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ARTICLE

Effects of genotype and food on naltrexone exposure in adolescents

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Abstract

Naltrexone (NTX), an opioid antagonist metabolized by aldo-keto reductase 1C4 (AKR1C4), is prescribed for psychiatric conditions like eating disorders with variable response. Systemic exposure is highly variable in adults, yet no data exist in children. The purpose of this study was to evaluate NTX exposure in adolescents with eating disorders. Adolescents aged 12–21 years with eating disorders underwent postdose blood sampling in the fasted and/or fed state. NTX and primary active metabolite, 6- β -naltrexol, were determined by ultra-high performance liquid chromatography tandem mass spectrometry. Pharmacokinetic parameters were determined by non-compartmental analysis. DNA was genotyped for *AKR1C4* missense mutations associated with decreased activity (rs3829125 and rs17134592). Linear mixed effects modeling was performed. In 21 participants, aged 16.9 ± 1.9 years (15–21 years), 81% female participants, maximum concentration (C_{\max}) was 90.4 ± 129 nM/mg/kg, area under the concentration-time curve from zero to infinity ($AUC_{0-\infty}$) was 166 ± 154 nMh/mg/kg, and varied 63-fold and 21-fold, respectively. Compared with wildtype, those with *AKR1C4* allelic variations ($n = 7$) displayed 3.2-fold higher $AUC_{0-\infty}$, four-fold higher C_{\max} and delayed time to T_{\max} . Linear mixed effects modeling demonstrated a large effect of genotype on $AUC_{0-\infty}$ (Cohen's $d = 2.3$) and C_{\max} (Cohen's $d = 1.4$). Food effect was large for $AUC_{0-\infty}$ (Cohen's $d = 2.6$), but highly variable and failed to reach significance for C_{\max} . The respective model accounted for 82% of the variance in NTX $AUC_{0-\infty}$ and 46% of the variance in C_{\max} . NTX systemic exposure is highly variable in adolescents with eating disorders and modulated, in part, by *AKR1C4* genotype and food intake. These findings may, in part, explain the large degree of interindividual variability observed response to NTX.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Naltrexone (NTX), an opioid antagonist metabolized by the cytosolic enzyme AKR1C4, is widely used across the lifespan for compulsive and impulsive

*Affiliations at the time work was completed.

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conditions including eating disorders. Therapeutic response is variable ranging from 40% to 80%. Systemic exposure is also highly variable in adults, yet no data exist in children.

WHAT QUESTION DID THIS STUDY ADDRESS?

What is the systemic exposure to NTX in adolescents and what is the impact of food and genotype on the interindividual variability in exposure?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Systemic exposure to NTX, a commonly used opioid antagonist, is highly variable which may contribute to variability in therapeutic response. Previously uncharacterized genetic variation in the drug metabolizing enzyme, AKR1C4, and a food effect contributes to this variability.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Individual level factors identified in this study are necessary to inform the design of exposure-response studies aimed at guiding model-informed individualized dosing in the future.

INTRODUCTION

Naltrexone (NTX) is a nonselective opioid antagonist used off-label to treat numerous pediatric conditions,¹ yet response is variable and systemic exposure in this population remains undefined. NTX targets reward-related alterations associated with impulsive and compulsive behaviors, like purging and binge eating seen in eating disorders.¹ NTX blocks opioid-mediated positive reinforcement, ideally making the behavior more easily extinguished. Eating disorders are associated with substantial morbidity (e.g., cardiac compromise, malnutrition, and substance use disorder) and mortality rates 2- to 6-fold higher than the general population.² Although NTX is a promising treatment for eating disorders, one in three adolescents fail to respond.³

Variability in drug exposure may be an underlying contributor to variability in response. NTX exposure is highly variable in adults (>10 fold), although no pharmacokinetic (PK) data exist in adolescents. By extension, the optimal dose to treat eating disorders remains unknown⁴⁻⁷ resulting in doses ranging from 50 to 400 mg being used to reduce eating disorder behaviors.¹ Individual level factors that may impact exposure include age, genetics, and disease-related changes in disposition pathways.

Naltrexone is primarily metabolized by aldo-keto reductase 1C4 (AKR1C4), with minor contributions from other cytosolic enzymes.^{1,8} AKR1C4 is expressed primarily in the liver and is involved in endogenous processes, like lipid metabolism.⁹ Variability along these endogenous pathways may contribute to differential *AKR1C4* regulation and, in turn, alter NTX metabolism. For example, *AKR1C4* expression is induced by bile acid metabolites via liver X receptor (LXR).¹⁰ AKR1C4 activity is inhibited by

fatty acids at physiologically relevant plasma concentrations.^{11,12} Both bile acid metabolites and fatty acid concentrations change in response to food intake.¹³⁻¹⁵ Together, this suggests the possibility of acute dietary modulation of AKR1C4; however, the clinical relevance is unknown. There are little data regarding a food effect on NTX systemic exposure as no fed/fasted bioequivalence or well-controlled dietary studies are available.

Other factors to consider that could impact AKR1C4 activity and NTX metabolism include ontogeny and genetic variation. Although developmental differences impacting the expression and activity of various drug metabolizing enzymes (e.g., CYP2D6, CYP2C19, and CYP3A) are well-documented,¹⁶ age appears to have a limited impact on AKR1C4 activity.¹⁷ Genetic variation in *AKR1C4* may lead to altered NTX metabolism. In vitro data links two co-occurring AKR1C4 missense mutations, S145C and L311V, to decreased enzyme activity.^{17,18} The impact of these variants has yet to be investigated in patients; however, they may be a relevant contributor to variability given global allele frequencies of 5%–50%.^{19,20}

Finally, disease-related changes that effect relevant disposition pathways may contribute to altered drug exposure. Patients with eating disorders demonstrate reduced gastrointestinal (GI) transit time in active disease and an altered microbiome.²¹⁻²³ Oral NTX undergoes extensive first pass metabolism (bioavailability: 5%–40%) and readily crosses cell membranes via passive diffusion.¹ Thus, delayed GI transit time could alter the rate and extent of absorption. UDP-glucuronosyltransferase (UGT) enzymes (e.g., UGT2B7) are involved in glucuronide formation of both NTX and 6- β -naltrexol.²⁴ In adults, enterohepatic recycling from bacterial cleavage of the glucuronide and reabsorption of the active compound into systemic

circulation has been seen.^{25,26} Animal studies suggest that enterohepatic recycling may be impacted by an altered microbiome, but more evidence is needed in humans.²⁷ Thus, pathophysiologic delayed GI transit time and altered microbiome in the eating disorder population could alter the rate and extent of absorption.

