CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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<u>NON-CLINICAL REVIEW(S)</u>

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

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Product:	MOUNJARO (Tirzepatide/LY3298176)
Indication:	Adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus
Applicant:	Eli Lilly and Co
Review Division:	Division of Diabetes, Lipid Disorders, and Obesity
Reviewer:	Elena Braithwaite
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1 Executive Summary

1.1 Introduction

Mounjaro (tirzepatide, LY3298176) was developed by Eli Lilly and Co as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus (T2DM).

1.2 Brief Discussion of Nonclinical Findings

LY3298176 is a 39 amino acid peptide agonist of the glucose-dependent insulinotropic polypeptide receptor (GIPR) and glucagon-like peptide 1 receptor (GLP-1R), which demonstrated binding to both receptors in humans and nonclinical species and minimal to no agonist activity at the glucagon receptor (GCGR) or the glucagon-like peptide 2 receptor (GLP-2R). In rats fed a high fat/high sucrose diet and diet induced obese (DIO) mice, LY3298176 caused decreases in food intake and body weight, and improved glucose tolerance. Subcutaneously administered LY3298176 also reduced fat mass, fat-free mass, plasma cholesterol, and increased metabolic rates to a greater extent when compared a GLP-1 receptor agonist in DIO mice. The contribution of both GIPR and GLP-1 in regulation of glucose tolerance was demonstrated in GIPR null mice and GLP-1R null mice. LY3298176 improved glucose tolerance in the GIPR and GLP-1R null mice and enhanced glucose-dependent insulin secretion in islets isolated from these mice. Additionally, LY3298176 stimulated cAMP production and lipolysis in mature adipocytes that express the GIPR and not the GLP-1R. Due to the dual function of the LY3298176 peptide, two established pharmacological class designations were selected to describe the pharmacologic activities, namely ‘glucose-dependent insulinotropic polypeptide receptor agonist’ and ‘glucagon-like peptide-1 receptor agonist’.

The peptide is tethered to a long chain acyl moiety to promote binding to albumin and increase plasma half-life. Consistent with its ability to bind albumin, LY3298176 was highly protein bound in monkey, rat, and human plasma and preferentially distributed to the plasma compartment of whole blood. Small amounts were found to cross the blood brain barrier in quantitative whole body autoradiography studies in male rats. The primary metabolic pathway in rats, monkeys, and humans, consists of proteolytic cleavage of the peptide backbone, β-oxidation of the C20-fatty diacid moiety and amide hydrolysis. No parent drug was found in the urine or feces in any species.

Like other peptide agonists of GLP-1R, subcutaneously administered LY3298176 produced increased heart rates in male monkeys at clinically relevant exposures after single and repeat dose studies (up to 6 months). LY3298176 caused an increase in blood pressure only in a single dose study. No LY3298176-related effect on QTc interval occurred in monkeys with single or repeated dosing.

The toxicity profile of LY3298176 was evaluated after repeated dosing in rats and monkeys. Most findings in rats and monkeys were consistent with those observed with the GLP-1R agonist class of pharmaceuticals and have been observed previously with peptide-based GIPR agonists in DIO mice (decreased food consumption and reduced body weight/body weight gain) (Mroz et al. 2018). In rats, minimal to slight thyroid C-cell hyperplasia occurred at clinical exposures and are findings that have been previously observed with the GLP-1R agonist class of pharmaceuticals. C-cell hyperplasia observed in the 6-month study progressed to thyroid neoplasms in the 2-year rat carcinogenicity study. In monkeys, body weight loss was dose limiting at clinically relevant exposures, suggesting that co-agonism at GLP-1R and GIPR contributed to the overall magnitude of the food consumption and body weight responses observed compared to those typically observed with GLP-1R agonists. It should be noted that excessive LY3298176-dependent weight loss is due to exaggerated pharmacology occurring in non-diabetic, non-obese, healthy animals.

The potential of LY3298176 to induce tumors during long term clinical use was assessed in a 2-year rat carcinogenicity study and a 6-month transgenic RasH2 mouse study. Consistent with the results of carcinogenicity studies with other GLP-1R agonists in rodents in 2-year studies, LY3298176 caused C-cell tumors in the rat carcinogenicity study at clinical exposures. A statistically significant increase in thyroid C-cell adenomas was observed in males and females, and a statistically significant increase in thyroid C-cell adenomas and carcinomas combined was observed in males and females at all doses examined. Due to species-specific differences in GLP-1R expression and activation, the relevance of these findings to humans is unclear. While no association with GLP-1R agonism and clinical incidence of C-cell tumors has been established to date, a boxed warning has been included in labeling, noting a potential risk for thyroid C-cell tumors, which is consistent with long-acting GLP-1R agonist class labeling. No LY3298176-related neoplastic findings occurred in RasH2 transgenic mice after twice weekly dosing for 6 months. While GLP-1R agonists typically induce C-cell tumors in wild type mice with lifetime dosing, this result is consistent with that of dulaglutide, the other GLP-1R agonist tested in the transgenic rasH2 mouse model.

LY3298176 was tested in a comprehensive battery of assessments in rats and rabbits to evaluate all stages of reproduction and development. To evaluate the safety of treatment from mating to implantation, a combined fertility and embryonic development study was conducted in rats. No LY3298176 effects were observed on male fertility. At clinically relevant exposures, an increased number of female rats experienced prolonged estrous cycles or persistent/prolonged diestrus at all doses examined, and decreases in the numbers of corpora lutea, implantation sites, and viable embryos, which was likely secondary to the pharmacodynamic-related effects on food consumption and decreased body weight gain related to LY3298176. An embryo-fetal development study was conducted to evaluate effects of treatment on pregnant females and the development of the embryo and fetus during organogenesis. As expected from the pharmacodynamic activity of LY3298176, embryo-fetal development studies showed findings related to reduced food consumption and body weight gain in both pregnant rats and rabbits at clinically relevant exposures. Reductions in food consumption and body weight gain are known to be associated with reduced/delayed ossification and/or reduced fetal weight in rats (Fleeman et al. 2005) and fetal loss/abortion in rabbits

(Matsuzawa et al. 1981). However, several of the findings in rats that occurred at clinically relevant exposures could not be directly attributed to reduced maternal body weight, including increases in the incidences of external, visceral, and skeletal malformations and variations in rat fetuses that exceeded concurrent and historical control incidences (litter %) and generally occurred across different litters. Uncertainty regarding the clinical relevance of these findings can be adequately communicated in product labeling. Notably, potentially drug-related imbalances in malformations have been seen in other GLP-1R agonist reproduction studies, which suggests that inclusion of these findings in the risk summary section of pregnancy labeling is warranted. In a pre- and postnatal development study in rats to evaluate effects in the period from implantation through weaning, lower mean body weights in F1 pups from dams treated with LY3298176 were likely due to the pharmacodynamic reductions in food consumption and body weight in the maternal animals at clinically relevant exposures that persisted throughout lactation. The reduction in pup weight was considered adverse at the highest dose and weight reductions were observed from birth or soon thereafter through postnatal day 91.

1.3 Recommendations

1.3.1 Approvability

The nonclinical data support market approval of LY3298176 as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus.

1.3.3 Labeling

Changes recommended by nonclinical are indicated in red.

(b) (4)

1 Page of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

(b) (4)
[redacted]

2 Drug Information

2.1 Drug

CAS Registry Number
2023788-19-2

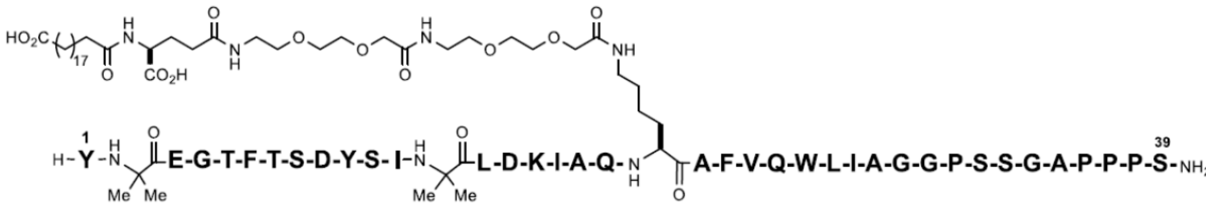
Generic Name
Tirzepatide

Code Name
AD-60212, ALN-60212, ALN-PCSSC, PCSSC

Chemical Name
L-Serinamide, L-tyrosyl-2-methylalanyl-L-α-glutamylglycyl-L-threonyl-Lphenylalanyl-L-threonyl-L-seryl-L-α-aspartyl-L-tyrosyl-L-seryl-L-isoleucyl-2-methylalanyl-L-leucyl-L-α-aspartyl-L-lysyl-L-isoleucyl-L-alanyl-L-glutaminyl-*N*6-[(22*S*)-22,42-dicarboxy-1,10,19,24-tetraoxo-3,6,12,15-tetraoxa-9,18,23-triazadotetracont-1-yl]-Llysyl-L-alanyl-L-phenylalanyl-L-valyl-L-glutaminyl-L-tryptophyl-L-leucyl-L-isoleucyl-Lalanylglycylglycyl-L-prolyl-L-seryl-L-serylglycyl-L-alanyl-L-prolyl-L-prolyl-L-prolyl

Molecular Formula/Molecular Weight
$C_{225}H_{348}N_{48}O_{68}$
4810.52 Da (monoisotopic mass)
4813.45 Da (average mass IUPAC 2007)

Structure or Biochemical Description



LY3298176 is a 39-amino acid synthetic peptide. It consists of a peptide component based on the GIP sequence containing 2 non-coded amino acids (aminoisobutyric acid, Aib) in positions 2 and 13, a C-terminal amide, and the Lys residue at position 20 is attached to a 1,20-eicosanedioic acid via a linker which consists of a γ-Glu and two 8-amino-3,6-dioxaoctanoic acids. The secondary structure of LY3298176 is predominantly α-helical, the tertiary structure is consistent with a natively folded peptide, and LY3298176 reversibly self-associates with a monomer-trimerhexamer equilibrium under native conditions.

Pharmacologic Class
Glucose-dependent insulinotropic polypeptide receptor agonist and glucagon-like peptide 1 receptor agonist

2.3 Drug Formulation

Table 1: Composition of Tirzepatide (LY3298176) Injection

Ingredient	Quantity (mg) per Syringe						Function	Reference to Standards
	2.5 mg/0.5 mL	5 mg/0.5 mL	7.5 mg/0.5 mL	10 mg/0.5 mL	12.5 mg/0.5 mL	15 mg/0.5 mL		
Active Ingredient								
Tirzepatide	2.5	5	7.5	10	12.5	15	Active ingredient	See Section 3.2.S.4.1, Specification
Other Ingredients								
Dibasic Sodium Phosphate Heptahydrate	[redacted] (b) (4)							USP-NF
Sodium Chloride	[redacted]							USP-NF, Ph.Eur.
Hydrochloric Acid Solution, [redacted] (b) (4)	[redacted]							USP-NF, Ph.Eur.
Sodium Hydroxide Solution [redacted] (b) (4)	[redacted]							USP-NF, Ph.Eur.
Water for Injection	[redacted]							USP-NF, Ph.Eur.

Abbreviations: Ph.Eur. = European Pharmacopoeia; q.s. = quantity sufficient; USP-NF = United States Pharmacopoeia National Formulary

Table copied from the Applicant's submission (**Table 3.2.P.1.2-1**).

2.4 Comments on Novel Excipients

There are no novel excipients in the tirzepatide (LY3298176) formulation.

2.5 Comments on Impurities/Degradants of Concern

Two repeat dose toxicity studies (2 weeks in duration) were conducted in rats to qualify impurities, primarily degradation products, that were detected in clinical batches but were not present in toxicology lots at similar levels in pivotal repeat dose toxicity studies [redacted] (b) (4) These impurities were evaluated by administering 3 mg/kg of Toxicology Batches C224435-2020-0004 (Study # 8420235) or C224435-2018-0018 (Study # 8394210) to rats by twice weekly subcutaneous injection for two weeks. Findings observed in these "impurity qualification" studies were similar to those seen in previously conducted toxicology studies suggesting that these impurities do not affect the pharmacodynamic activity of LY3298176.

Table 2: Exposure Multiples for Impurities of Concern

(b) (4) [redacted]

Based on 60 kg individual and a maximum recommended human dose of 15 mg/week. [2] LY3298176 (3 mg/kg) was administered twice weekly (5 total doses) by subcutaneous injection to Sprague Dawley rats.

Two extractables [redacted] (b) (4) were identified at levels above the FDA-calculated analytical evaluation threshold (b) (4) [redacted] These extractables are not predicted to be genotoxic and are subject to a limit of (b) (4) [redacted] µg/day based on the safety concern threshold. Therefore, no extractables or leachables of toxicological significance were identified.

2.6 Proposed Clinical Population and Dosing Regimen

Patients with type 2 diabetes mellitus (≥18 years of age) should start with a 2.5 mg subcutaneous injection once weekly. After 4 weeks, the dose should be increased to 5 mg once weekly. If needed to achieve individual treatment goals, dose increases can be made in 2.5 mg increments after a minimum of 4 weeks on the current dose up to a maximum dose of 15 mg once weekly.

2.7 Regulatory Background

On September 15, 2021, Eli Lilly and Co. submitted an original new drug application (NDA) for tirzepatide (also known as Mounjaro or LY3298176) for use as an adjunct to diet and exercise to improve glycemic control in adults with Type 2 Diabetes Mellitus (T2DM). Eli Lilly submitted a priority review voucher for this application.

4 Pharmacology

4.1 Primary Pharmacology

Ligand-mediated activation of GLP-1 or GIP receptors, both members of the classical G-protein coupled receptor (GPCR) family, allows Gs-alpha to function as a guanine nucleotide exchange factor that catalyzes the release of guanosine diphosphate (GDP) from Gs-alpha. Activation and engagement of G protein coupled receptors results in recruitment of multiple intracellular proteins (e.g., β-arrestin, adenylyl cyclase) that activate distinct signaling pathways, facilitate incorporation of adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP), promote receptor internalization (sequestration)/recycling/downregulation, and enhance glucose-dependent insulin secretion. While receptor internalization is important for signaling, both plasma-membrane-localized and internalized receptors have been implicated in signaling (Fletcher MM et al. 2018).

In vitro Studies

The peptide component of LY3298176 is based on the GIP sequence, which is attached to a 1,20-eicosanedioic acid moiety attached via a linker. The long-chain acyl moiety promotes albumin binding and reduces target receptor binding.

LY3298176 was evaluated in a series of in vitro studies demonstrating its ability to bind GLP-1 and GIP receptors across species and activate second messenger pathways that resulted in accumulation of intracellular cAMP, recruitment of guanosine triphosphate (GTP) and β-arrestin-2 and receptor internalization. The contribution of the GLP-1 and GIP receptors, individually, to the functional agonist activity of LY3298176 and its ability to promote glucose tolerance was demonstrated by LY3298176-mediated glucose-dependent insulin secretion in pancreatic islets isolated from both GIPR and GLP-1R null mice. The ability of LY3298176 to stimulate cAMP and lipolysis via GIPR activity was also shown using human adipocytes that express GIPR but not GLP-1R.

