8-4-2024

Adrenal Suppression From Vamorolone and Prednisone in Duchenne Muscular Dystrophy: Results From the Phase 2b Clinical Trial

Alexandra Ahmet

Rebecca Tobin

Utkarsh J. Dang

Raoul Rooman

Michela Guglieri

See next page for additional authors

The Journal of Clinical Endocrinology & Metabolism, 2024, **00**, 1–11
https://doi.org/10.1210/clinem/dgae521
Advance access publication 4 August 2024
Clinical Research Article

Adrenal Suppression From Vamorolone and Prednisone in Duchenne Muscular Dystrophy: Results From the Phase 2b Clinical Trial

Alexandra Ahmet,[1,2] Rebecca Tobin,[3] Utkarsh J. Dang,[3] Raoul Rooman,[4] Michela Guglieri,[5] Paula R. Clemens,[6] Eric P. Hoffman,[7] and Leanne M. Ward,[1,2]; on behalf of the CINRG Vamorolone 004 Investigators

[1]Division of Endocrinology, Children's Hospital of Eastern Ontario and Department of Pediatrics, University of Ottawa, Ottawa, Ontario K1H 8L1, Canada
[2]Children's Hospital of Eastern Ontario Research Institute, Ottawa, Ontario K1H 8L1, Canada
[3]Department of Health Sciences, Carleton University, Ottawa, Ontario K1S 5B6, Canada
[4]PendoCon bv, Putte 2580, Belgium
[5]John Walton Muscular Dystrophy Research Centre, Newcastle University and Newcastle Hospital National Health Service Foundation Trust, Newcastle NE1 3BZ, UK
[6]Department of Neurology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA
[7]Department of Pharmaceutical Sciences, Binghamton University—State University of New York, Binghamton, New York 13790, USA

Correspondence: Leanne Ward, MD, FRCPC, Children's Hospital of Eastern Ontario, 401 Smyth Rd., Ottawa, ON, K1H 8L1, Canada. Email: lward@cheo.on.ca.

Abstract

Context: Vamorolone, a novel "dissociative" steroid, demonstrated similar efficacy in muscle function relative to prednisone 0.75 mg/kg/day but improved linear growth and bone turnover markers in a randomized trial of pediatric Duchenne muscular dystrophy (DMD).

Objectives: To determine the frequency of adrenal suppression (AS) induced by vamorolone and prednisone in pediatric DMD and to assess cortisol thresholds using a monoclonal antibody immunoassay.

Methods: Post hoc analysis of cortisol levels was performed on data from a randomized, double-blind, placebo- and prednisone-controlled 24-week trial of vamorolone with a 24-week crossover extension. Morning and ACTH-stimulated cortisol levels were measured using the Elecsys II immunoassay, with AS defined as a stimulated cortisol of <500 nmol/L ("historical threshold") and <400 nmol/L ("revised threshold").

Results: Mean age at enrolment was 5.41 ± 0.86 years (n = 118). At week 24, the proportion of participants with AS using the historical and revised cortisol thresholds, respectively, were as follows: prednisone 0.75 mg/kg/day = 100% (25/25) and 92.0% (23/25); vamorolone 6 mg/kg/day = 95.2% (20/21) and 90.5% (19/21); vamorolone 2 mg/kg/day = 84.2% (16/19) and 47.5% (9/19); and placebo = 20.0% (4/20) and 0% (0/20). Morning and peak ACTH-stimulated cortisol were strongly correlated in steroid-treated boys (Spearman correlation week 48 = 0.83).

Conclusion: AS after vamorolone and prednisone was frequent and vamorolone-associated AS appeared dose-dependent. A lower stimulated cortisol threshold may be appropriate when using a monoclonal assay. We recommend hydrocortisone for glucocorticoid stress dosing in patients receiving vamorolone.