Given the variability in response to NTX seen in adolescents with eating disorders, known variability in exposure in adults, and concurrent pathophysiology with the potential to impact drug disposition, it is imperative to define systemic exposure in this clinical population. To address this knowledge gap, this study aims to define the PKs of NTX in adolescents with eating disorders. Findings from this study are a first step in understanding the exposure-response relationship and will aid in the untangling of factors associated with NTX response and nonresponse.

MATERIALS AND METHODS

Study design

Data are presented from two prospectives, open label studies in adolescents with eating disorders characterized by binge eating and/or purging according to the Diagnostic and Statistical Manual of Mental Disorders – 5th edition (DSM-V; e.g., anorexia nervosa-binge/purge, bulimia nervosa, and binge eating disorder),²⁸ as diagnosed by their treating clinician (e.g., adolescent medicine provider or psychologist). The studies were approved by the Institutional Review Boards at Children's Mercy Hospital and the University of Kansas Medical Center.

Fasted cohort

Adolescents aged 13–21 years, as defined above, were prescribed oral NTX as part of their clinical care (dosages ranged from 25 to 100 mg) and completed an observational PK study visit at steady-state (≥ 4 days of prescribed dose). After ≥ 4 h of fasting, participants had blood drawn predose and over a 12-h time course following their NTX dose. Participants were allowed to eat their first meal 2 h after taking NTX and drink water ad lib. Participants could repeat the PK study visit if their prescriber initiated a NTX dose change.

Fed cohort

Adolescents aged 14–21 years, as defined above, who were not currently taking NTX (within ≥ 4 weeks), on a stable regimen if taking other medications (no dose/drug changes

≥ 4 weeks), had no opioid exposure in the past 7 days, had no prior hypersensitivity reaction to NTX, and were not pregnant were eligible for enrollment. NTX 50 mg was administered orally 2 h after eating a standardized meal designed by an eating disorder nutritionist (525 calories, 28% fat, 55% carbohydrate, and 18% protein). Participants ate their next standardized meal 1 h after administration of NTX. Blood was drawn predose and over a 7-h time course. The PK sampling scheme was abbreviated based on modeling of preliminary data from the fasted cohort.

For both cohorts, blood was collected in lavender EDTA vacutainers and then centrifuged at $1000 \text{ g} \times 10 \text{ min}$ for plasma separation. Plasma was aliquoted into cryovials and stored at -80°C until ultra-high performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) analysis.

Disease status/severity

The Clinical Global Impressions – Improvement (CGI-I) scale and the Eating Pathology Symptom Inventory (EPSI) were used as a proxy for disease status at time of study visit for fasted and fed conditions, respectively.

Clinical Global Impressions

Clinical Global Impressions-Severity (CGI-S) and Clinical Global Impressions-Improvement (CGI-I) scales are widely used in psychopharmacologic research to describe overall case severity and clinical improvement.²⁹ CGI-S and CGI-I were determined independently by two investigators (authors J.T. and M.V.) via electronic health record (EHR) review, with consensus reached through discussion (author M.V. is an eating disorder clinical expert and S.S. is the principal investigator). In the fasted cohort ($n = 13$), CGI-S was measured prior to NTX initiation to contextualize the clinical presentation requiring NTX therapy. CGI-I was measured at time of study visit to describe overall eating disorder treatment response at that timepoint. One participant was omitted from CGI-I do to lack of EHR documentation (NTX was initiated and the study was completed during an inpatient stay, and outpatient/ongoing care was obtained outside of the institution, thus no relevant EHR documentation was available).

Eating Pathology Symptom Inventory

The EPSI is a validated self-reported measure of symptoms and behaviors associated with eating disorder pathology.^{30,31} Participants in the fed cohort ($n = 8$) completed

an electronic version of the EPSI (copyright clearance obtained). Self-reported responses were compared with published normative data³² as a proxy for symptom burden/disease severity at the time of study day.

UPLC-MS/MS analysis and analytical method validation

Materials

Naltrexone (NTX), 6-beta-naltrexol (6βN), deuterium-labeled naltrexone (NTX-D3), and deuterium labeled 6-beta-naltrexol (6βN-D3) certified reference materials were purchased from Sigma Aldrich (St. Louis, MO). Ammonium hydroxide, optima grade methanol, and optima grade formic acid were also purchased from Sigma Aldrich. Pooled human plasma ($N = 50$, mixed sex) with ethylenediaminetetraacetic acid (EDTA) as anticoagulant was obtained from BioIVT (Westbury, NY).