The Applicant examined LY3298176's ability to bind cloned GLP-1R and GIPR. In vitro radioligand binding assays in the presence of 0.1% bovine serum albumin indicated that LY3298176 had a higher affinity for the human and monkey GIPR when compared to the GLP-1R. But the reverse was true in rodents where LY3298176 had a higher affinity for the rat and mouse GLP-1R when compared to the GIPR. In the absence of albumin, the binding affinity of LY3298176 for cloned GIPR and GLP-1R were higher when compared to studies where albumin was present indicating that albumin binding reduces LY3298176 binding to both GLP-1 and GIP receptors. Overall, LY3298176 demonstrated more potent binding affinity to human GIPR when compared to human GLP-1R both in the absence and presence of albumin. But LY3298176 binding to these receptors was less potent than the native ligands GLP-1 and GIP at their respective receptors in the presence of albumin.

Table 3: In Vitro Receptor Binding Affinity of LY3298176 for Cloned Human, Monkey, Rat, and Mouse GIP and GLP-1 Receptors

Receptor	Presence of 0.1% BSA	Receptor Binding Affinity, Ki, nM (SEM, n)			
		Human	Monkey	Rat	Mouse
GIPR	+	4.02 (0.37, n = 6)	1.48 (0.38, n = 6)	386 (69, n = 7)	646 (138, n = 7)
	-	0.37 (0.04, n = 3)	0.32 (0.05, n = 3)	--	--
GLP-1R	+	378 (52, n = 7)	391 (60, n = 7)	129 (13, n = 7)	88.4 (5.2, n = 7)
	-	2.88 (0.31, n = 5)	5.08 (0.41, n = 4)	1.40 (0.37, n = 4)	0.84 (0.06, n = 4)

Table prepared by Patricia Brundage. Studies conducted in the absence of BSA contained 0.1% Bacitracin for non-specific blocking to allow affinity determination.

To evaluate the functional efficacy and potency of LY3298176, a series of in vitro assays were conducted to evaluate LY3298176's ability to promote GTP and β-arrestin recruitment, induce cAMP, and influence receptor internalization.

Recruitment of guanosine triphosphate to the stimulatory G-protein subunit Gs-alpha of the GLP-1 and GIP receptors was evaluated by measuring functional recruitment of non-hydrolyzable [^{35}S]-GTPγS to the stimulatory Gs-alpha subunit protein using preparations of purified membranes from HEK293 cells stably over expressing either recombinant human GLP-1R or human GIPR and antibody-capture scintillation proximity assay methodology. EC_{50} values for LY3298176 at the GLP-1R (0.617 nM) and GIPR (0.379 nM) were 2.6- and 3.8-fold lower than EC_{50} values, respectively, for positive control ligand peptides.

To investigate the ability of LY3298176 to facilitate recruitment of β-arrestin-2, CHO cells expressing Pro-Link-tagged receptors and enzyme-acceptor-tagged β-arrestin-2 were incubated with positive control peptides or LY3298176. Interactions between receptors and β-arrestin-2 were measured by luminescence. LY3298176 was not successful at promoting the interaction between the GLP-1R and β-arrestin-2 (EC_{50} > 10,500 μM); but detectable interactions were observed between the GIPR and β-arrestin-2 (EC_{50} = 2.3 nM).

To examine agonist-mediated receptor internalization, HEK293 cells stably expressing human GLP-1R and GIPR were tagged at the N-terminus with 3X-HA (human hemagglutinin) and the C-terminus with enhanced green fluorescent protein. Changes in surface receptor expression were examined following treatment with positive control native ligand or LY3298176. LY3298176 caused intracellular translocation of both GLP-1R (EC_{50} = 101.9 nM and E_{max} of 43.6%) and GIPR (EC_{50} = 18.1 nM and E_{max} of 102.7%) that was confirmed by confocal microscopy.

In HEK cell lines, cellular cAMP assays revealed that LY3298176 stimulated intracellular cAMP production in cells expressing the GIPR and GLP-1R. cAMP induction was most robust in cell lines expressing GIPR when compared to cells expressing GLP-1R. LY3298176-mediated cAMP induction appeared to be specific to the GLP-1 and GIP receptors as cells expressing low levels of the glucagon receptor (GCGR) or GLP-2 receptor (GLP-2R) showed very weak induction, or no induction in the presence of albumin.

Several in vitro studies were conducted to demonstrate the individual contribution of each receptor to the functional agonist activity of LY3298176. Differentiated, mature human adipocytes (which endogenously express the GIPR but not the GLP-1R) treated with either LY3298176 (EC_{50} = 0.119 nM) or native human GIP (LSN2838382) (EC_{50} = 5.37 nM) were able to stimulate cAMP production (as measured by a homogeneous time resolved fluorescence or HTRF cAMP assay kit that detects changes in cAMP accumulation in response to Gs coupled GPCR activation or inhibition) in the absence of albumin. When free glycerol was measured as a surrogate for lipolysis, LY3298176 (EC_{50} = 0.0118 nM) and LSN838382 (EC_{50} = 0.24 nM) stimulated production of free glycerol in adipocytes as a result of GIPR activation.

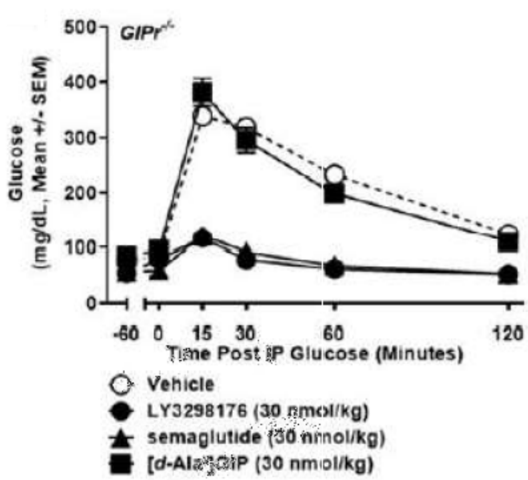
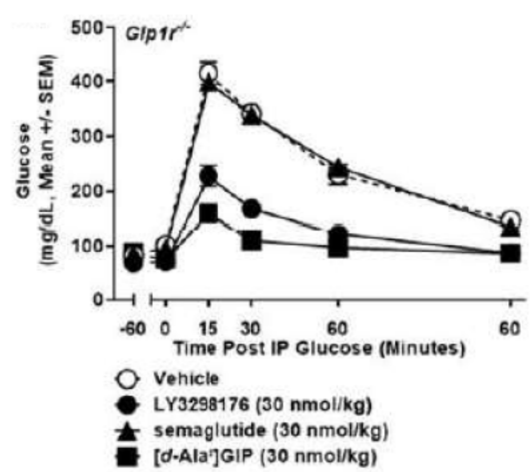
When pancreatic islets isolated from wild-type, GIPR null or GLP-1R null mice were treated with glucose and 0.1% BSA supplementation in the absence or presence of increasing levels of LY3298176 (10-1000 nM), LY3298176 dose-dependently enhanced insulin secretion from wild-type, GIPR knockout and GLP-1R knockout mouse pancreatic islets with EC_{50} values of 32 nM, 69 nM and 244 nM, respectively.

<u>In vivo Studies</u>
LY3298176 was evaluated in normal rats and diabetic animal models of disease to demonstrate its role in reducing body weight, decreasing food consumption, improving glucose tolerance, and promoting deoxyglucose uptake in muscle and adipose tissue that was not mediated by weight loss alone. In diet induced obese (DIO) mice, LY3298176 also appears to be more potent than semaglutide at decreasing fat mass and increasing metabolic rates. Its ability to promote insulin sensitivity may involve increased metabolism of branched chain amino acids (BCAAs) in brown adipose tissue (BAT). GLP-1R or GIPR null mice were also evaluated to unveil the role of both the GLP-1 and GIP receptors in LY3298176-mediated glucose tolerance. The role GLP-1R and GIPR individually play in LY3298176-mediated reductions in body weight/food consumption/fat mass and increases in metabolic rate was not established in the in vivo studies.

Normal male Wistar rats subcutaneously administered a single dose of LY3298176 (0.1, 0.3, 1, 3, and 10 nmol/kg) experienced dose-dependent increases in insulin secretion following an intravenous glucose injection that reached statistical significance at ≥1 nmol/kg with an ED_{50} of 0.87 nmol/kg. Similarly, wild-type, normal, fasted C57BL/6 mice subcutaneously administered LY3298176 (0.01, 0.1, 1, 10, or 100 nmol/kg) experienced statistically significant decreases in glucose AUC at doses ≥ 0.1 nmol/kg LY3298176 that resulted in dose-dependent improvements in glucose tolerance as determined by an intraperitoneal glucose tolerance test (ipGTT).

Subcutaneously administered LY3298176 (30 nmol/kg) dosed 17 to 18 hours prior to glucose challenge produced improvements in glucose tolerance in both GIPR and GLP-1R null mice as measured by a statistically significant decrease in blood glucose AUC levels. To further investigate the roles that GIPR and GLP-1R play individually in glucose tolerance, LY3298176 exposure in null background mice was combined with exposure to GLP-1R and GIPR analogs. In GIPR null mice, LY3298176 and the GLP-1R agonist semaglutide (30 nmol/kg) individually decreased blood glucose AUC to similar levels; but subcutaneous dosing of the DPP4-resistant GIP analogue [d-Ala^2] GIP did not improve glucose tolerance in GIP null mice. Additionally, GLP-1R null mice treated with LY3298176 or [d-Ala^2] GIP (30 nmol/kg) experienced similar decreases in blood glucose AUC, while subcutaneous semaglutide (30 nmol/kg) exposure did not improve glucose tolerance in GLP-1R null mice. Finally, the role of GIPR and GLP-1R was investigated in wild-type mice. When LY3298176 or semaglutide (30 nmol/kg) was administered alone by subcutaneous injection, decreases in blood glucose levels were observed. However, when LY3298176 or semaglutide (30 nmol/kg) was co-administered with the GLP-1R antagonist Jant-4 (1000 nmol/kg, dose intraperitoneally), Jant-4 blocked semaglutide's ability to reduce blood glucose levels but only partially impacted LY3298176's ability to reduce blood glucose levels. These studies indicate that LY3298176 improves glucose tolerance through a mechanism mediated by both GIP and GLP-1 receptors in mice.

Figure 1: LY3298176 improves glucose tolerance in an ipGTT in the absence of the GIP receptor and in the absence of the GLP-1 receptor

GIP receptor null mice	GLP-1 receptor null mice
	

Wild-type mice

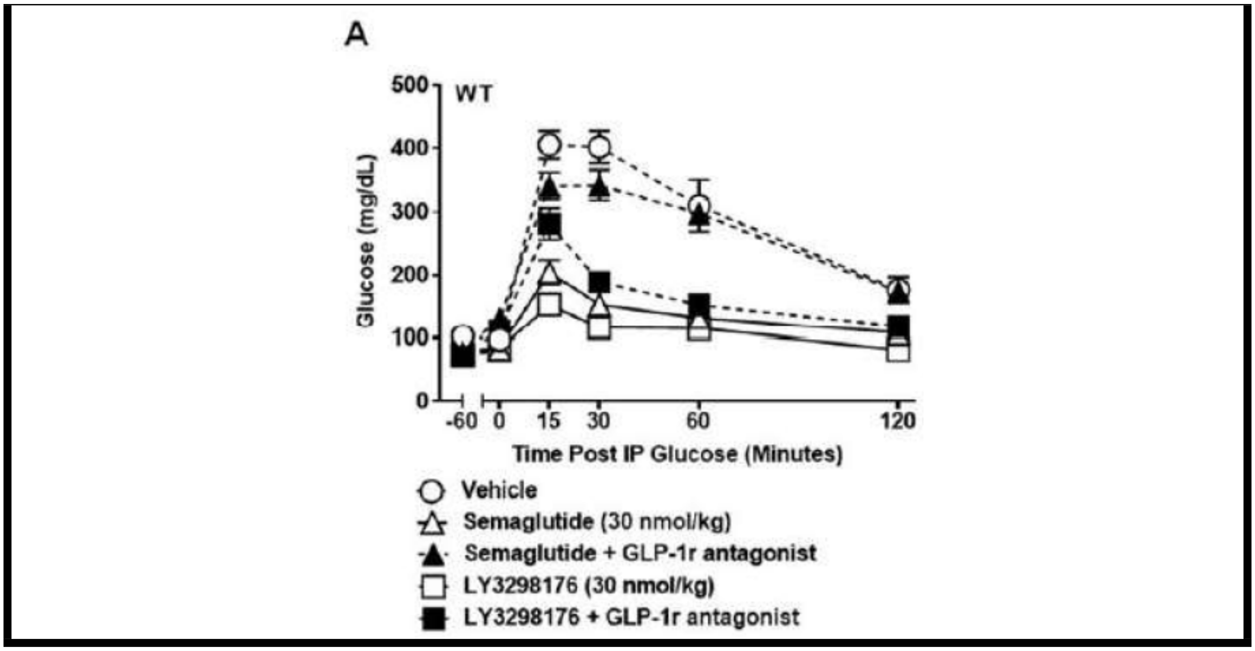


Figure modified from the Applicant's submission (Figures 2, 3, and 4). Blood glucose levels from 0 to 120 minutes post glucose dose challenge in GIP receptor null mice (top left). Blood glucose levels from 0 to 120 minutes post glucose dose in GLP-1 receptor null mice (top right). Data are presented as mean of 5 mice per group. Blood glucose levels from 0 to 120 minutes post glucose dose in wild-type mice administered semaglutide or LY3298176 either alone or in combination with the GLP-1 receptor antagonist Jant-4 (1000 nmol/kg). Data are presented as mean of 6 mice per group.

Male Long Evans rats that were fed a high fat/high sucrose diet for 4 weeks and then subcutaneously administered LY3298176 for 14 days were fasted overnight and subject to a euglycemic clamp study. In this study, chronic dosing of LY3298176 (10 nmol/kg/day) resulted in decreased food consumption that correlated with a decrease in body weight. After steady state was reached (blood glucose levels and glucose utilization rates were reasonably constant), LY3298176 exposure resulted in a statistically significant increase in the glucose infusion rate (used as an index of whole-body insulin sensitivity) and increased 2-deoxyglucose uptake in muscle and adipose tissue that was observed along with a trend toward decreasing endogenous glucose production. Finally, chronic LY3298176 administration resulted in reduced fasted, plasma triglycerides during the clamp period.