Key Words: adrenal suppression, adrenal insufficiency, prednisone, vamorolone, Duchenne muscular dystrophy

Abbreviations: DMD, Duchene muscular dystrophy; HPA, hypothalamic-pituitary-adrenal; LSM, least square means; MMRM, mixed model for repeated measures; CRH, corticotrophin-releasing hormone.

Hypothalamic-pituitary-adrenal axis (HPA) suppression ("adrenal suppression") is an underrecognized, iatrogenic side effect of therapeutic doses of glucocorticoids (1). Glucocorticoids have been the mainstay of therapy for boys with Duchenne muscular dystrophy (DMD), putting them at risk of morbidity and mortality from adrenal suppression (2-4). Adrenal suppression may be asymptomatic or have nonspecific symptoms but, if unrecognized or improperly managed, can lead to adrenal crisis and death during times of physiological stress (1, 5). In boys with DMD, nonspecific signs and symptoms of adrenal insufficiency, such as fatigue and weakness, are particularly difficult to recognize because they are also characteristic of the underlying condition. Given the potential life-threatening nature of adrenal crisis from adrenal suppression, an in-depth understanding of the risk and characteristics of adrenal suppression related to prednisone and vamorolone in DMD is required to inform the implementation of these therapies in clinical practice.

Glucocorticoids are an effective therapy for many inflammatory and immunologic conditions and are part of the standards of care for DMD (4). However, their long-term use is associated with significant side effects including, but not

Received: 1 May 2024. **Editorial Decision:** 23 July 2024. **Corrected and Typeset:** 26 August 2024

limited to, poor linear growth, excess weight gain, delayed puberty, bone fragility due to osteoporosis, hyperglycemia, hyperlipidemia, and adrenal suppression (6). Therefore, attempts have been made to create analogues that dissociate the beneficial activity from some of the adverse effects (7-9).

Vamorolone is a novel anti-inflammatory steroid that lacks a hydroxyl group at the C11 position of the steroid skeleton. This modification prevents a hydrogen bond with the Asn564 residue in the glucocorticoid receptor (10). Consequently, compared to prednisone or deflazacort, the vamorolone glucocorticoid receptor complex has less affinity for coactivator proteins and is therefore a much weaker activator of gene transcription, which mediates some of the glucocorticoid-related side effects (9, 11). However, the transrepression activity (which mediates most of the anti-inflammatory activity), is conserved; this renders vamorolone a dissociative (or biased) ligand for the glucocorticoid receptor (9). In addition, the lack of a hydroxyl group on C11 prevents a hydrogen bond forming with the N770 residue of the mineralocorticoid receptor (12). In preclinical experiments, vamorolone behaved as a mineralocorticoid receptor antagonist, in contrast to prednisone and deflazacort (12). Lastly, vamorolone is not a substrate for 11-beta-hydroxysteroid dehydrogenases I and II due to the lack of the C11 hydroxyl group; this is important, because 11-beta-hydroxysteroid dehydrogenases I and II have been shown to mediate some of the adverse effects of prednisone (13-15).

In a recent randomized, placebo- and prednisone-controlled clinical trial in boys with DMD (NCT03439670), vamorolone showed comparable efficacy in improving muscle function relative to prednisone, without a negative effect on linear growth velocity and bone turnover markers (16, 17). Both prednisone and vamorolone treatment groups showed a high incidence of adrenal suppression using the standard threshold of <500 nmol/L (proportion of participants on vamorolone 6 mg/kg with adrenal suppression = 95% [20/21]; vamorolone 2 mg/kg = 86% [18/21]; prednisone 0.75 mg/kg = 100% [26/26]), although the placebo group also showed an unexpected incidence of adrenal suppression (20% [4/20]) (16). The loss of growth stunting with vamorolone compared with traditional corticosteroids was also seen in 2.5-year long-term extension studies (18).