Analytical method validation

The analytical method for determination of NTX and 6βN was developed and validated based upon the US Food and Drug Administration guidance.³³ Deuterium-labeled analytes served as internal standards (IS). Linear calibration curves consisted of plotting the peak area of the analyte divided by that of the IS versus the analyte concentration. Chromatography was performed on a Waters Acquity UPLC system (Waters, Milford, MA). A Raptor Biphenyl analytical column (1.8 μm, 2.1 × 100 mm) with a matching precolumn held at 60°C was used for separation (Restek, Bellefonte, PA). Mobile phases A and B consisted of 0.1% formic acid in water and 0.1% formic acid in methanol, respectively. The liquid chromatography gradient proceeded from 20 to 40% B over 3 min at a flowrate of 0.3 ml/min. All analyses were performed on a Waters Xevo TQ-XS triple quadrupole instrument equipped with an electrospray ion (ESI) source in multiple reaction monitoring (MRM) mode. The operating conditions were as follows: positive ESI polarity, capillary voltage 3.0 kV, cone voltage 10 V, cone gas 150 L/h, desolvation gas 800 L/h, desolvation temperature 450°C, and source temperature 150°C. The following MRM transitions were used to quantify the analytes: 342.3 → 270 for NTX, 344.3 → 254 for 6βN, 345.3 → 270 for NTX-D3 (IS), and 347.3 → 254 for 6βN-D3 (IS).

A 10-point calibration curve with both analytes in plasma matrix was prepared over the linear range of 0.1–100 nM. Plasma matrix standards and quality controls (QCs) were prepared by diluting standard solutions

20-fold into plasma. Within study (batch) QCs were prepared at three concentration levels 0.3, 3, and 80 nM. During method validation, lower limit of quantitation (LLOQ) QCs were included as well. Method accuracy and precision were within acceptable limits according to the FDA guidelines for a bioanalytical assay (accuracy 85%–115% of nominal and coefficient of variation [CV] < 15%; LLOQ accuracy 80%–120% of nominal and CV < 20%).

Plasma sample preparation

Oasis HLB 96-well μElution plates (Waters) were used to remove proteins and other compounds found in plasma that may compromise the sensitivity of the UPLC-MS/MS analysis. The plate was preconditioned with 200 μl methanol per well and washed twice with 200 μl water per well. A positive pressure manifold was used to move washes, sample, and elution buffer through the plate. Plasma (200 μl, standards, QCs, or samples) was mixed in a microcentrifuge tube with 0.3% formic acid in water with 5 nM IS (100 μl) and then centrifuged at 13,000 g for 10 min. Following centrifugation, 250 μl of the supernatant was then applied to the preconditioned plate. The samples were moved through the plate with gentle positive pressure followed by a wash of 200 μl 5% methanol and 5% NH₄OH in water. Samples were eluted from the plate into a fresh 96-well collection plate using two elution steps: 100 μl of 2% formic acid in 90% methanol/water, followed by 100 μl 100% methanol. The eluted samples were evaporated to dryness under nitrogen and then reconstituted in 150 μl 50% methanol/water. Participant samples were analyzed in triplicate with an acceptance threshold of ≤15% CV.

Within study cross-validation

A second method was developed and validated to lower the required plasma volume from 200 to 100 μl and expand the linear calibration curve for 6βN. Changes from the original method are outlined below: 6βN calibration curve 1–1000 nM with batch QCs of 3, 30, and 800 nM. Plasma (100 μl) was mixed with 50 μl of 0.3% formic acid in water with 10 nM IS and then centrifuged at 13,000 g for 10 min. Then, 125 μl of the supernatant was applied to preconditioned HLB plate. The wash and elution steps remained the same, but the final reconstitution solution was changed to 25% methanol/water. The gradient was changed to 10–30% B over 3 min at a flowrate of 0.35 ml/min. Cross-validation with the original method was performed according to the FDA guidance. We re-analyzed 10% of the participant samples selected to cover the low

to high concentration range. Ninety percent of our re-analyzed samples were within $\pm 20\%$ of the nominal value, exceeding the 67% recommended by the FDA, and indicating valid agreement.

Genotype analysis

DNA was extracted from whole blood using Qiagen AllPrep Mini kit (Qiagen, Germantown, MD). The presence of non-synonymous *AKR1C4* coding region single nucleotide polymorphisms (SNPs) rs3829125 and rs17134592, also referred to by their amino acid substitutions, S145C and L311V, respectively, was determined by Taq-Man genotype assays (Thermo Fisher Scientific, Waltham, MA; C__25595999_20; C__33709410_20). *AKR1C4* S145C and L311V are associated with reduced naltrexone biotransformation in vitro, but the impact of these variants on NTX systemic exposure has not been explored in patients.¹⁷ DNA samples obtained from Coriell were used as positive controls. All assays were performed in triplicate.

Pharmacokinetic analysis

PK parameters were estimated by noncompartmental analysis of NTX and 6- β -naltrexol plasma concentrations using Kinetica 5.0 (Thermo Fisher Scientific) and subsequently normalized to mg/kg dose. Maximum plasma concentration (C_{\max}) and the time to C_{\max} (T_{\max}) were observed directly from the concentration versus time data. Terminal elimination rate (λ_z) was calculated from linear regression of log-linear plasma concentration–time curves. The area under the plasma concentration versus time curve during the sampling period (AUC_{0-t}) was calculated using the mixed log-linear rule and extrapolated ($C_{\text{last}}/\lambda_z$) to infinity ($AUC_{0-\infty}$).

Statistical analysis

Naltrexone and 6- β -naltrexol plasma concentrations and PK parameters were log transformed to reduce skew. SPSS version 24 (IBM, Armonk, NY) was used for descriptive statistics, Student's *t*-test (independent, two-tailed) and Spearman's rho. To understand fixed effects (genotype and cohort) and random effects (potential within-participant correlations) on systemic exposure ($AUC_{0-\infty}$ and C_{\max}), linear mixed effects modeling was done in R (R studio, Boston, MA) using lmerTest and EMAtools packages. Parameters were estimated by the restricted maximum likelihood method and degrees-of-freedom were

computed by Kenward-Roger's method for the small sample size. The FDA draft guidance was followed to calculate equivalence between fed and fasted conditions.³⁴

RESULTS

Participant demographics

Twenty-one PK visits (notated as “observations”) were completed by 12 unique participants. Two participants completed two fasted PK visits each. Seven participants completed PK study visits in both the fasted and fed conditions. Table 1 lists participant demographics. When evaluated independently, the fasted and fed cohorts did not differ by age, body mass index (BMI), BMI z-score, weight, or mg/kg dose ($p > 0.05$). Participants were concurrently taking a variety of medications, some known to involve CYP450 enzymes (e.g., CYP2D6 and CYP2C19) and UGT enzymes. Self-reported substance use was recorded at each study visit. The majority of participants reported no use of nicotine (64%, $n = 14$), alcohol (56%, $n = 12$), or cannabis (64%, $n = 14$).