Figure 2: Blood glucose and glucose infusion rate during a euglycemic clamp study in fasted Long Evans diet-induced insulin resistant rats treated with LY3298176

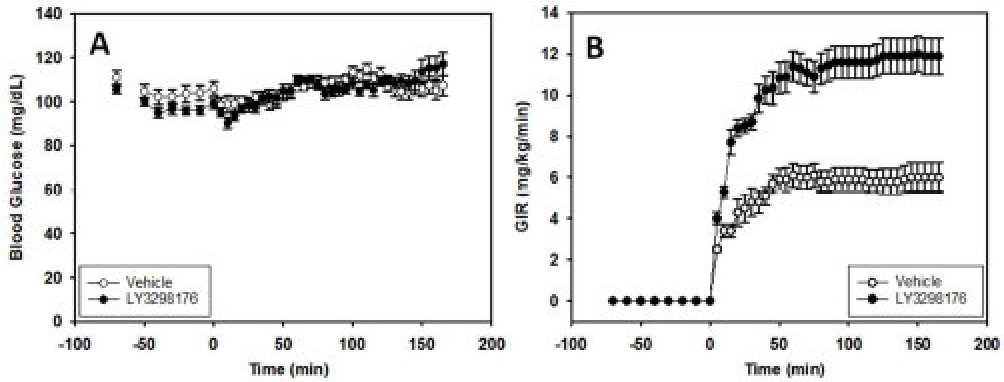


Figure copied from the Applicant's submission (Figure 3). Blood glucose (A) and glucose infusion rate (B) during a euglycemic clamp study of fasted Long Evans diet-induced insulin resistant rats treated with vehicle or LY3298176.

Similar studies conducted in DIO C57BL/6 mice showed that subcutaneous administration of LY3298176 (10 nmol/kg/day) resulted in decreased food intake, a statistically significant decrease in body weight, a statistically significant increase in glucose infusion rate (under steady state conditions), and a statistically significant increase in 2-deoxyglucose uptake in muscle and adipose tissue when compared to controls. LY3298176 (10 nmol/kg) also produced greater decreases in food intake, body weight, and increases in glucose infusion rate when compared to 10 nmol/kg semaglutide after subcutaneous administration for 14 days. Finally, both LY3298176 and semaglutide exposure resulted in a trend toward decreasing endogenous glucose production.

To examine whether the greater effects in LY3298176 -treated mice were due to increased body weight loss when compared to semaglutide, a second study was conducted in DIO mice comparing a lower dose of LY3298176 (3 nmol/kg) with semaglutide (10 nmol/kg) and a pair-fed control group after chronic subcutaneous administration for 14 days. In this scenario, similar decreases in food consumption and body weight were observed in all treated and pair-fed groups when compared to controls. Under steady state conditions with similar decreases in body weight and food consumption, LY3298176 -treated mice still experienced significantly increased glucose infusion rates and 2-deoxyglucose uptake in muscle and adipose tissue when compared to controls, semaglutide treated- and pair-fed mice.

Figure 3: Cumulative food intake and cumulative body weight of DIO C57/BL6 mice treated with semaglutide, LY3298176, or pair-feeding

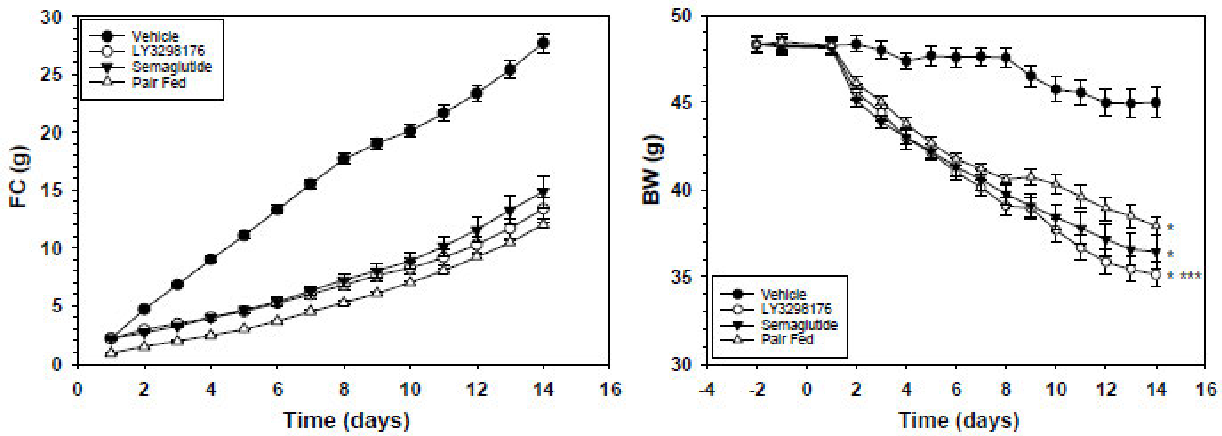


Figure copied from the Applicant's submission (Figure 7). Cumulative food intake (left) and cumulative body weight (right) of DIO C57/BL6 mice treated with vehicle (n = 15), semaglutide (n = 14), LY3298176 (n = 15), or pair-feeding (n = 14).BW = body weight, FC = food consumption. Data shown are mean ± standard error of the mean (SEM). Statistical analysis (*p<.05 versus vehicle, ***p<.05 LY3298176 versus pair-fed) was completed by Modified Krushal-Wallis test followed by Bonferroni adjustment for multiple comparisons.

To determine LY3298176's influence on metabolism and body composition, male DIO mice were subcutaneously administered LY3298176 (10, 30 or 100 nmol/kg) or the long-acting GLP-1 analog semaglutide (LSN3119036, 30 nmol/kg) every 3 days for 15 days. LY3298176 caused reductions in fat mass, fat-free mass, plasma cholesterol and liver triglycerides that were greater than those seen with semaglutide.

Figure 4: Effect of chronic treatment with LY3298176 on body weight, fat mass, and fat-free mass in DIO mice

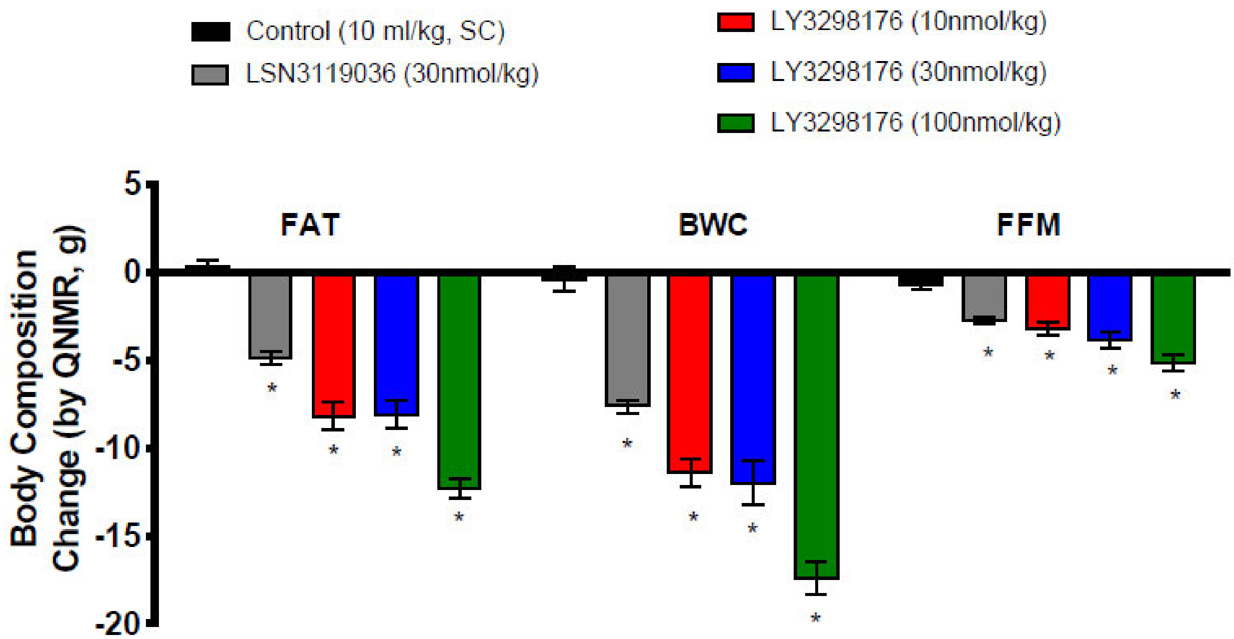


Figure copied from the Applicant's submission (Figure 3) The effect of chronic treatment with LY3298176 on fat mass (FAT), body weight change (BWC), and fat-free mass (FFM) in DIO mice. LSN3119036 (30 nmol/kg/every 3 days) is semaglutide, a long-acting GLP-1 analog. *p<.05, compared to vehicle group (one-way ANOVA, Dunnett's). Values are presented as means ± SEM with n = 5.

Figure 5: Plasma cholesterol (mg/dL) following chronic treatment with LY3298176 in DIO mice

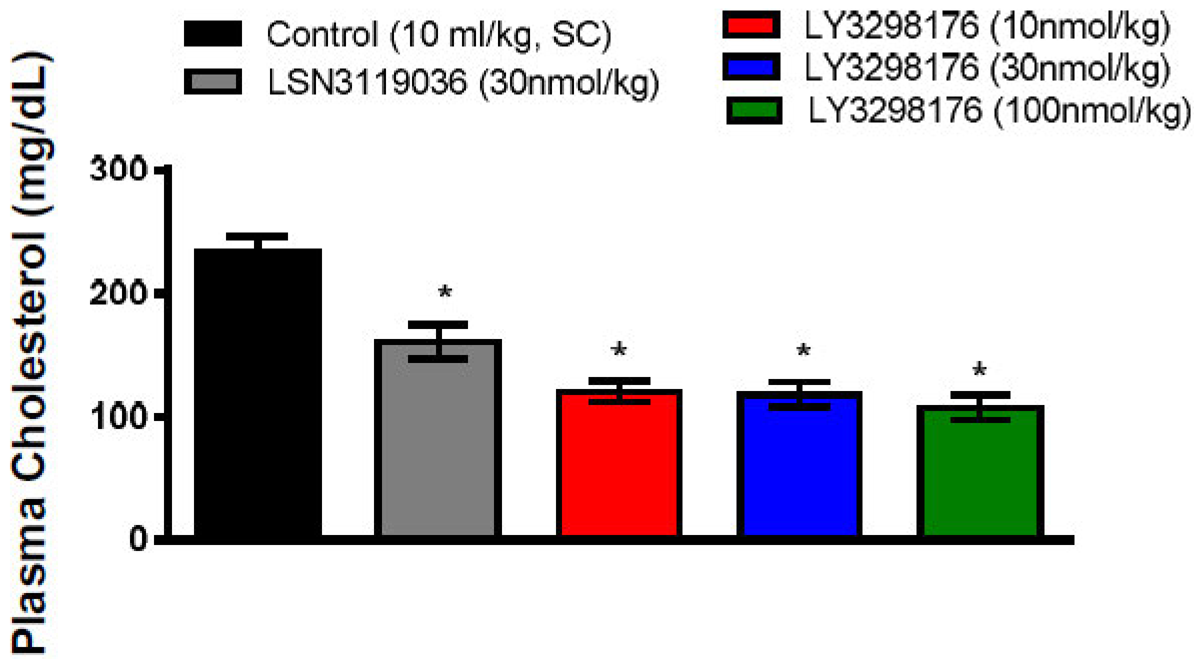


Figure copied from the Applicant's submission (Figure 5). Plasma cholesterol (mg/dL) following chronic treatment with vehicle, or LY3298176 (10, 30, or 100 nmol/kg/every 3 day) to DIO mice. LSN3119036 (30 nmol/kg/every 3 days) is semaglutide, a long-acting GLP-1 analog. *p<.05, compared to vehicle group (one-way ANOVA, Dunnett's). Values are presented as means ± SEM with n = 5.

Figure 6: Liver triglyceride (mg/g tissue) content following chronic LY3298176 treatment in DIO mice

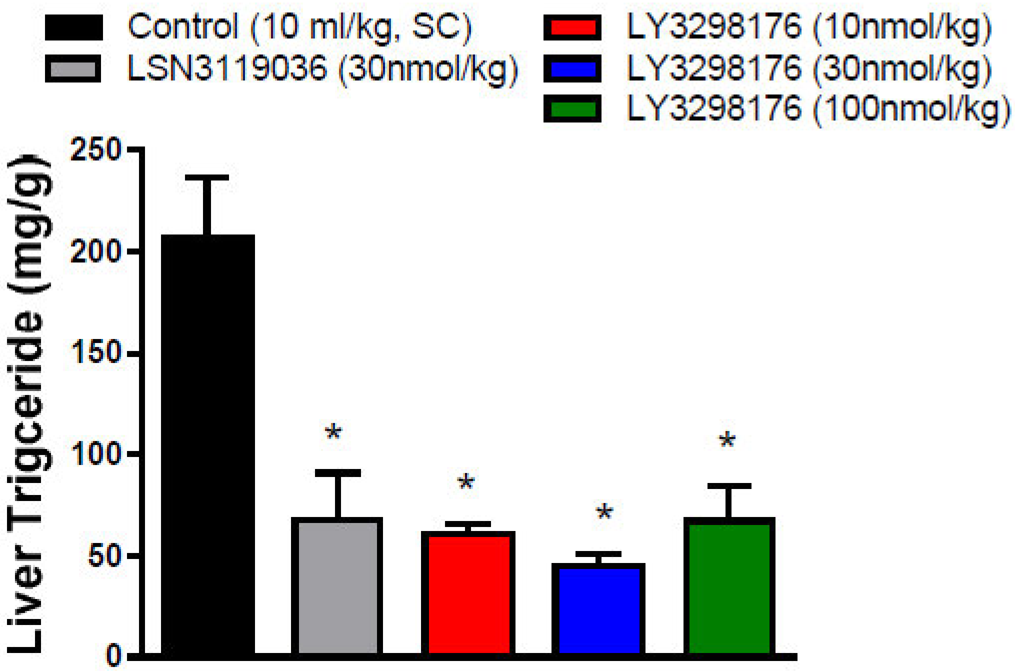


Figure copied from the Applicant's submission (Figure 6) Liver triglyceride (mg/g tissue) content following chronic treatment with vehicle, or LY3298176 (10, 30, or 100 nmol/kg/every 3 day) to DIO mice. LSN3119036 (30 nmol/kg/every 3 days) is semaglutide, a long-acting GLP-1 analog. *p<.05, compared to vehicle group (one-way ANOVA, Dunnett's). Values are presented as means ± SEM with n = 5.

DIO mice given LY3298176 (10 nmol/kg) or semaglutide (LSN3119036, 30 nmol/kg) every 3 days for 22 days also experienced decreased respiratory exchange ratios during the first week of dosing when food intake was most reduced suggesting fat was the predominant fuel source. There was also a sustained increase in heat exchange ratio caused by LY3298176 starting in Week 2 of dosing, which may indicate an increase in metabolic rate.

