied, since previous studies examining various mechanisms of weight loss, including caloric restriction, elicited a reduction in retroperitoneal fat pad weight. In response to food restriction, the retroperitoneal fat pad weight was 37% lower than that of ad lib-fed mice. Interestingly, despite this difference in retroperitoneal fat mass, no changes were observed in the plasma concentration of anacetrapib, or in the mass of anacetrapib in adipose tissue, where the loss of fat mass was likely balanced by an increase in anacetrapib concentration, such that the total amount of anacetrapib in the fat pad remained unchanged. Fat mass loss in response to caloric restriction is associated with a shrinkage of the lipid droplet, but not a reduction in adipocyte

**FIGURE 6** Effect of food restriction on eWAT and rWAT weight and tissue levels of anacetrapib. N = 30 DIO mice were placed on food restriction (N = 15) or ad lib feeding (N = 15) for 28 days, and N = 5 animals per group were euthanized for tissue collection on days 7, 14, and 28 as described in Methods. Anacetrapib mass was calculated as described in Methods. Data points represent means ± SEM. ***P < .001 vs ad lib-fed group.
cell number (Kosteli et al.), so the lack of an effect of fat mass loss on anacetrapib levels in adipose or plasma suggests that the lipid droplet has a large capacity for collecting and retaining anacetrapib. Examination of the retroperitoneal and epididymal fat also enabled the reproducible quantitation of fat mass (ie, each depot could be completely removed, weighed, and analyzed). Importantly, adipose tissue from other regions was not studied, and it cannot be ruled out that the pharmacokinetic phenomena described in the current study might be different in other fatty regions.

The phenomenon of anacetrapib distributing into and retention/slow egress from adipose tissue is thought to be driven primarily by the high lipophilicity of anacetrapib (LogD 7.1), and the neutrality of the molecule.12 The specific molecular interactions responsible for such a strong attraction and retention of anacetrapib have not been identified and require further study, however, the tissue distribution and pharmacokinetic observations of anacetrapib across preclinical and clinical studies may provide clues to help understand other molecules which display a depot effect in adipose tissue.

The results from the current study support the large body of non-clinical and clinical data which indicate that long-term treatment with anacetrapib is safe and well tolerated. Looking more closely at adipose tissue, the site where anacetrapib has been shown to be preferentially distributed and retained, our results also support the notion that the accumulation of anacetrapib in the lipid droplet of adipose tissue is not associated with significant alteration in adipose function in DIO wild-type mice. The lack of effect of rapid weight loss on anacetrapib levels in adipose tissue and plasma suggests that the driving force for retaining anacetrapib in adipose tissue is quite strong, and resistant to the influence of rapid mobilization of lipid stores from adipose.

CONFLICT OF INTEREST
At the time of completion of the manuscript, all authors were employees of Merck, Sharpe & Dohme, Corp., a subsidiary of Merck & Co., Inc, Kenilworth, NJ USA and potentially own stock and/or hold stock options in Merck & Co., Inc, Kenilworth, NJ USA.

AUTHORS CONTRIBUTIONS
Johns, Wang, Rosa, Hubert, and Chen participated in research design. Wang, Rosa, Hubert, Xu, and Chen conducted experiments. Xu contributed new reagents or analytic tools. Johns, Wang, Rosa, Hubert, Xu, and Chen performed data analysis. Johns, Bateman, and Blaustein wrote or contributed to the writing of the manuscript.

ORCID
Douglas G. Johns https://orcid.org/0000-0002-8726-8514

REFERENCES
1. Bloomfield D, Carlson GL, Sapre A, et al. Efficacy and safety of the cholesteryl ester transfer protein inhibitor anacetrapib as monotherapy and coadministered with atorvastatin in dyslipidemic patients. Am Heart J. 2009;157:352-360.
2. Cannon CP, Shah S, Dansky HM, et al.; Determining the Efficacy and Tolerability Investigators. Safety of anacetrapib in patients with or at high risk for coronary heart disease. N Engl J Med. 2017;363:2406-2415.
3. Bowman L, Hopewell JC, Chen F, et al.; HP53/TIMI55-REVEAL Collaborative Group. Effects of anacetrapib in patients with atherosclerotic vascular disease. N Engl J Med. 2017;377:1217-1227.
4. Barter PJ, Caulfield M, Eriksson M, et al. Effects of torcetrapib in patients at high risk for coronary events. N Engl J Med. 2007;357:2109-2122.
5. Johns DG, Duffy J, Fisher T, Hubbard BK, Forrest MJ. On- and off-target pharmacology of torcetrapib: current understanding and implications for the structure activity relationships (SAR), discovery and development of cholesteryl ester transfer protein (CETP) inhibitors. Drugs. 2012;72:491-507.
6. Schwartz GG, Olsson AG, Abt M, et al. Effects of dalcetrapib in patients with a recent acute coronary syndrome. N Engl J Med. 2012;367:2089-2099.
7. Lincoff AM, Nicholls SJ, Riesmeyer JS, et al. Evacetrapib and cardiovascular outcomes in high-risk vascular disease. N Engl J Med. 2017;376:1933-1942.
8. Gotto AM Jr, Cannon CP, Li KS, et al.; DEFINE Investigators. Evaluation of lipids, drug concentration, and safety parameters following cessation of treatment with the cholesteryl ester transfer protein inhibitor anacetrapib in patients with or at high risk for coronary heart disease. Am J Cardiol. 2014;113:76-83.
9. Kosteli A, Sugaru E, Haemmerle G, et al. Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue. J Clin Invest. 2010;120(10):3466-3479.
10. Krishna R, Gheyas F, Liu Y, et al. Chronic administration of anacetrapib is associated with accumulation in adipose and slow elimination. Clin Pharmacol Ther. 2017;102:832-840.
11. Hartmann G, Kumar S, Johns D, et al. Disposition into adipose tissue determines accumulation and elimination kinetics of the cholesteryl ester transfer protein inhibitor anacetrapib in mice. Drug Metab Dispos. 2016;44:428-434.
12. Johns DG, LeVoci L, Krsmanovic M, et al. Characterization of anacetrapib distribution into the lipid droplet of adipose tissue in mice and human cultured adipocytes. Drug Metab Dispos. 2019;7:227-233.
13. Bloom JD, Dutia MD, Johnson BD, et al. Disodium (R, R)-5-[2-[2-(3-chlorophenyl)-2-hydroxyethyl]-amino]propyl]-1,3-benzodioxole-2,2-dicarboxylate (CL 316,243). A potent beta-adrenergic agonist virtually specific for beta 3 receptors. A promising anti diabetic and antiobesity agent. J Med Chem. 1992;35:3081-3084.
14. Scheja L, Heese B, Zitzer H, et al. Acute-phase serum amyloid A as a marker of insulin resistance in mice. Exp Diabetes Res. 2008;2008:230837.
15. Wormstedt Asertholm I, Tao C, Morley TS, et al. Adipocyte inflammation is essential for healthy adipose tissue expansion and remodeling. Cell Metab. 2014;20:103-118.
16. Zhou H, Li Z, Hojjati MR, et al. Adipose tissue-specific CETP expression in mice: impact on plasma lipoprotein metabolism. J Lipid Res. 2006;47:2011-2019.

How to cite this article: Johns DG, Wang S-P, Rosa R, et al. Impact of drug distribution into adipose tissue on function: The cholesteryl ester transfer protein (CETP) inhibitor anacetrapib as a test case. Pharmacol Res Perspect. 2019:e00543. https://doi.org/10.1002/prp2.543