Disease severity/status

In the fasted cohort, CGI-S prior to starting NTX was 6.6 ± 0.5 (range 6–7, severely ill to most extremely ill). Participants completed the fasted study day within 6 months of starting NTX (mean 2.2 ± 1.6 months after starting NTX) and CGI-I at PK visit was 2.1 ± 1.0 (range 1–4, very much improved to no change). No relationship was observed between CGI-S prior to NTX initiation and NTX AUC or C_{\max} . In the fed cohort, mean EPSI scores on the binge eating, cognitive restraint, purging, restricting, and excessive exercise subscales were similar to normative mean values from college students and below mean values reported in a historical eating disorder cohort.³² More participants were on stool softeners at the fasted visit compared with the fed visit, suggesting that constipation/delayed GI transit may be present in the early, fasted cohort.

Pharmacokinetic parameters

Individual plasma concentration versus time curves ($n = 21$) are depicted in Figure 1. Table 2 lists the PK parameters for the combined cohort ($n = 21$) and within genotype groups. There was no statistically significant correlation between dose (mg or mg/kg) and absolute exposure ($r = 0.28$, mg and $AUC_{0-\infty}$ nMh; $r = 0.01$, mg and C_{\max} nM; $r = 0.24$, mg/kg and $AUC_{0-\infty}$ nMh; and $r = -0.06$, mg/kg and C_{\max} nM).

TABLE 1 Participant characteristics

Demographics	Fasted cohort (n = 13)	Fed cohort (n = 8)	Combined (n = 21)
Age, year, mean \pm SD (range)	16.3 \pm 1.5 (15–19)	17.8 \pm 2.1 (16–21)	16.9 \pm 1.9 (15–21)
Weight, kg, mean \pm SD (range)	67.2 \pm 11.6 (45–83)	68.2 \pm 11.9 (47–85)	67.5 \pm 11.4 (45–85)
BMI, mean \pm SD (range)	25.0 \pm 4.0 (17.4–30.9)	25.8 \pm 4.3 (18.4–32.5)	25.3 \pm 4.0 (17.4–32.5)
BMI z-score, mean \pm SD (range)	0.80 \pm 1.2 (–1.9 to 1.9)	0.73 \pm 1.2 (–1.3 to 1.9)	0.82 \pm 1.1 (–1.9 to 1.9)
Naltrexone dose, mg/kg, mean \pm SD (range)	1.0 \pm 0.5 (0.5–2.0)	0.8 \pm 0.2 (0.59–1.1)	0.92 \pm 0.39 (0.56–2.0)
AKR1C4 S145C/L311V, n (%)	3 (23%)	4 (50%)	7 (33%)
Gender identity, n (%)			
Female	10 (77%)	7 (88%)	17 (81%)
Male	1 (8%)	–	1 (5%)
Other (self-described)	2 (15%)	1 (13%)	3 (14%)
Self-described race and ethnicity, n (%)			
Asian	1 (8%)	1 (8%)	2 (10%)
White	8 (62%)	6 (75%)	14 (57%)
More than one race	2 (15%)	1 (13%)	3 (14%)
Unknown/not reported	1 (8%)	1 (13%)	2 (10%)
Hispanic	5 (38%)	3 (38%)	8 (38%)
Substance use, n (%)			
Nicotine, daily	1 (8%)	2 (25%)	3 (14%)
Alcohol, regularly ^a	4 (31%)	3 (38%)	7 (33%)
Marijuana, regularly ^a	3 (23%)	1 (13%)	4 (19%)
Concurrent medications, n (%)			
SSRI/SNRI	11 (85%)	7 (88%)	
Atypical antipsychotics	4 (31%)	2 (25%)	
Other psychotropics (TeCA, stimulant)	1 (8%)	1 (13%)	
Hormonal contraceptives	3 (23%)	3 (38%)	
H2blocker/PPI	6 (46%)	1 (13%)	
Stool softeners	9 (69%)	–	
Probiotics	1 (8%)	1 (13%)	
Pancrelipase ^b	2 (15%)	–	
Psychiatric diagnoses, n (%)			
Anorexia nervosa, binge-purge	10 (77%)	8 (100%)	
Bulimia nervosa	1 (8%)	–	
OSFED – binge/purge specified	2 (15%)	–	
Major depressive disorder	11 (85%)	7 (88%)	
Anxiety disorders	7 (54%)	8 (100%)	
NSSI	4 (31%)	–	
PTSD	1 (8%)	2 (25%)	
Bipolar disorder	–	1 (13%)	
ADHD	–	2 (25%)	
Autism spectrum	1 (8%)	1 (13%)	

Abbreviations: ADHD, attention deficit hyperactivity disorder; AKR1C4, aldo-keto reductase 1C4; BMI, body mass index; H2, histamine; NSSI, non-suicidal self injury; OSFED, other specified feeding or eating disorder; PPI, proton pump inhibitor; PTSD, post-traumatic stress disorder; SNRI, serotonin/norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TeCA, tetracyclic antidepressant.

^aRegularly defined as weekly or monthly use. No one reported daily alcohol or marijuana use.

^bOff-label symptomatic treatment of eating disorder.