Figure 7: Effect of chronic LY3298176 treatment on metabolic rate in DIO mice

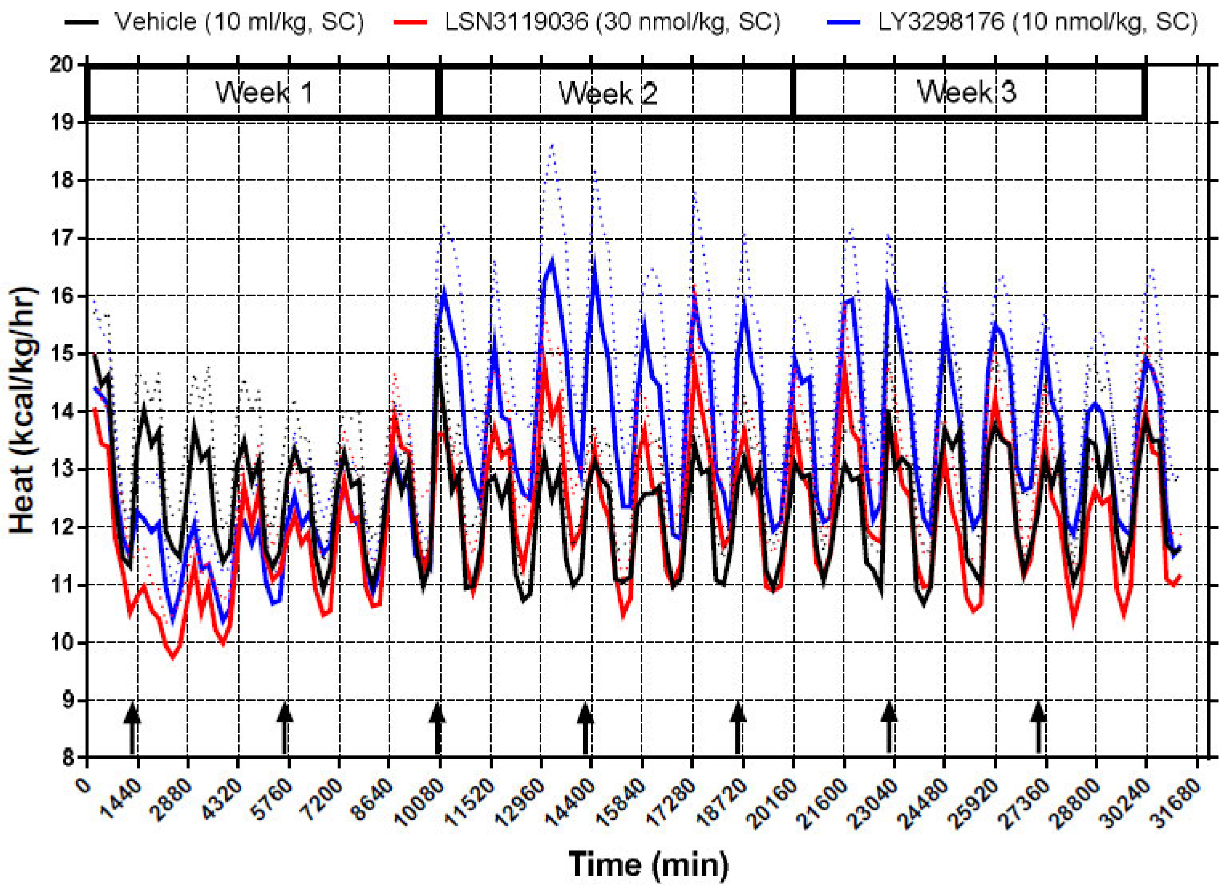


Figure copied from the Applicant's submission (Figure 10) The effect of chronic treatment with LY3298176 (LY3298176) (blue line; 10 nmol/kg/every 3 days) on metabolic rate in DIO mice. LSN3119036 (red line; 30 nmol/kg/every 3 days) is semaglutide, a long-acting GLP-1 analog. The arrows on x-axis indicate the time of injection, which were done on Day 1, 4, 7, 10, 13, 16, and 19. Values are presented as means ± SEM with n = 6. Standard Error of Mean is presented as dotted lines over solid lines.

GLP-1 has been shown previously to play a role in slowing gastric emptying. To examine LY3298176's ability to influence gastric emptying, overnight fasted DIO mice were administered LY3298176 (3, 10, 30, and 100 nmol/kg), or the positive control semaglutide (LSN3119036, 30 nmol/kg) by subcutaneous injection. The next day mice were given a semi liquid diet via oral gavage. Mice given ≥10 nmol/kg LY3298176 showed a statistically significant reduction in semi-liquid gastric emptying (evaluated as the percent of remaining food in the stomach). In a published study, DIO mice subcutaneously administered a single dose of LY3298176 (0.3, 1, 3,10 or 30 nmol/kg) before a 16 hour overnight fast also experienced a delay in gastric emptying at doses ≥ 1 nmol/kg. However, after chronic administration of LY3298176 (1, 3 and 10 nmol/kg) for a duration of 14 days, the inhibitory effect on gastric emptying was reportedly not observed (Urva et al. 2020).

Since brown adipose tissue (BAT) is metabolically active and has the capacity to lower circulating metabolites, a literature source has suggested that dysregulation of branched-chain amino acid (BCAA) catabolism in BAT may lead to increased circulating plasma levels of BCAAs (Yoneshiro et al. 2019). Ultimately defective BCAA metabolism in BAT can result in development of diet-induced obesity and glucose intolerance (Yoneshiro et al. 2019). To investigate the effect of LY3298176 on BAT catabolism and circulating levels of BCAAs and pathway-related metabolites, DIO and insulin resistant mice were treated with LY3298176 (3 or 10 nmol/kg) for 14 days. In BAT, statistically significant increases in glutamic acid (3 and 10 nmol/kg) and BCAAs isoleucine, leucine, and valine (3 and 10 nmol/kg) were observed after exposure to LY3298176. Branched chain keto acid (BCKAs) in BAT were not determined due to insufficient sample quantity. Conversely, in plasma, a statistically significant reduction in circulating levels of glutamic acid (10 nmol/kg), BCAAs isoleucine (3 and 10 nmol/kg), leucine (10 nmol/kg) and valine (3 or 10 nmol/kg) were observed after subcutaneous exposure to LY3298176. There was also a statistically significant reduction in the BCKA ketoleucine (10 nmol/kg). These findings may indicate that LY3298176 can activate pathways in BAT associated with catabolism of BCAA that stimulate uptake of BCAA, increase clearance of circulating BCAAs from the plasma and improve insulin sensitization.

Table 4: BCAAs, Glutamic Acid, and BCKAs Changes Associated with LY3298176

	Plasma		Brown Adipose Tissue (BAT)	
Metabolite	3 nmol/kg	10 nmol/kg	3 nmol/kg	10 nmol/kg
Glutamic Acid	1.00	0.75*	4.05*	2.81*
Isoleucine	0.74*	0.70*	1.90*	1.93*
Leucine	0.80 (p = .07)	0.77*	1.87*	1.90*
Valine	0.77*	0.76*	1.78*	1.83*
Ketoisoleucine	0.71	0.67	n.a.	n.a.
Ketoleucine	0.75	0.66*	n.a.	n.a.
Ketovaline	n.d.	n.d.	n.a.	n.a.

Abbreviations: ANOVA = analysis of variance, BCAAs = branched-chain amino acids, BCKAs = branched-chain ketoacids, n.a = not available, n.d = not detected.

Data presented as fold change between LY3298176 and vehicle groups. *Unadjusted p-value ≤.05 versus vehicle by one-way ANOVA. Number of plasma (n = 5) and BAT (n = 6) samples was the same for vehicle and the LY3298176 groups.

n.a = BCKAs in BAT were not monitored due to insufficient sample quantity.

Table copied from the Applicant's submission (Table 1).

4.2 Secondary Pharmacology

Dedicated secondary pharmacology studies were not conducted as LY3298176 appears to be highly specific and did not show significant off-target organ toxicity in repeat dose toxicology studies.

4.3 Safety Pharmacology

Neurological examinations, body temperature, and respiratory parameters were examined in the one-month monkey study and no significant drug-related findings were noted up to a dose of 0.5 mg/kg/week (2X the MRHD).

To examine the effects of LY3298176 on the cardiovascular system, a hERG assay was conducted as well as single and multiple dose toxicology studies in monkeys. LY3298176 did not have a statistically significant impact on hERG current at concentrations up to 300 μM (the IC_{50} for the inhibitory effect of LY3298176 on hERG potassium current was not calculated but was estimated to be greater than 300 μM). After a single subcutaneous administration of LY3298176 (0.15 or 0.5 mg/kg), increased heart rates were observed at both doses in male telemetry-instrumented conscious cynomolgus monkeys. At the 0.15 mg/kg dose, increased diastolic and mean arterial pressure and reduced pulse pressure were observed. Sustained increases in heart rate were also recorded at the 0.15 mg/kg dose. During chronic studies of 1- or 6-months in duration, subcutaneous administration of LY3298176 (0.05, 0.15 or 0.5 mg/kg) in unanesthetized monkeys resulted in a trend toward higher heart rates using jacketed external telemetry.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

METHODS

LY3298176 concentrations were determined by validated liquid chromatography with tandem mass spectrometry (LC/MS) methods. Due to rapid in vivo catabolism, unstable radiolabeled linkages, and recycling of amino acids into non-drug related proteins/peptides, distribution studies with radiolabeled proteins can be difficult to interpret. With that caveat, the highest concentrations of LY3298176 were found at the injection site and in the kidney.

Table 5: Pharmacokinetic Parameters of LY3298176 in Male Rats Following a Single Subcutaneous Administration of [14C]- LY3298176

Dose	3 mg/kg (133 µCi/kg)		
Parameter	**Radioactivity in Blood**	**Radioactivity in Plasma**	**Tirzepatide in Plasma**
C_{max} (ng eq/g or ng/mL)	8340	16300	19600
T_{max} (hr)	6.00	6.00	6.00
$t_{1/2}$ (hr)	57.2	25.6	9.03
$AUC_{0\text{-}t}$ (ng-eq•hr/g or ng•hr/mL)	284000	551000	477000
$AUC_{0\text{-}\infty}$ (ng-eq•hr/g or ng•hr/mL)	285000	551000	478000

Abbreviations: C_{max} = maximum observed plasma concentration T_{max} = time to reach C_{max}; $t_{1/2}$ = half-life; $AUC_{0\text{-}t}$= area under the plasma concentration-time curve from time 0 to last measurable time point; $AUC_{0\text{-}\infty}$= area under the plasma concentration-time curve from time 0 to infinity; eq = equivalents [^{14}C]tirzepatide.

Note: Data are n = 3animals/time point.

Table copied from the Applicant’s submission (Table 2.6.4.4.)

Table 6: Pharmacokinetic Parameters of Total Radioactivity in Selected Tissues from Male Pigmented Rats Following a Single Subcutaneous Dose of 3 mg/kg [14C]- LY3298176

Matrix or Tissue	$AUC_{0\text{-}t}$ (ng-eq•hr)/g	$AUC_{0\text{-}\infty}$ (ng-eq•hr/g)	C_{max} (ng-eq/g)	T_{max} (hr)	$t_{1/2}$ (hr)
Arterial wall	264949	NC	4710	12	NC
Blood	249473	251847	7430	12	30.6
Bone	7823	9884	248	12	40.7
Brain	6044	7045	105	12	28.8
Cecum	269421	272548	5220	48	25.0
Dose site	21155752	21170570	202000	1.0	88.5
Eye uveal tract	171232	181844	3040	12	147
Fat (abdominal)	14263	16636	297	12	28.7
Fat (brown)	85333	92439	1550	12	53.1
Intervertebral ligaments	389908	422161	4830	12	246
Kidney	1449494	1461997	11600	48	46.6
Liver	272516	277770	4530	12	27.4
Lung	194817	197845	4590	12	32.5
Pancreas	104393	108146	2070	12	34.7
Skin (pigmented)	96869	102938	1360	24	42.6
Stomach	91268	93473	2220	12	32.9
Urinary bladder	303046	310937	5150	48	30.7

Abbreviations: AUC = area under the plasma concentration-time curve from time 0 to last measurable time point (t) or to infinity (∞); C_{max} = maximum observed concentration; NC = not calculated; T_{max} = time to C_{max}; $t_{1/2}$ = half-life.

Table copied from the Applicant’s submission (Table 2.6.4.14.)

Table 7: Cumulative Elimination of Radioactivity (Mean ± SD) from Male Rats Following a Single Subcutaneous Dose of 3 mg/kg [^{14}C]- LY3298176

Route of Elimination	Intact Rat N = 4 %	Bile-Duct Cannulated Rat N = 4 %
Urine	45.0 ± 2.3	47.9 ± 3.1
Feces	47.1 ± 0.8	7.49 ± 3.13
Bile	NA	47.1 ± 3.6
Total[a]	112 ± 2	110 ± 1

Abbreviations: N = number of animals; NA = not applicable; SD = standard deviation.

[a] The total also includes cage rinse, cage wash, cage wipe, urine wipe, feces wipe, volatiles, expired air, expired air backup, carcass and in bile-duct cannulated rats includes bile cannula and jacket rinses.

Note: The collection interval was 336 hours in intact rats and bile-duct cannulated rats.

Table copied from the Applicant's submission (Table 2.6.4.17.)

Table 8: Cumulative Elimination of Radioactivity (Mean ± SD) from Male Monkeys Following a Single Subcutaneous Dose of 0.5 mg/kg [^{14}C]- LY3298176

Route of Elimination	Monkey N = 4 %
Urine	49.4 ± 4.6
Feces	35.0 ± 2.5
Total[a]	97.2 ± 1.8

Abbreviations: N = number of animals; SD = standard deviation.

[a] The total also includes cage rinse, wash, wipe, and debris and urine wipe.

Note: The collection interval was 672 hours in monkeys.

Table copied from the Applicant's submission (Table 2.6.4.18.)

ABSORPTION

The pharmacokinetic profile of LY3298176 was similar in mice, rats, rabbits, and monkeys following subcutaneous dosing. In all species examined, LY3298176 was absorbed into the plasma after both single and repeated subcutaneous administration (T_{max} values ranged between 4 and 24 hours and the elimination half-life ranged between 55 to 103 hours). Bioavailability in monkeys was 83%, which is comparable with the bioavailability in humans (absolute bioavailability of a 5 mg subcutaneous dose of LY3298176 in healthy participants was approximately 80% from clinical study 18F-MC-GPGE). Systemic exposure to LY3298176 (as measured by plasma C_{max} and $AUC_{0\text{-}t}$ values) increased with increasing dose. Accumulation of LY3298176 was minimal after repeated administration in mice, rats, and monkeys (<2-fold). For all species examined, similar levels of systemic exposure were observed in males and females indicating that sex did not have a significant impact on absorption.