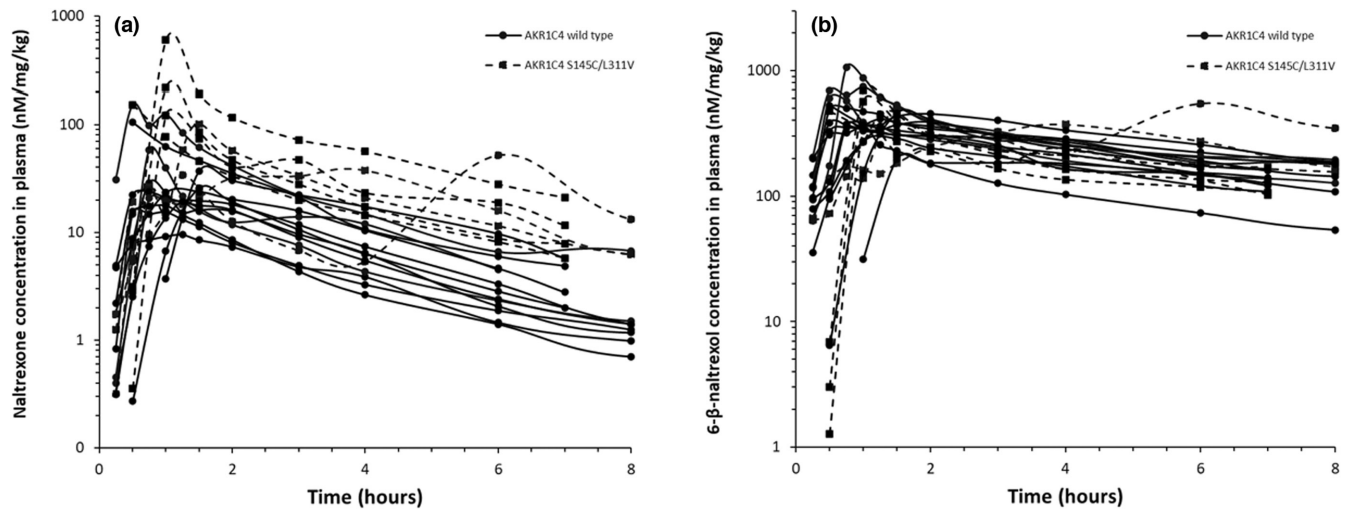


FIGURE 1 Concentration time curves for each observation ($n = 21$) labeled by *AKR1C4* genotype. (a) Naltrexone. (b) 6- β -Naltrexol.

TABLE 2 Pharmacokinetic parameters for naltrexone and 6- β -naltrexol

	AUC _{0-∞} (nM h per mg/kg)	C _{max} (nM per mg/kg)	T _{max} (h)	T _{1/2} (h)
Naltrexone				
Combined	165.8 ± 154.2 (34.0–727)	90.4 ± 129 (9.50–596)	1.3 ± 1.3 (0.5–6)	2.0 ± 0.4 (1.5–2.9)
AKR1C4 wild type	96.2 ± 94.5 (34–241)	44.3 ± 42.3 (9.50–151)	1.0 ± 0.4 (0.5–1.5)	2.0 ± 0.4 (1.6–2.9)
AKR1C4 S145V/L311V	305 ± 191 (177–727)	183 ± 192 (37.4–596)	2.1 ± 2.0 (0.5–6)	2.1 ± 0.3 (1.5–2.4)
6- β -naltrexol				
Combined	3871 ± 1723 (1595–8402)	489 ± 184 (197–1067)	1.5 ± 1.3 (0.5–6)	8.3 ± 3.4 (2.4–12)
AKR1C4 wild type	3961 ± 1883 (1830–8402)	484 ± 214 (197–1067)	1.1 ± 0.5 (0.5–2)	10 ± 6.2 (4.1–28)
AKR1C4 S145V/L311V	3752 ± 1482 (1595–5916)	500 ± 118 (343–691)	2.1 ± 2.1 (0.5–6)	8.3 ± 3.4 (2.4–12)

Note: Combined, $n = 21$; AKR1C4 wild type, $n = 14$; AKR1C4 S145/L311V, $n = 7$.

Abbreviations: AUC_{0-∞}, area under the concentration-time curve from zero to infinity; C_{max}, maximum concentration; T_{1/2}, terminal half-life; T_{max}, time to maximum concentration.

The two *AKR1C4* SNPs were in complete linkage disequilibrium in our sample (co-occurring 100% of the time). Compared with wildtype ($n = 14$), those with heterozygous *AKR1C4* allelic variation ($n = 7$) displayed 3.2-fold higher NTX AUC_{0-∞}, 4.2-fold higher naltrexone C_{max}, and delayed T_{max} (Table 2). Wide variability was seen within each group (Figure 2). In contrast to NTX, 6 β N PK parameters were not substantially affected by genotype (Table 2 and Figure 3), yet variability remained within groups, although to a lesser extent than NTX. There were no individuals that were homozygous for the *AKR1C4* SNPs.

Average exposure in NTX also differed with feeding status (Figures 2 and 3); however, there was substantial variability within each cohort for AUC_{0-∞} 110 ± 72.5 versus 257 ± 209 nM h/mg/kg, $p = 0.030$ and to a greater extent with C_{max} 48 ± 42 versus 160 ± 189 nM/mg/kg ($p = 0.050$). The 90% confidence interval for the ratio of the population geometric means between fed and fasted conditions was 123%–398% for AUC_{0-∞} and 117%–617% for C_{max}. NTX

T_{max} was similar in fed and fasted state (fasted 1.5 ± 1.6, range 0.5–6 h vs. fed 1.1 ± 0.4, range 0.5–1.5 h). Compared with the fasted state, 6 β N C_{max} did not significantly differ in the fed state (fasted 517 ± 198, range 373–1067 nM/mg/kg vs. fed 443 ± 160, range 197–691 nM/mg/kg; Figure 3). The 6 β N T_{max} was similar to NTX (fasted 1.7 ± 1.6, range 0.5–6 h vs. fed 1.1 ± 0.4, range 0.5–1.5 h).

Main effects on naltrexone exposure determined by linear mixed effects modeling

Linear mixed effects modeling was selected to model random (i.e., repeated observations in participants) and fixed (i.e., genotype, fed/fasted) effects on each measure of naltrexone exposure. For C_{max}, the variance of the random effect was zero so estimates from the linear fixed effects model are shown. The models accounted for 82% of the

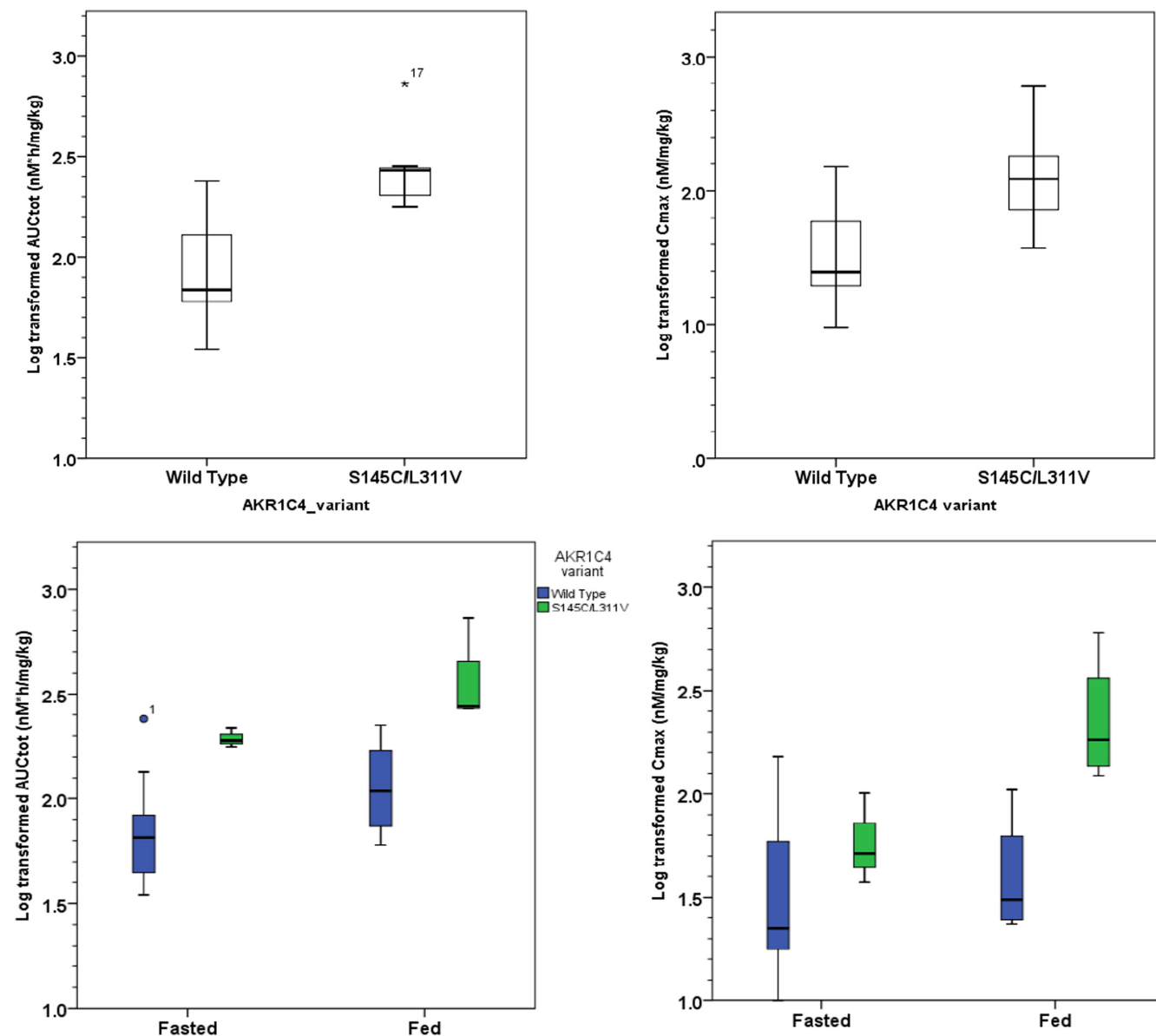


FIGURE 2 Naltrexone exposure by genotype and cohort \times genotype. Individuals with AKR1C4 variants (var) were heterozygous. There were no individuals that were homozygous for the AKR1C4 variants. Fasted WT $n = 10$, Fasted Var $n = 3$, Fed WT $n = 4$, Fed Var $n = 4$. AUC, area under the concentration-time curve; C_{\max} , maximum concentration; WT, wild type.

variance in NTX $AUC_{0-\infty}$ (nMh per mg/kg) and 46% of the variance in naltrexone C_{\max} (nM per mg/kg; Tables 3 and 4). *AKR1C4* genotype demonstrates a large effect on $AUC_{0-\infty}$ (Cohen's $d = -2.3$) and C_{\max} (Cohen's $d = -1.4$), such that those without the variant have substantially reduced exposure. Compared with the fasted state, the fed state also has a large effect on $AUC_{0-\infty}$ (Cohen's $d = 2.6$). The food effect on C_{\max} is widely variable but still large (Cohen's $d = 0.86$).

DISCUSSION

Our findings present the first comprehensive PK dataset, to our knowledge, for NTX in adolescents. We define the

extent of variability in systemic exposure to NTX and the primary active metabolite, 6- β -naltrexol, and describe person-level factors associated with variability. In adolescents with eating disorders, systemic exposure is largely influenced by a food effect and AKR1C4 genotype. These findings are an important step toward understanding NTX dose-exposure-response relationships and facilitating precision dosing to optimize treatment outcomes.