DISTRIBUTION

LY3298176 was highly protein bound (99.15% in monkey plasma and 97.70% in rat plasma) and preferentially distributed to the plasma compartment of the blood consistent with its ability to bind albumin. Findings in animals were comparable with what is seen in humans as protein binding of fluorescently labeled LY3298176 in human plasma ranged from 98.64% to 99.30% with a mean of 99.06%. Quantitative whole body autoradiography studies where a single subcutaneous administration of [^{14}C]-LY3298176 in nonpigmented, Sprague Dawley or pigmented, Long Evans male rats was given revealed that the highest levels of radioactivity were detected at the injection site and kidney. Small amounts of LY3298176 were able to cross the blood brain barrier as radioactivity was detected in the brain and spinal cord. Radioactivity declined over time and was below the limit of quantitation for most tissues 672 hours post-dose.

METABOLISM

In vivo, the primary metabolic pathway in rats and monkeys included proteolytic cleavage of the peptide backbone, β-oxidation of the C20-fatty diacid moiety and amide hydrolysis. Thirteen metabolites were detected in rats, 11 metabolites were detected in monkeys, and all human plasma metabolites were identified in rat and monkey plasma. Similar to animals, the primary metabolic pathways that contributed to the clearance of LY3298176 included proteolytic cleavage of the peptide backbone, β-oxidation of the C20-fatty acid moiety, and amide hydrolysis.

Figure 8: Proposed Metabolic Pathway of LY3298176 in Rats and Monkeys

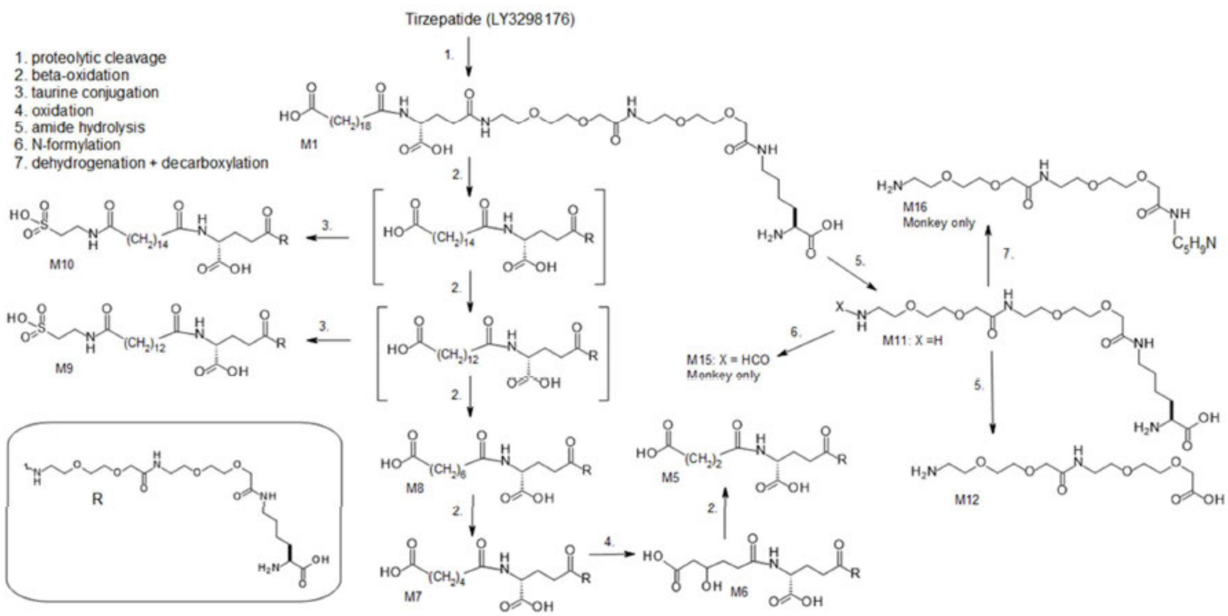


Figure copied from the applicant's submission.

ELIMINATION

LY3298176 was cleared by metabolism (no parent compound was detected in urine or feces) in rats and monkeys. Metabolites were eliminated almost equally in the urine (~45%) and feces (~47%) in rats, and the excretion of LY3298176-associated radioactivity in bile duct cannulated rats established that hepatobiliary excretion is a route of elimination. In male cynomolgus monkeys, excretion was ~49% in the urine and ~35% in feces. A similar elimination profile was seen in humans. After a single dose of [^{14}C]-LY3298176 (approximately 2.9 mg) in healthy males, approximately 50% of the administered radioactivity was recovered in urine, while approximately 21% was recovered in feces. LY3298176 was eliminated by metabolism with no intact LY3298176 observed in urine or feces (Study 18F-MC-GPHX).

5.2 Toxicokinetics

Table 9: Toxicokinetics of LY3298176 in 001178-W (Wild Type) Mice Following Twice-Weekly Subcutaneous Administration of LY3298176 at Doses of 1, 3, or 10 mg/kg in a Carcinogenicity Study for 26 Weeks

	Administered Dose (mg/kg)					
	1		3		10	
Parameter	Male (N = 3)	Female (N = 3)	Male (N = 3)	Female (N = 3)	Male (N = 3)	Female (N = 3)
Day 1						
C_{max} (ng/mL)	3500	3880	13500	11400	41100	39900
SD C_{max} (ng/mL)	612	206	624	321	5870	7740
$AUC_{0\text{-}96hr}$ (ng•hr/mL)	109000	102000	359000	332000	1220000	1140000
SEM $AUC_{0\text{-}96}$ (ng•hr/mL)	11600	4530	12600	7700	39900	41600
Day 176						
C_{max} (ng/mL)	5270	5660	15500	18500	45700	53100
SD C_{max} (ng/mL)	598	417	819	700	6990	2290
$AUC_{0\text{-}96hr}$ (ng•hr/mL)	166000	171000	484000	498000	1520000	1530000
SEM $AUC_{0\text{-}96}$ (ng•hr/mL)	6280	4310	14600	6350	61200	51500

Abbreviations: $AUC_{0\text{-}96hr}$ = area under the plasma concentration-time curve during the dosing interval; C_{max} =maximum observed plasma concentration; SD = standard deviation; SEM = standard error of the mean.

Note: Values represent three animals/sex/group/time point; PK parameters were generated from a composite analysis from sparse sampling to enable a complete plasma profile in the mice.

Table copied from the applicant's submission (Table 2.6.4.8.)

Table 10: Toxicokinetic Parameters of LY3298176 in Sprague Dawley Rats Following Twice-Weekly Subcutaneous Administration of 0.5, 1.5, or 3 mg/kg LY3298176 for 6 Months

	Administered Dose (mg/kg)					
	0.5		1.5		3	
Parameter	Male (N = 3)	Female (N = 3[a])	Male (N = 3)	Female (N = 3)	Male (N = 3)	Female (N = 3[a])
Day 1						
C_{max} (ng/mL)	1130	1020	3610	3040	13800	5470
SD C_{max} (ng/mL)	65.1	59.7	625	721	4000	839
$AUC_{0\text{-}96hr}$ (ng•hr/mL)	44400	33800	113000	92600	279000	173000
SEM $AUC_{0\text{-}96hr}$ (ng•hr/mL)	1790	1140	8680	4450	17000	10000
Day 176						
C_{max} (ng/mL)	1040	1310	3320	3950	5580	6980
SD C_{max} (ng/mL)	89.5	45.1	605	749	1290	352
$AUC_{0\text{-}96hr}$ (ng•hr/mL)	45600	62300	128000	156000	257000	302000
SEM $AUC_{0\text{-}96hr}$ (ng•hr/mL)	2670	2870	7030	7270	11000	12500

Abbreviations: $AUC_{0\text{-}96hr}$ = area under the plasma concentration-time curve during the dosing interval; C_{max} = maximum observed plasma concentration; N = number of animals per time point; SD = standard deviation; SEM = standard error of the mean.

[a]Values represent 3 animals/sex/group, except for 0.5 and 3 mg/kg females on Day 176 at the 4-hour postdose and predose collections, respectively, where N=2, due to outlier exclusions (excluded data were outside ±3 SD of other data in males and females collected at the same time point). PK parameters were generated from a composite analysis from sparse sampling to enable a complete plasma profile in the rat.

Table copied from the applicant's submission (Table 2.6.4.9.)

Table 11: Toxicokinetic Parameters of LY3298176 in Sprague Dawley Rats Following Twice-Weekly Subcutaneous Administration of LY3298176 at Doses 0.15, 0.5, or 1.5 mg/kg in a Carcinogenicity Study

	Administered Dose (mg/kg)					
	0.15		0.5		1.5	
Parameter	Male (N = 3)	Female (N = 3)	Male (N = 3)	Female (N = 3)	Male (N = 3)	Female (N = 3)
Day 1						
C_{max} (ng/mL)	567	524	1380	1480	5660	4020
SD C_{max} (ng/mL)	53.5	125	367	235	1490	1100
$AUC_{0\text{-}96hr}$ (ng•hr/mL)	13700	14300	42000	44900	157000	135000
SEM $AUC_{0\text{-}96hr}$ (ng•hr/mL)	459	772	3210	2490	10700	6050
Day 358						
C_{max} (ng/mL)	341	398	1050	1140	3060	3370
SD C_{max} (ng/mL)	80.9	67.5	175	56.9	294	310
$AUC_{0\text{-}96hr}$ (ng•hr/mL)	16600	18600	49200	53300	130000	159000
SEM $AUC_{0\text{-}96hr}$ (ng•hr/mL)	894	585	3080	3120	4650	5790

Abbreviations: $AUC_{0\text{-}96hr}$ = area under the plasma concentration-time curve during the dosing interval; C_{max} = maximum observed plasma concentration; N = number of animals per time point; SD = standard deviation; SEM = standard error of the mean.

Note: Values represent 3 animals/sex/group/time point.; PK parameters were generated from a composite analysis from sparse sampling to enable a complete plasma profile in the rat.

Table copied from the applicant's submission (Table 2.6.4.10.)

Table 12: Mean (± Standard Deviation) Toxicokinetic Parameters of LY3298176 in Pregnant Sprague Dawley Rats Following Twice-Weekly Subcutaneous Administration of 0.02, 0.1, or 0.5 mg/kg LY3298176 Beginning on Gestation Day 6

	Administered Dose (mg/kg)					
	0.02 (N = 2)		0.1 (N = 3)		0.5 (N = 4)	
Parameter	Mean	SD	Mean	SD	Mean	SD
Gestation Day 6						
$AUC_{0\text{-}last}$ (ng•hr/mL)	NA	NA	8570	1220	53200	12700
C_{max} (ng/mL)	26.2	NA	329	66.9	1740	331
Gestation Day 17						
$AUC_{0\text{-}last}$ (ng•hr/mL)	821	393	4010	1490	35800	3650
C_{max} (ng/mL)	52.2	17.0	174	46.6	1480	287

Abbreviations: $AUC_{0\text{-}last}$ = area under the plasma concentration-time curve from the time of dosing to the sampling time of the last observed concentration; C_{max} = maximum observed plasma concentration; N = number of animals; NA = not applicable; SD = standard deviation.

Table copied from the applicant's submission (Table 2.6.4.11.)

Table 13: Mean (± Standard Deviation) Toxicokinetic Parameters of LY3298176 in Pregnant New Zealand White Rabbits Following Weekly Subcutaneous Administration of 0.01, 0.03, or 0.1 mg/kg LY3298176 on Gestation Days 7 and 14

	Administered Dose (mg/kg)					
	0.01 (N = 4)		0.03 (N = 3-4)		0.1 (N = 4)	
Parameter	Mean	SD	Mean	SD	Mean	SD
Gestation Day 7						
$AUC_{0\text{-}168hr}$ (ng•hr/mL)	3220	334	14000	959	55500	3360
C_{max} (ng/mL)	54.3	8.91	192	6.85	632	69.7
Gestation Day 14						
$AUC_{0\text{-}168hr}$ (ng•hr/mL)	3560	450	14700	2450	56400	2380
C_{max} (ng/mL)	46.6	2.92	159	37.0	567	14.2

Abbreviations: $AUC_{0\text{-}168hr}$ = area under the plasma concentration-time curve during the dosing interval; C_{max} = maximum observed plasma concentration; N = number of animals; SD = standard deviation.

Table copied from the applicant's submission (Table 2.6.4.12.)

Table 14: Toxicokinetic Parameters of LY3298176 in Monkeys Following Weekly Subcutaneous Administration of 0.05, 0.15, or 0.5 mg/kg LY3298176 for 6 months

	Administered Dose (mg/kg)					
	0.05		0.15		0.5	
Parameter	Male (N = 4)	Female (N = 4)	Male (N = 4)	Female (N = 4)	Male (N = 7)	Female (N = 7)
Day 1						
Mean C_{max} (ng/mL)	306	336	1140	1070	4590	4800
SD C_{max} (ng/mL)	21.1	84.6	214	85.8	995	615
Mean $AUC_{0\text{-}168hr}$ (ng•hr/mL)	26200	28300	91100	86500	333000	340000
SD $AUC_{0\text{-}168hr}$ (ng•hr/mL)	2370	4420	11100	10200	45200	27700
Day 176						
Mean C_{max} (ng/mL)	389	438	1340	1210	4190	4580
SD C_{max} (ng/mL)	15.0	128	250	42.4	605	1030
Mean $AUC_{0\text{-}168hr}$ (ng•hr/mL)	34000	34100	121000	102000	342000	331000
SD $AUC_{0\text{-}168hr}$ (ng•hr/mL)	3540	19300	18300	11200	19000	62000

Abbreviations: $AUC_{0\text{-}168hr}$ = area under the plasma concentration-time curve during the dosing interval; C_{max} = maximum observed plasma concentration; N = number of animals; SD = standard deviation.

Table copied from the applicant's submission (Table 2.6.4.13.)

6 General Toxicology

6.2 Repeat-Dose Toxicity

Pivotal repeat dose toxicity studies of 6 months in duration were conducted in rats and monkeys.

Rats subcutaneously administered LY3298176 (0, 0.5, 1.5, or 3 mg/kg) twice weekly for a duration of 6 months experienced reversible, statistically significant, and dose-dependent decreases in food consumption that were associated with decreases in mean body weight and mean body weight gains when compared to control rats, consistent with other members of the GLP-1R agonist drug class. Histopathological findings in the pancreas included zymogen depletion and persistent lobular atrophy that correlated with persistent decreases in amylase. Zymogen granule depletion is likely due to GLP-1R mediated pharmacodynamic activity. Lobular atrophy is a common finding in Sprague Dawley rats (Chadwick et al. 2014). No lobular atrophy was observed in the 2-year rat carcinogenicity study. These observations taken together question the clinical relevance of this finding. In the spleen, decreases in extramedullary hematopoiesis that were associated with changes in red cell parameters are likely due to LY3298176-mediated decreases in body weight and food consumption (i.e., malnutrition). Thyroid C-cell hyperplasia was also noted at the high dose. The NOAEL for this study was conservatively set at 1.5 mg/kg (1-fold the MRHD) based on findings of minimal to slight thyroid C-cell hyperplasia at the highest dose examined. All doses examined resulted in levels of exposure that were similar to those found in the clinic based on AUC values. But the findings noted are typical for the GLP-1R agonist class of drugs and are thought to be monitorable in a clinical setting or are potentially rodent specific findings.