Weight and dose-normalized NTX exposure demonstrated substantial variability from participant to participant in both C_{\max} and AUC (63- and 21-fold, respectively). Exposure to the primary active metabolite was also variable but to a lesser degree than the parent drug, which is consistent with adult data.^{4,5,25} NTX is a nonselective

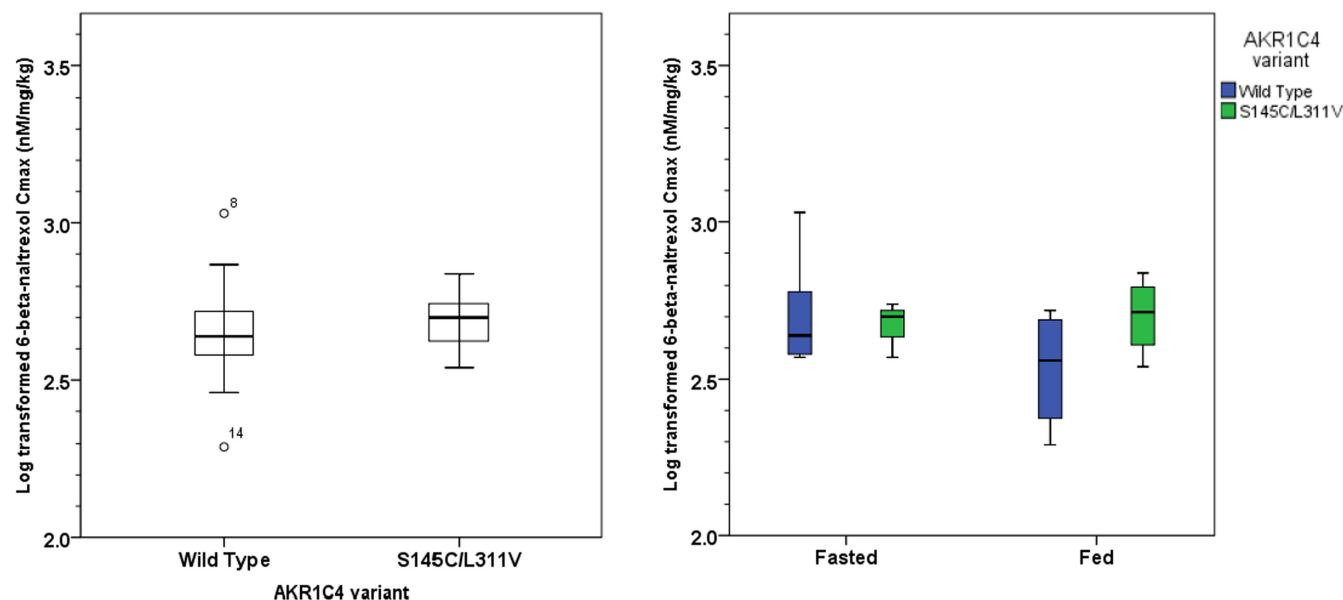


FIGURE 3 The 6- β -naltrexol exposure by genotype and cohort \times genotype. Individuals with AKR1C4 variants (var) were heterozygous. There were no individuals that were homozygous for the AKR1C4 variants. Fasted WT $n = 10$, Fasted Var $n = 3$, Fed WT $n = 4$, Fed Var $n = 4$. C_{\max} , maximum concentration; WT, wild type.

TABLE 3 Linear mixed effect model of naltrexone C_{\max}

Predictors	Effects of individual factors on naltrexone exposure – AUC		
	Estimates	CI	<i>p</i> value
(Intercept)	2.43	1.85–3.01	<0.001
AKR1C4 WT	–0.43	–0.72 to –0.15	0.005
Fed state	0.27	0.11–0.44	0.003
Random effects			
σ^2	0.02		
τ_{00} UniquePt	0.03		
ICC	0.55		
N_{UniquePt}	12		
Observations	21		
Marginal R^2 / conditional R^2	0.589/0.815		

Note: *p* value <0.05 in bold.

Abbreviations: AUC, area under the concentration-time curve; CI, confidence interval; ICC, intraclass correlation coefficient.

opioid antagonist, capable of binding and blocking the μ , κ , and δ opioid receptors with waning affinity, respectively. These receptors have divergent activity from euphoria (μ) and positive affect (δ) to dysphoria and negative affect (κ). Due to complex neurocognitive interplay, the extent of blockade at each receptor may contribute to the overall response in the patient. Although saturation of μ opioid receptor occurs with standard dosing, the extent of κ and δ receptor blockade is sensitive to alterations in systemic exposure.^{35,36} In adolescents with eating disorders, the daily dose of NTX is often titrated to reach desired response

(e.g., reduced purging and/or binge eating), reaching up to 400 mg in some studies.^{1,37} Although no systemic exposure target for NTX yet exists, it is plausible that a delicate balance of opioid receptor antagonism corresponding with a specific exposure window is needed for response.

Our data support a food effect on NTX exposure, contributing to variability. Compared with the fasted condition, NTX $AUC_{0-\infty}$ was significantly increased in the fed state in our study. NTX C_{\max} was also elevated, but wide variability seen among participants and limited sample size likely precluded statistical significance. The 90%

TABLE 4 Linear mixed effect model of naltrexone C_{\max}

Predictors	Effects of individual factors on naltrexone exposure – C_{\max}		
	Estimates	CI	<i>p</i> value
(Intercept)	2.14	1.28–3.00	<0.001
AKRIC4 WT	–0.50	–0.85 to –0.15	0.008
Fed state	0.29	–0.05 to 0.63	0.087
Random effects			
σ^2	0.12		
τ_{00} UniquePt	0.00		
N_{UniquePt}	12		
Observations	21		
Marginal R^2 /conditional R^2	0.461/NA		

Note: *p* value <0.05 in bold.