Figure 9: Mean Body Weights in Male Rats

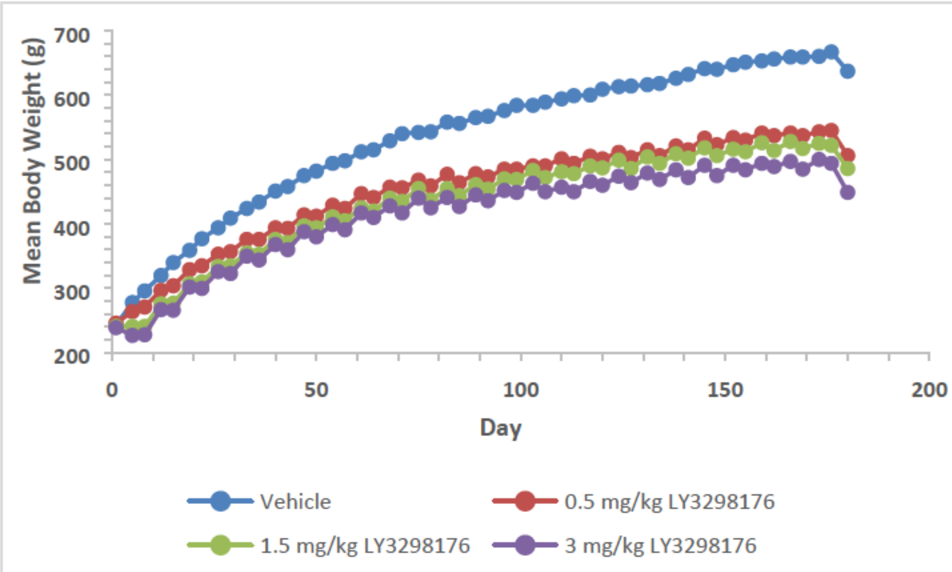


Figure prepared by Elena Braithwaite.

Figure 10: Mean Body Weights in Female Rats

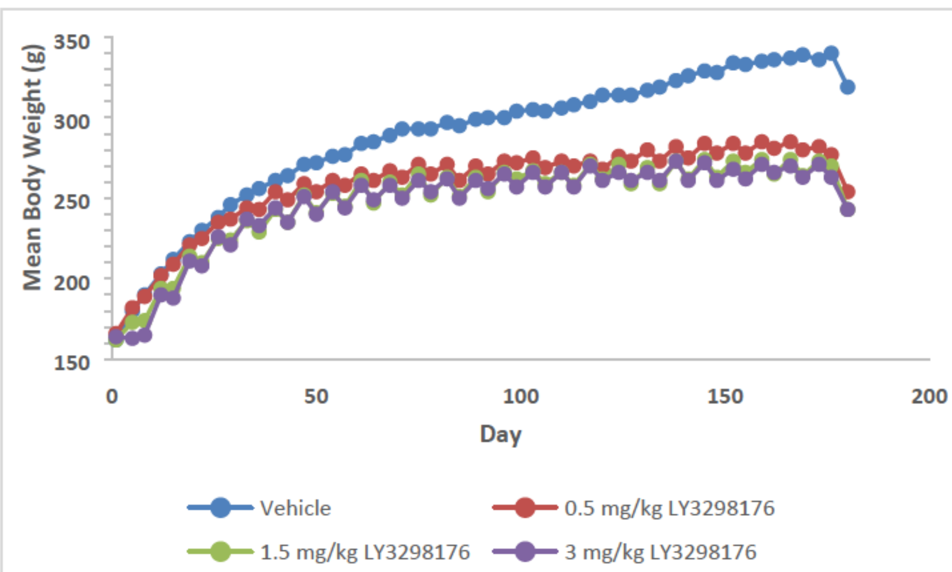


Figure prepared by Elena Braithwaite.

Table 15: Incidence and Severity of LY3298176-Related Microscopic Findings in the Pancreas of Rats– Terminal Sacrifice

	LY3298176							
Sex	Males				Females			
Dose Level (mg/kg)	0	0.5	1.5	3	0	0.5	1.5	3
Pancreas								
Number Examined	15	15	14	14	15	15	15	15
Atrophy, lobular								
Minimal	6	6	4	5	3	3	6	9
Slight	0	1	0	1	0	0	0	0
Depletion, zymogen								
Minimal	0	1	3	4	0	2	3	4
Slight	0	1	3	2	0	0	0	0

Table modified from the applicant's submission (Text Table 4.5).

Table 16: Incidence and Severity of LY3298176-Related Microscopic Findings in the Pancreas of Rats– Recovery Sacrifice

	LY3298176			
Sex	Males		Females	
Dose Level (mg/kg)	0	3	0	3
Pancreas				
Number Examined	5	5	5	5
Atrophy, lobular				
Minimal	4	4	2	3
Slight	0	0	0	1

Table copied from review by Jessica Hawes under IND 128801 (entered into DARRTS on 10/03/2018).

Table 17: Incidence and Severity of LY3298176-Related Thyroid C-cell Hyperplasia – Terminal Sacrifice

Test Article	(dosage)	1	2	3	4				
LY3298176	mg LY3298176/kg	0	0.5	1.5	3				
Tissue/ Observation	Group/Subgroup/Sex:	1/1/M	2/1/M	3/1/M	4/1/M	1/1/F	2/1/F	3/1/F	4/1/F
	Number of Animals:	15	15	14	14	15	15	15	15
Thyroid	Number Examined:	15	0	0	14	15	0	0	15
	Unremarkable:	15	0	0	11	15	0	0	13
		0	0	0	1	0	0	0	0
Hyperplasia, C-cell									
	finding not present -	15	0	0	11	15	0	0	13
	minimal 1	0	0	0	3	0	0	0	1
	slight 2	0	0	0	0	0	0	0	1
	Total Incidence:	0	0	0	3	0	0	0	2

Table modified from the applicant's submission (Text Table 6.5).

In a 6-month repeat dose toxicology study where monkeys were subcutaneously administered LY3298176 (0, 0.05, 0.15, or 0.5 mg/kg/week), similar findings typical of drugs in the GLP-1R agonist class were observed. Reversible and dose-dependent decreases in food consumption that resulted in decreased body weights and body weight gains were observed. Decreased pancreatic zymogen granules were seen in females (0.5 mg/kg or 1-fold the MRHD) resulting from GLP-1R agonist activity in healthy animals. In addition, increased heart rates throughout dosing that did not reach statistical significance were observed in males at levels of exposure ≥0.5-fold the MRHD. This finding is consistent with the observation that GLP-1Rs have been identified in heart sinoatrial node myocytes in monkeys (Pyke et al. 2014). LY3298176 also had non-adverse effects on the gastrointestinal system that included vomitus in males during the first two weeks of dosing (1-fold the MRHD) and sporadic findings of minimal to slight hemorrhage in the cecum or stomach that were observed at all doses but were not dose-related and effected females at a greater incidence than males (≥0.1-fold the MRHD). Five monkeys administered 0.5 mg/kg/week LY3298176 (1-fold the MRHD) required veterinary intervention due to test article-related weight loss. Therefore, the NOAEL was 0.15 mg/kg/week, which occurred at exposure levels achieved in the clinic. All findings noted are expected with the GLP-1R agonist class of pharmaceuticals. A 6-month duration was chosen for the non-rodent toxicity study because findings in the 1-month toxicology studies in both species were consistent with exaggerated pharmacology, target organ toxicity was not identified in the 1-month studies, and no off-target toxicity was expected based on the specificity of the peptide. Although LY3298176 is a synthetic peptide less than 40 amino acids in length that contains a non-peptide sidechain and would typically be subject to the guidelines outlined in ICH M3(R2), ICH S2(R1) and ICH M7, principles outlined in ICH S6 could be applied in this situation as longer duration toxicology studies would not be expected to identify additional toxicities.

Figure 11: Mean Body Weights in Male Monkeys

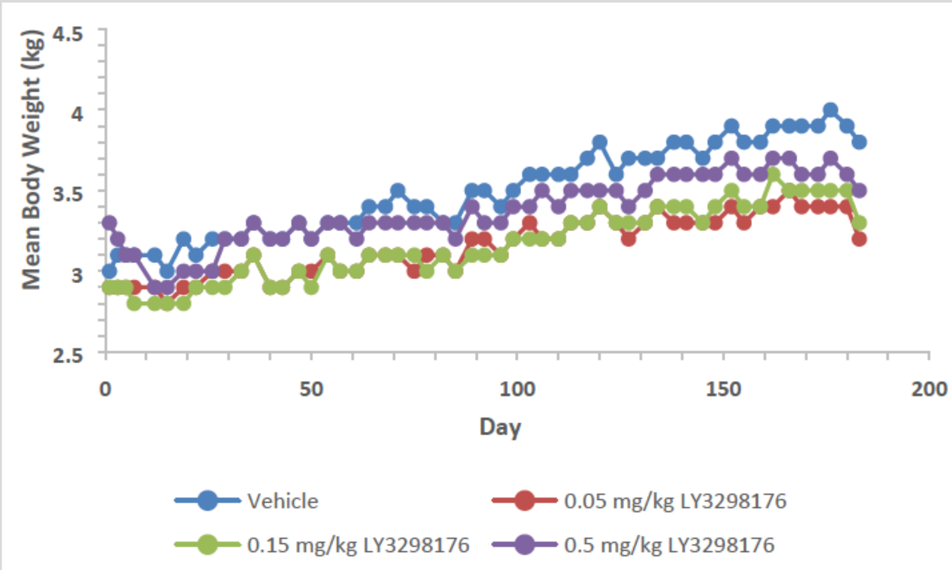


Figure prepared by Elena Braithwaite.

Figure 12: Mean Body Weights in Female Monkeys

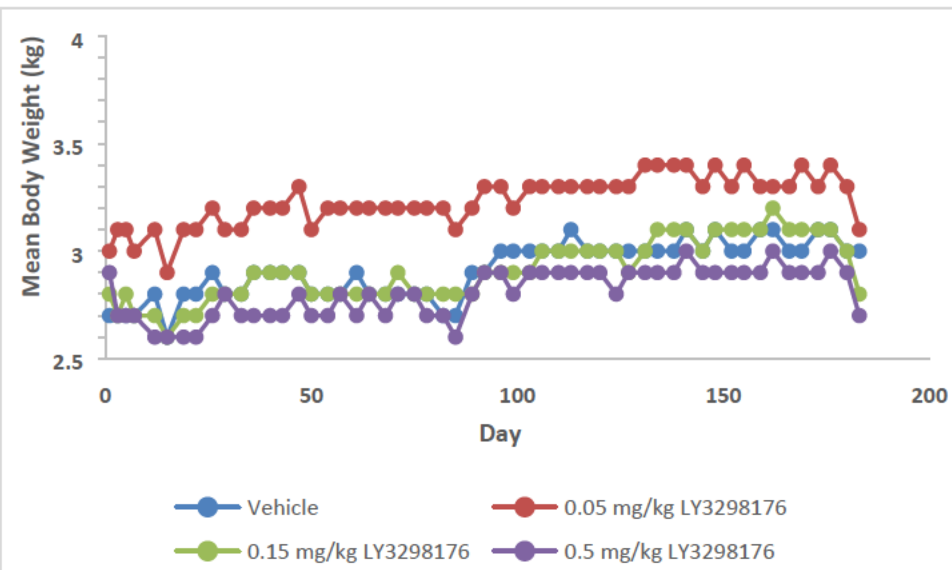


Figure prepared by Elena Braithwaite.

Table 18: Mean Heart Rates in the 6-Month Monkey Study

Heart Rate (beats/minute)																
Dose (µg/kg)	Males								Females							
	Pre-Test		Day 8		Day 78		Day 169		Pre-Test		Day 8		Day 78		Day 169	
	Block 1	Block 2	Block 1	Block 2	Block 1	Block 2	Block 1	Block 2	Block 1	Block 2	Block 1	Block 2	Block 1	Block 2	Block 1	Block 2
0	116 (111-122)	104 (95-109)	121 (226-129)	99 (97-101)	123 (116-133)	102 (100-105)	123 (117-131)	109 (103-113)	114 (111-118)	100 (98-104)	119 (110-128)	100 (98-102)	125 (118-145)	104 (100-107)	121 (115-137)	115 (107-118)
0.05	126 (119-131)	111 (107-114)	127 (122-139)	111 (106-115)	129 (120-143)	110 (104-112)	122 (109-137)	104 (96-108)	134 (121-154)	115 (112-119)	**139*** (133-147)	121 (117-125)	133 (128-139)	**122*** (118-124)	**145*** (139-158)	130 (125-134)
0.15	135 (127-143)	113 (109-115)	136 (133-141)	125 (122-128)	139 (135-144)	**129*** (125-131)	140 (137-145)	**131*** (123-133)	122 (110-145)	101 (99-105)	**130*** (113-157)	109 (102-113)	130 (119-143)	115 (110-117)	127 (117-146)	114 (108-121)
0.5	109 (105-115)	103 (99-107)	123 (120-128)	128 (118-135)	130 (125-134)	**131*** (120-137)	131 (121-139)	124 (119-129)	117 (105-129)	103 (100-103)	126 (121-134)	118 (114-124)	**142*** (136-145)	**137*** (131-142)	**146*** (138-158)	131 (125-138)
% Change from Pre-Test																
0	-	-	+4.3%	-4.8%	+6.0%	-1.9%	+6.0%	+4.8%	-	-	+4.4%	0%	+9.6%	+4.0%	+6.1%	+15.0%
0.05	-	-	+0.8%	0%	+2.4%	-0.9%	-3.2%	-6.3%	-	-	+3.7%	+5.2%	+0.7%	+6.1%	+8.2%	+13.0%
0.15	-	-	+0.7%	+10.6%	+3.0%	+14.2%	+3.7%	+15.9%	-	-	+6.6%	+7.9%	+6.6%	+13.9%	+4.1%	+12.9%
0.5	-	-	+12.8%	+24.3%	+19.3%	+27.2%	+20.2%	20.4%	-	-	+7.7%	+14.6%	+21.4%	+33.0%	+24.8%	+27.2%

Block 1 = 1 to 7 hours postdose
Block 2 = 9 to 19 hours postdose, corresponding to T_{max}
Bold font = * p value < 0.05
Outside pre-test range
Greater % change than controls: ♂ > +6.0%, ♀ > +15.0%
( ) = range of individual mean values at each time point within the block

Table prepared by Jessica Hawes (IND 128801, entered into DARRTS on 10/03/2018 – Table 6).

Table 19: Incidence of LY3298176-Related Decrease in Pancreatic Zymogen Granules – Terminal Sacrifice

Test Article	(dosage)	1	2	3	4				
LY3298176	mg/kg	0	0.05	0.15	0.5				
Tissue/ Observation	Group/Sex: Number of Animals:	1/M 4	2/M 4	3/M 4	4/M 4	1/F 4	2/F 4	3/F 4	4/F 4
Pancreas	Number Examined:	4	4	4	4	4	4	4	4
	Unremarkable:	2	3	2	3	1	2	1	0
Zymogen granules, decreased									
	finding not present -	4	4	4	4	4	4	4	2
	minimal 1	0	0	0	0	0	0	0	2
	Total Incidence:	0	0	0	0	0	0	0	2

Table modified from the applicant's submission (Text Table 5.3).

7 Genetic Toxicology

LY3298176 contains novel non-peptide sidechains. Therefore, to examine its genotoxic potential, an in vivo rat bone marrow micronucleus assay was performed. LY3298176 tested negatively for genotoxicity in an in vivo rat bone marrow micronucleus assay. Although a maximum tolerated dose was not established in this study, the highest dose examined did provide a 2.4-fold exposure multiple to the MRHD based on AUC and a 4-fold exposure multiple to the MRHD based on C_{max}.

8 Carcinogenicity

In a 2-year carcinogenicity study, Sprague-Dawley rats were subcutaneously administered LY3298176 (0, 0.15, 0.5, or 1.5 mg/kg/week or 0.12-, 0.36-, or 1.02-fold the MRHD). Dose-dependent and statistically significant increases in the incidence of C-cell neoplasms (adenomas and combined adenomas and carcinomas) were observed in the thyroid gland of LY3298176-treated dose groups. Pairwise comparisons revealed a statistically significant increase in the incidences of C-cell adenomas (at doses ≥0.5 mg/kg twice weekly) and combined C-cell adenomas and carcinomas (at all doses examined) in the thyroid of male rats when compare to the vehicle control group. In females, pairwise comparisons showed a statistically significant increase in the incidences of C-cell adenomas and combined C-cell adenomas and carcinomas for all dose groups examined. While the human relevance of GLP-1R agonist-induced C-cell tumor formation in rodents is still not clear, species differences in GLP-1 receptor expression and action in the thyroid potentially indicate greater rodent sensitivity to this effect. In rodents, GLP-1Rs localized to C-cells stimulated calcitonin release, up-regulation of calcitonin gene expression, and subsequently C-cell hyperplasia. In contrast, humans and cynomolgus monkeys had low GLP-1R expression in thyroid C-cells, and GLP-1R agonists did not activate adenylate cyclase or generate calcitonin release in primates (Knudsen et al. 2010). Due to low survival rates in control rats, all surviving rats across groups were sacrificed prior to the scheduled terminal sacrifice at Week 104 (surviving female rats were sacrificed at Week 84 and surviving males were sacrificed at Week 87). Pituitary adenomas were the most common cause of deaths and moribund condition in both control and LY3298176-treated rats and is consistent with previous studies showing that pituitary adenomas are a common spontaneous tumor in Sprague Dawley rats with an incidence ranging between 31 and 70% (Tennekes, Gembardt et al. 2004). Premature euthanasia was consistent with the general stopping criteria for carcinogenicity studies and was conducted with concurrence from the Executive Carcinogenicity Assessment Committee (09/08/2020). Dose-dependent decreases in body weight that correlated with decreased food consumption were observed over the duration of the study. Decreases in body weight reached statistical significance at all doses examined starting on Dosing Day 8 or 50 for males and females, respectively, and generally became more severe over the duration of the study.

Table 20: Tumor Types with P-Values ≤ 0.05 for Dose Response Relationship or Pairwise Comparisons between Treated Groups and Control Groups in Rats

Sex	Organ Name	Tumor Name	0 mg Vehicle Cont (N=60) P - Trend	0.15 mg Low (N=60) P - VC vs. L	0.5 mg Med (N=60) P - VC vs. M	1.5 mg High (N=60) P - VC vs. H
Male	Thyroid	B-Adenoma, C-cell	6/60 (27) <0.0001*	19/60 (37) 0.0169@	29/60 (41) 0.0001*	38/60 (46) <0.0001*
		M-Carcinoma, C-cell	0/60 (24) 0.4799	4/60 (29) 0.0811	8/60 (30) 0.0056*	2/60 (26) 0.2653
		B-Adenoma/ M-Carcinoma, C-cell	6/60 (27) <0.0001*	20/60 (37) 0.0099*	33/60 (43) <0.0001*	39/60 (46) <0.0001*
Female	Thyroid	B-Adenoma, C-cell	4/59 (24) 0.0006*	19/60 (35) 0.0035*	30/60 (40) <0.0001*	31/60 (43) <0.0001*
		M-Carcinoma, C-cell	1/59 (22) 0.0561	3/60 (26) 0.3708	2/60 (25) 0.5489	6/60 (30) 0.1129
		Adenoma/ Carcinoma, c-cell	5/59 (24) 0.0004*	20/60 (35) 0.0055*	30/60 (40) <0.0001*	34/60 (45) <0.0001*

Table copied from the Statistical Review and Evaluation by Malick Mbodj. & X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
*: Statistically significant at 0.005 and 0.025 level for common and rare tumor or 0.01 and 0.05 level for common and rare tumors for tests of dose response relationship and pairwise comparison, respectively.
@: Not statistically significant at 0.01 for common tumors for pairwise comparison.

In a 26-week carcinogenicity study, RasH2 transgenic mice subcutaneously administered LY3298176 (0, 1, 3, or 10 mg/kg twice weekly) did not have increased incidences of neoplastic findings when analyzed by trend or pairwise comparisons. In contrast, a statistically significant increase in the incidences of malignant lymphoma in the hematolymphoid system, benign squamous cell papilloma in the skin/subcutis, and benign, squamous cell papilloma in the non-glandular stomach were observed in the group receiving N-methyl-N-nitrosourea (75 mg/kg) as a positive control, indicating that the RasH2 model worked as intended. Similar to findings in the rat carcinogenicity study, statistically significant decreases in body weight were observed in LY3298176-treated mice at all doses examined, which prompted the applicant to offer DietGel as a dietary supplement during the study.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Fertility and early embryonic-development studies were conducted in male and female rats where LY3298176 (0, 0.5, 1.5, or 3.0 mg/kg) was subcutaneously administered twice weekly. Male rats, dosed with LY3298176 for 4 weeks prior to mating, throughout mating and continuing until euthanasia at mid-pregnancy, did not experience adverse changes in reproductive parameters (mating index, male fertility index, male copulation index, and mean precoital interval) despite the animals experiencing adverse decreases in body weight when compared to control rats at all dose levels (≥0.3 times the MRHD). Female rats were dosed with LY3298176 from 2 weeks prior to mating, throughout cohabitation, and until gestation day (GD) 6. Decreases in food consumption and weight loss were observed at all doses during the pre-mating period (≥0.5 mg/kg or ≥0.3 times the MRHD). Additionally, an increased number of animals were observed at all doses with prolonged estrous cycles or persistent/prolonged diestrus. Decreases in the numbers of corpora lutea, implantation sites, and viable embryos were also observed (≥1.5 mg/kg or ≥1 time the MRHD). These findings were likely secondary to the pharmacodynamic-related effects on food consumption and body weight.

9.2 Embryonic Fetal Development

Embryo-fetal development (EFD) studies were conducted in rats and rabbits. In rats, pregnant dams were subcutaneously administered LY3298176 (0, 0.02, 0.1, or 0.5 mg/kg) on GD 6, 10, 13, and 17. On GD 20, statistically significant decreases in dam body weights (↓6-13%) and adverse decreases in weight gains (↓12-26%) compared to control rats were observed at doses ≥0.1 mg/kg (0.07 times the MRHD). Uterine weights were significantly decreased at 0.5 mg/kg (0.45 times the MRHD). Decreases in fetal weights (↓18-20%) and increases in the incidences of external (fetal anasarca, proboscis-like nose, microstomia), visceral (double aorta), and skeletal (rib anomaly) malformations, and visceral (major blood vessel variation) and skeletal (reduced ossification of the vertebral arches, unossified sternebra(e), and reduced ossification of the skull) variations in fetuses and litters were observed in the 0.5 mg/kg dose group (0.45 times the MRHD). While fetal anasarca has been previously observed in fetuses born to dams on a feed restricted diet, the incidences of malformations (proboscis-like nose double aorta, rib anomaly) exceeded ranges observed in historical controls from the contract research organization. A statistically significant increase in the incidence of major blood vessel variation was also observed that exceeded historical control ranges. These findings were observed in fetuses from different litters; therefore, the increased incidence of malformations after LY3298176 maternal exposure appear to be drug related. Therefore, the NOAEL for maternal toxicity was 0.02 mg/kg (0.03 times the MRHD) based on LY3298176-mediated adverse decreases in mean body weight observed at higher doses. The NOAEL for embryo-fetal development was 0.1 mg/kg (0.07 times the MRHD) based on lower mean fetal body weights and increased incidences of malformations and developmental variations observed at higher doses.

Figure 13: Maternal Body Weights in Rats

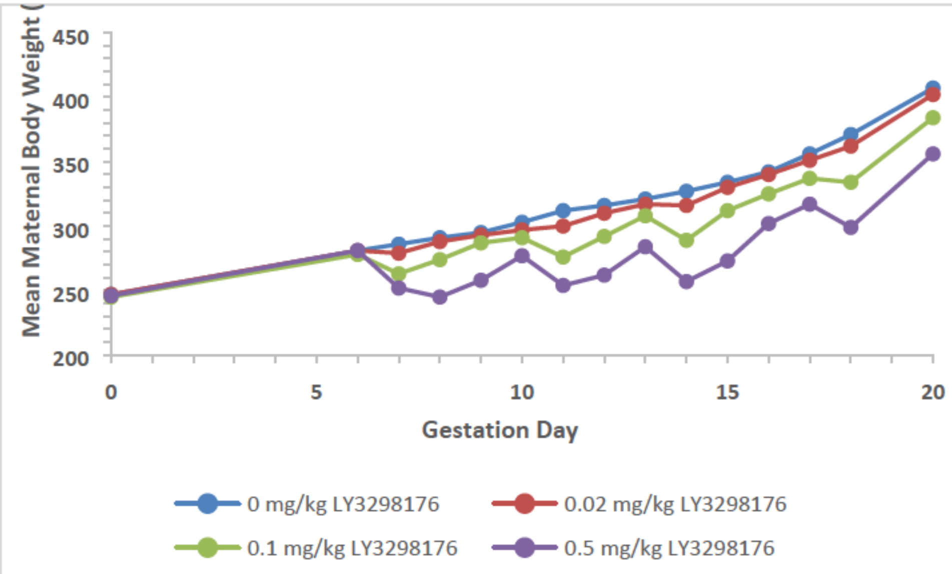


Figure prepared by Elena Braithwaite.

Table 21: Summary of Gestation Body Weights, Body Weight Changes and Uterine Weights in Rats

Dose (mg/kg):	0[a]	0.02	0.1	0.5
Body weight (g;%)				
Gestation Day 7	286	279 (-2.4)	263 (-8.0)**	252 (-11.9)**
Gestation Day 8	291	288 (-1.0)	274 (-5.8)**	245 (-15.8)**
Gestation Day 9	295	293 (-0.7)	287 (-2.7)	258 (-12.5)**
Gestation Day 10	303	297 (-2.0)	291 (-4.0)**	277 (-8.6)**
Gestation Day 11	312	300 (-3.8)*	276 (-11.5)**	254 (-18.6)**
Gestation Day 12	316	310 (-1.9)	292 (-7.6)**	262 (-17.1)**
Gestation Day 13	321	317 (-1.2)	308 (-4.0)**	284 (-11.5)**
Gestation Day 14	327	316 (-3.4)	289 (-11.6)**	257 (-21.4)**
Gestation Day 15	334	330 (-1.2)	312 (-6.6)**	273 (-18.3)**
Gestation Day 16	342	340 (-0.6)	325 (-5.0)**	302 (-11.7)**
Gestation Day 17	356	351 (-1.4)	337 (-5.3)**	317 (-11.0)**
Gestation Day 18	371	362 (-2.4)	334 (-10.0)**	299 (-19.4)**
Gestation Day 20	407	402 (-1.2)	384 (-5.7)**	356 (-12.5)**
Body weight change (g)				
Gestation Days 6-10	23	16**	13**	-4**
Gestation Days 6-7	5	-2**	-15**	-29**
Gestation Days 10-13	17	20	17	8**
Gestation Days 13-14	6	-1**	-19**	-28**
Gestation Days 13-18	50	44	27**	15**
Gestation Days 18-20	36	40	50**	57**
Gestation Days 6-18	90	81*	56**	18**
Uterine weight (g)	85.6	86.6	83.5	68.8**
Net body weight (g)	321.5	315.5	300.9**	287.2**
Net body weight gain (g)	74.7	68.8	55.7**	41.1**

[a] Vehicle control.

*p<0.05. **p<0.01.

% = Percent difference from the vehicle control group.

Table modified from the applicant's submission

Table 22: Fetal Weights in Rats

GROUP:	0 MG/KG	0.02 MG/KG	0.1 MG/KG	0.5 MG/KG
MALE FETAL WEIGHTS (g)				
MEAN	4.1	4.0	4.0	3.3**
% DIFFERENCE		-2.4	-2.4	-19.5
S.D.	0.30	0.21	0.29	0.43
S.E.	0.06	0.04	0.06	0.09
N	24	25	25	24
FEMALE FETAL WEIGHTS (g)				
MEAN	3.8	3.9	3.8	3.1**
% DIFFERENCE		2.6	0.0	-18.4
S.D.	0.25	0.23	0.29	0.47
S.E.	0.05	0.05	0.06	0.10
N	24	25	25	24
COMBINED FETAL WEIGHTS (g)				
MEAN	4.0	3.9	3.9	3.2**
% DIFFERENCE		-2.5	-2.5	-20.0
S.D.	0.29	0.21	0.26	0.44
S.E.	0.06	0.04	0.05	0.09
N	24	25	25	24

PROPORTIONAL (%) DATA COMPARED USING DUNN'S TEST
FETAL WEIGHTS COMPARED USING DUNN'S TEST
MODIFIED STATISTICS USED. * INDICATES PARAMETRIC ANALYSIS AND + INDICATES NON-PARAMETRIC ANALYSIS.
** = Significantly different from the control group at 0.01

Table modified from the applicant's submission (Table S11).