Abbreviations: CI, confidence interval; C_{\max} , maximum concentration; NA, not applicable.

confidence interval for the ratio of the geometric means between fed and fasted conditions for both $AUC_{0-\infty}$ and C_{\max} fell outside of the 80%–125% equivalence limits established by the FDA. There are no prior reports of head-to-head comparison of fed versus fasted conditions to our knowledge. However, there are limited data from adult studies that describe PK parameters in the fed or fasted condition.^{4–6,25,35} The mean fed $AUC_{0-\infty}$ (nMh) in our study was greater than twofold that reported by Wall and colleagues²⁵; however, their study was limited to four adult men. The mean fed C_{\max} (110 nM) in our study was similar to the high end of the range reported by Weerts and colleagues (47.3–97.4 nM).³⁵ The exposure parameters in the fasted condition in our study are comparable those reported in adults.^{4–6}

A strength of our design is the ability to compare exposure across timepoints and fed/fasted conditions in the same individual. The PK study in the fed condition always occurred at a later date than the PK study in the fasted condition, thus other factors may have contributed to exposure alterations, such as reduced disease severity with improved GI transit time (known to be prolonged in active disease). However, improved GI transit time in a healthier participant would be expected to shorten T_{\max} and decrease AUC. We observed the opposite effect on AUC (substantially increased rather than decreased) with minimal impact on T_{\max} . This suggests that feeding status rather than disease severity was the prominent driver of altered NTX exposure between these two cohorts. Co-occurring medication changes were minimal between study visits and not expected to substantially impact exposure (Table 1).

Another substantial contributor to exposure variability was *AKRIC4* genotype. Participants who were heterozygous for two co-occurring missense mutations (S145C/L311V) associated with reduced enzymatic activity¹⁸

displayed higher systemic exposure compared with those without this variant. These findings are consistent with in vitro evidence in human liver donor samples which demonstrates a large effect of these *AKRIC4* missense mutations on NTX metabolism, in an allele-dose-dependent manner (heterozygous standardized β –0.73; homozygous standardized β –2.66).¹⁷ Our data are the first to describe the in vivo impact of *AKRIC4* genetic variation on NTX metabolism. Investigations into these two *AKRIC4* SNPs, including our study, suggest they occur in complete linkage disequilibrium, despite being ~12 kb apart on exon 4 and exon 9 (GRCh38.p12), with global allele frequencies ranging from 5% to 50%.^{17,19,38} Population-dependent allele frequencies demonstrate higher prevalence in the Americas (~20%), followed by European (~12%), Asian (~10%), and African (~3%) populations.²⁰

AKRIC4 is expressed almost exclusively in the liver with both endogenous and exogenous functions. *AKRIC4* plays a role in cholesterol/bile acid metabolism, deactivation of testosterone and progesterone, detoxification of carcinogens like nicotine-derived nitrosamine ketone (NNK), and metabolism of chemotherapeutic agents, daunorubicin and doxorubicin.⁹ *AKRIC4* expression is regulated by $LXR\alpha$, which is activated by bile acid metabolites.¹⁰ *AKRIC4* is not only involved in cholesterol metabolism, but also regulated by it, at least in part, through a complex feedback loop.³⁹ Negative feedback of cholesterol metabolism leading to reduced bile acid metabolites in the liver upstream of $LXR\alpha$ may contribute to reduced *AKRIC4* expression and altered NTX metabolism. Post-prandial changes in fatty acid concentrations may also contribute to altered *AKRIC4* activity^{11,14} and the food effect detected in this study. Sex-dependent effects on *AKRIC4* activity as a function of testosterone and progesterone exposure are not expected due to the supraphysiologic concentrations

required for inhibition.⁴⁰ There are no known drug–drug interactions between NTX and other medications taken concurrently by our participants (Table 1). NTX and common co-prescribed medications (e.g., selective serotonin reuptake inhibitors and proton pump inhibitors in this study) do not share metabolic pathways, yet limited data available precludes the ability to predict drug–drug interactions with any certainty. This study focused on two *AKR1C4* SNPs with strong in vitro evidence of potential consequence in humans.^{17,18} Future studies may explore additional *AKR1C4* variants predicted to impact enzymatic activity, but that was outside the scope of this work.

Our study was not designed to ascertain the impact of age on NTX exposure, as our sample focused on an age range consistent with disease onset and the target population to meet the primary objective. Evidence from in vitro studies in human liver donors suggest that age likely has a limited impact on NTX metabolism (standardized $\beta = 0.42$ per decade of life).¹⁷ Other individual variables, like weight and BMI, did not appear to have a substantial impact on exposure, but our sample size may have precluded the detection of a small effect. The proxy measures of disease status provide insight into the current participant condition at the time of study visit, using provider documentation (CGI-I) and self-reported assessment (EPSI); however, these two measures are not directly comparable. The small, primarily female sample limits generalizability of these findings. Future studies are warranted to confirm the impact of food and genotype on NTX variability.

These data identify the role of *AKR1C4* genetic variability in NTX exposure in adolescents and suggest the presence of a food effect. Defining the individual level factors that alter drug exposure is a necessary step in the development of a precision therapeutics approach. Future studies linking exposure with response may lead to identification of an exposure target. Our findings will enable informed drug dosing and exposure optimization with the long-term goal of ultimately leading to predictable clinical response and improved health.

AUTHOR CONTRIBUTIONS

S.L.S., W.N., J.T., M.V., W.A., and S.A.-R. wrote the manuscript. S.L.S., S.A.-R., M.V., and W.A. designed the research. S.L.S., W.N., and J.T. performed the research. S.L.S., W.N., S.A., and M.V. analyzed the data.

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

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