Table 23: Summary of Noteworthy Malformations and Variations in Rat Fetuses

Dose (mg/kg):	0a	0.02	0.1	0.5	WIL HC mean (range)
External developmental malformations:					
Absolute No. (% per litter)					
Fetal anasarca	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)	0.00 (0.00-0.24)
Proboscis-like nose	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	0.00 (0.00-0.00)
Microstomia	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	0.00 (0.00-0.29)
Microphthalmia and/or anophthalmia	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	0.03 (0.00-0.58)
Visceral developmental malformations:					
Absolute No. (% per litter)					
Situs inversus	0 (0.0)	1 (0.3)	0 (0.0)	2 (0.6)	0.06 (0.00-1.14)
Interventricular septal defect	0 (0.0)	1 (0.3)	0 (0.0)	1 (0.3)	0.01 (0.00-0.33)
Lungs - lobular dysgenesis	0 (0.0)	1 (0.3)	0 (0.0)	1 (0.3)	0.05 (0.00-0.67)
Double aorta	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)	0.00 (0.00-0.00)
Visceral developmental variations:					
Absolute No. (% per litter)					
Major blood vessel variation	5 (1.6)	3 (0.8)	0 (0.0)	28 (8.7)*	0.13 (0.00-1.01)
Skeletal developmental malformations:					
Absolute No. (% per litter)					
Rib anomaly	0 (0.0)	0 (0.0)	0 (0.0)	6 (1.9)	0.06 (0.00-0.84)
Costal cartilage anomaly	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.6)	0.03 (0.00-1.67)
Bent limb bones	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.8)	0.02 (0.00-1.45)
Skeletal developmental variations:					
Absolute No. (% per litter)					
Sternebra(e) nos. 5 and/or 6 unossified	12 (3.5)	11 (3.1)	7 (1.8)	43 (14.1)	6.79 (0.70-19.86)
Reduced ossification of the 13th rib(s)	1 (0.2)	2 (0.5)	4 (1.1)	9 (2.7)	0.75 (0.00-2.80)
Cervical centrum no. 1 ossified	75 (22.7)	67 (18.7)	70 (19.8)	13 (3.8)**	19.23 (1.39-30.12)
Reduced ossification of the vertebral arches	1 (0.3)	0 (0.0)	1 (0.3)	9 (3.4)	0.36 (0.00-2.36)
Sternebra(e) nos. 1, 2, 3, and/or 4 unossified	0 (0.0)	0 (0.0)	0 (0.0)	26 (8.2)	0.25 (0.00-1.46)
Reduced ossification of the skull	1 (0.3)	0 (0.0)	0 (0.0)	8 (2.9)	0.29 (0.00-2.36)

All data are expressed as mean values for the group, except for malformation frequency.

a Vehicle control.

*p<0.05. **p<0.01.

Table copied from the applicant's submission.

In the rabbit EFD study, time-mated does were subcutaneously administered 0, 0.01, 0.03, or 0.1 mg/kg LY3298176 on GD 7 and 14. Mortalities and spontaneous abortions were observed at all doses examined and were associated with LY3298176-mediated decreases in food consumption and mean body weights (≥0.01 times the MRHD). Decreases in mean fetal weights (up to ↓8%) were observed at the highest dose examined (0.1 mg/kg or 0.23 times the MRHD) but did not reach statistical significance. Since LY3298176-related maternal toxicity was present at all doses, a NOAEL for maternal toxicity could not be determined. The NOAEL for embryo-fetal development was 0.03 mg/kg (0.06 times the MRHD) due to lower mean fetal body weights caused by the expected pharmacodynamic activity of LY3298176.

Figure 14: Maternal Body Weights in Rabbits

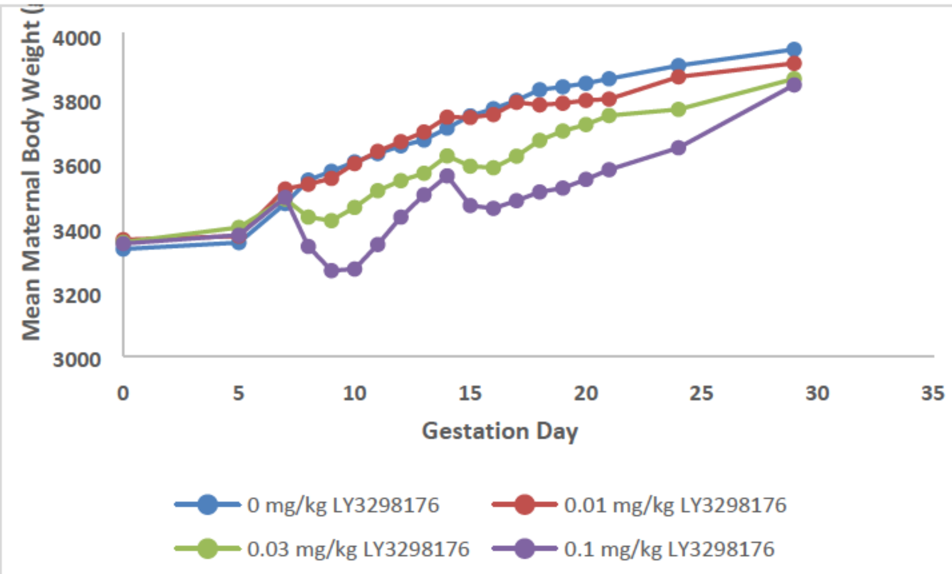


Figure prepared by Elena Braithwaite.

Table 24: Summary of Gestation Body Weight, Body Weight Changes and Uterine Weights in Rabbits

Dose (mg/kg):	0[a]	0.01	0.03	0.1
Mean body weight (g) (%)				
Gestation Day 7	3473	3518 (1.3)	3487 (0.4)	3492 (0.5)
Gestation Day 8	3546	3533 (-0.4)	3431 (-3.2)	3340 (-5.8)**
Gestation Day 9	3573	3551 (-0.6)	3420 (-4.3)	3265 (-8.6)**
Gestation Day 10	3602	3597 (-0.1)	3461 (-3.9)	3270 (-9.2)**
Gestation Day 11	3628	3635 (0.2)	3513 (-3.2)	3346 (-7.8)**
Gestation Day 12	3652	3665 (0.4)	3544 (-3.0)	3431 (-6.1)**
Gestation Day 13	3670	3695 (0.7)	3567 (-2.8)	3500 (-4.6)
Gestation Day 14	3707	3741 (0.9)	3621 (-2.3)	3559 (-4.0)
Gestation Day 15	3745	3740 (-0.1)	3589 (-4.2)	3467 (-7.4)**
Gestation Day 16	3767	3749 (-0.5)	3584 (-4.9)*	3458 (-8.2)**
Gestation Day 17	3793	3787 (-0.2)	3620 (-4.6)	3482 (-8.2)**
Gestation Day 18	3826	3779 (-1.2)	3669 (-4.1)	3509 (-8.3)**
Gestation Day 19	3835	3784 (-1.3)	3698 (-3.6)	3521 (-8.2)**
Gestation Day 20	3846	3793 (-1.4)	3718 (-3.3)	3548 (-7.7)**
Gestation Day 21	3860	3797 (-1.6)	3746 (-3.0)	3578 (-7.3)**
Gestation Day 24	3901	3866 (-0.9)	3765 (-3.5)	3646 (-6.5)*
Gestation Day 29	3951	3908 (-1.1)	3860 (-2.3)	3841 (-2.8)
Mean body weight change (g)				
Gestation Days 7–10	130	79	-26**	-223**
Gestation Days 10–14	105	144	161*	290**
Gestation Days 7–14	235	222	134*	67**
Gestation Days 14–17	85	46	-1**	-77**
Gestation Days 17–21	68	52	126	98
Gestation Days 14–21	153	103	125	34*
Gestation Days 7–21	388	306	259	105**
Gestation Days 21–29	90	94	111	212**
Mean gravid uterine weight (g)	500.9	508.6	503.2	459.4
Mean net body weight (g)	3449.7	3399.8	3356.5	3381.4
Mean net body weight change (g)	117.4	73.7	-0.4	13.1

[a] Vehicle control.

% = Percent difference from the vehicle control group.

*$p<0.05$. **$p<0.01$.

Table copied from the applicant's submission.

Table 25: Summary of Fetal Body Weights in Rabbits

Dose (mg/kg):	0[a]	0.01	0.03	0.1	CRL HC Mean (Range)[b]
Mean male fetal weights (g)	43.9	41.1	41.9	39.6	41.609 (38.220-44.758)
Mean female fetal weights (g)	41.8	41.0	39.6	39.1	40.625 (36.994-43.186)
Mean combined fetal weights (g)	42.7	41.0	41.1	39.3	41.151 (38.097-43.834)

All data expressed as mean values for the group

[a] Vehicle control.

[b] Charles River Ashland Historical Control Data (v2016.01).

Table modified from the applicant's submission

Table 26: Summary of Maternal Survival and Pregnancy Status

DOSE GROUP :	1 NO.	1 %	2 NO.	2 %	3 NO.	3 %	4 NO.	4 %
FEMALES ON STUDY	22		22		22		22	
FEMALES THAT ABORTED OR DELIVERED	0	0.0	0	0.0	1	4.5	2	9.1
FEMALES THAT DIED	0	0.0	3	13.6	1	4.5	2	9.1
FEMALES THAT ABORTED	0	0.0	1	33.3	0	0.0	0	0.0
NONGRAVID	0	0.0	0	0.0	0	0.0	0	0.0
GRAVID	0	0.0	2	66.7	1	100.0	2	100.0
FEMALES THAT WERE EUTHANIZED	0	0.0	0	0.0	0	0.0	1	4.5
NONGRAVID	0	0.0	0	0.0	0	0.0	0	0.0
GRAVID	0	0.0	0	0.0	0	0.0	1	100.0
FEMALES EXAMINED AT SCHEDULED NECROPSY	22	100.0	19	86.4	20	90.9	17	77.3
NONGRAVID	0	0.0	0	0.0	0	0.0	1	5.9
GRAVID	22	100.0	19	100.0	20	100.0	16	94.1
WITH RESORPTIONS ONLY	0	0.0	0	0.0	0	0.0	0	0.0
WITH VIABLE FETUSES	22	100.0	19	100.0	20	100.0	16	100.0
TOTAL FEMALES GRAVID	22	100.0	22	100.0	22	100.0	21	95.5

1- 0 MG/KG 2- 0.01 MG/KG 3- 0.03 MG/KG 4- 0.1 MG/KG

Table copied from the applicant's submission (Table S1).

9.3 Prenatal and Postnatal Development

In a pre- and postnatal development study, rats were subcutaneously administered LY3298176 (0, 0.02, 0.1, or 0.25 mg/kg) on GDs 6, 10, 13, 17, and 20, and on Lactation Days 4, 7, 11, 14, and 18. Pregnant dams experienced statistically significant, dose-related decreases in food consumption and decreases in body weight (4-7% at GD 20 and 3-5% at Lactation Day 18) throughout gestation and lactation at doses ≥0.1 mg/kg when compared to control rats. Maternal exposure to LY3298176 (≥0.1 mg/kg) resulted in lower mean body weights in F1 pups and adverse decreases in F1 pup body weight at the highest dose examined (0.25 mg/kg) from birth or soon thereafter through postnatal day 91.

Table 27: Summary of Maternal Body Weights – Gestation (g; % difference from control)

Gestation Day	0 mg/kg	0.02 mg/kg	0.10 mg/kg	0.25 mg/kg
0	222	224 (0.9)	225 (1.4)	221 (-0.5)
5	247	249 (0.8)	252 (2.0)	247 (0.0)
6	254	255 (0.4)	256 (0.8)	253 (-0.4)
10	276	276 (0.0)	273 (-1.1)	267 (-3.3)
13	290	287 (-1.0)	283 (-2.4)	272** (-6.2)
17	328	323 (-1.5)	316* (-3.7)	304** (-7.3)
20	369	363 (-1.6)	353* (-4.3)	342** (-7.3)

* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

Table copied from the applicant's submission (Text Table 19).

Table 28: Summary of Maternal Body Weights – Lactation (g; % difference from control)

Lactation Day	0 mg/kg	0.02 mg/kg	0.10 mg/kg	0.25 mg/kg
1	287	280 (-2.4)	273* (-4.9)	259** (-9.8)
4	304	302 (-0.7)	290* (-4.6)	281** (-7.6)
7	314	313 (-0.3)	296** (-5.7)	284** (-9.6)
11	318	318 (0.0)	298** (-6.3)	288** (-9.4)
14	321	320 (-0.3)	300** (-6.5)	292** (-9.0)
18	313	315 (0.6)	303 (-3.2)	296** (-5.4)
21	308	312 (1.3)	304 (-1.3)	301 (-2.3)

* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

Table copied from the applicant's submission (Text Table 22).

Table 29: Summary of Body Weights – Offspring Weights (g; % difference from control)

Postnatal Day	0 mg/kg	0.02 mg/kg	0.10 mg/kg	0.25 mg/kg
Males				
1	7.6	7.7 (1.3)	7.7 (1.3)	6.9** (-9.2)
4	11.4	11.5 (0.9)	11.3 (-0.9)	10.7 (-6.1)
7	17.9	17.9 (0.0)	16.9 (-5.6)	15.6** (-12.8)
10	24.7	24.6 (-0.4)	22.5* (-8.9)	20.2** (-18.2)
14	34.3	34.8 (1.5)	31.1* (-9.3)	27.7** (-19.2)
21	54.7	55.5 (1.5)	52.1 (-4.8)	47.0** (-14.1)
Females				
1	7.3	7.3 (0.0)	7.2 (-1.4)	6.8 (-6.8)
4	10.9	11.1 (1.8)	10.9 (0.0)	10.4 (-4.6)
7	17.1	17.4 (1.8)	16.3 (-4.7)	15.3** (-10.5)
10	23.7	24 (1.3)	21.8 (-8.0)	19.9** (-16.0)
14	33.1	33.8 (2.1)	30.6 (-7.6)	27.6** (-16.6)
21	52	54 (3.8)	51.1 (-1.7)	46.7** (-10.2)

* = Significantly different from the control group at 0.05 using Dunnett's test
** = Significantly different from the control group at 0.01 using Dunnett's test

Table copied from the applicant's submission (Text Table 23).

9.4 Juvenile Toxicology

In a juvenile toxicity study where rats were subcutaneously treated with 0, 0.15, 0.5, or 1.5 mg/kg LY3298176 every 3 days from post-natal day (PND) 21 through 84, adverse decreases in body weight that exceeded 10% (≥0.5 mg/kg in males and females), and decreased body weight gain that were associated with decreased food consumption were observed. Additionally, adipocyte atrophy in the skin/subcutis and changes in red blood cell parameters were noted. These findings are consistent with previously performed repeat dose toxicity studies of 1 and 6 months of duration in rats and are related to the pharmacological activity of the test article. Therefore, the NOAEL for development in the juvenile toxicity study was the highest dose examined (1.5 mg/kg).

12 Appendix/Attachments

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