Importantly, both morning cortisol and adrenocorticotropic hormone (ACTH)-stimulated cortisol concentrations were measured in the clinical trial setting using a centralized, newer monoclonal cortisol assay (16) (Supplementary Material) (19). While recent literature suggests the need for a redefined lower cortisol threshold for the diagnosis of adrenal insufficiency when using a monoclonal cortisol assay compared to the historical polyclonal assays (20-22), a pediatric threshold has not yet been defined. We now report a detailed, post hoc analysis of the adrenal function data from this trial, including both the analyses using a lower threshold for ACTH-stimulated cortisol (<400 nmol/L) (see Methods for rationale of threshold) and correlation of morning cortisol with ACTH-stimulated cortisol measures. Specifically, our data allowed us to compare the effect of 2 doses of vamorolone (2 and 6 mg/kg/day) and prednisone (0.75 mg/kg/day) administered daily in young boys with DMD on the HPA axis using both the classic ACTH-stimulated cortisol threshold of 500 nmol/L (18.1 ng/dL) and a lower, revised threshold of 400 nmol/L (14.5 ng/dL). Additional post hoc analyses included assessment of the timing of peak cortisol on standard-dose ACTH stimulation testing (30 vs 60 minutes) and examination of first-morning cortisol thresholds for prediction of cortisol levels on ACTH stimulation testing.

Methods

Study Design

The primary study was a randomized, double-blind, placebo- and prednisone-controlled trial of vamorolone in 121 (safety population period 1; n = 118) steroid-naïve, ambulatory boys, age 4 to <7 years of age with genetically confirmed DMD (clinicaltrials.gov NCT03439670). The full study protocol and statistical analysis have been published previously (16). Treatment period 1 (hereafter referred to as period 1) involved a 24-week comparison that included 4 treatment arms (vamorolone 2 m/kg/day, vamorolone 6 mg/kg/day, prednisone 0.75 mg/kg/day, and placebo), with primary efficacy and safety outcomes conducted between June 29, 2018, and February 24, 2021, as previously reported (16). While maintaining the blind, treatment period 2 (hereafter referred to as period 2) followed a tapered washout for the prednisone and placebo groups (ie, from 24 to 28 weeks). This 4-week washout period was then followed by 20 weeks of allocation to vamorolone 2 mg/kg/day or vamorolone 6 mg/kg/day plus continuation of the period 1 vamorolone groups. The clinical trial results from period 2 have been recently published (16, 17).

Participants

Within the multicenter trial (16), boys with genetically confirmed DMD who were 4 to younger than 7 years of age were enrolled. Boys were excluded if they had any previous or current systemic oral glucocorticoid exposure; boys with a history of topical or inhaled glucocorticoid use within the past 4 weeks were excluded unless anticipated to be treated on a stable dose throughout the duration of the study follow-up. Complete inclusion and exclusion criteria have been published elsewhere (16). The trial was approved by the competent ethics committee at each participating institution and was conducted in accordance with the International Conference on Harmonisation Guidelines for Good Clinical Practice and the World Medical Association Declaration of Helsinki.

Intervention

As previously described (16, 17), participants were randomized according to a 2:2:1:1:1:1 ratio to vamorolone 2 mg/kg per day for 48 weeks, vamorolone 6 mg/kg/day for 48 weeks, placebo (period 1) to vamorolone 2 mg/kg/day (period 2), placebo (period 1) to vamorolone 6 mg/kg/day (period 2), prednisone 0.75 mg/kg/day (period 1) to vamorolone 2 mg/kg/day (period 2), and prednisone 0.75 mg/kg/day (period 1) to vamorolone 6 mg/kg/day (period 2). After period 1, there was a blinded 4-week transition period for participants who received either placebo or prednisone, during which the dose of prednisone or placebo was tapered to 0. Participants who crossed over from prednisone or placebo received vamorolone at 2 or 6 mg/kg/day for the last 20 weeks in period 2. See Fig. 1 for a schematic design of the trial.

Adrenal Function Assessment

Adrenal function was assessed by measuring morning serum cortisol levels at day 1 (predose) and weeks 12, 24, 28, 40,

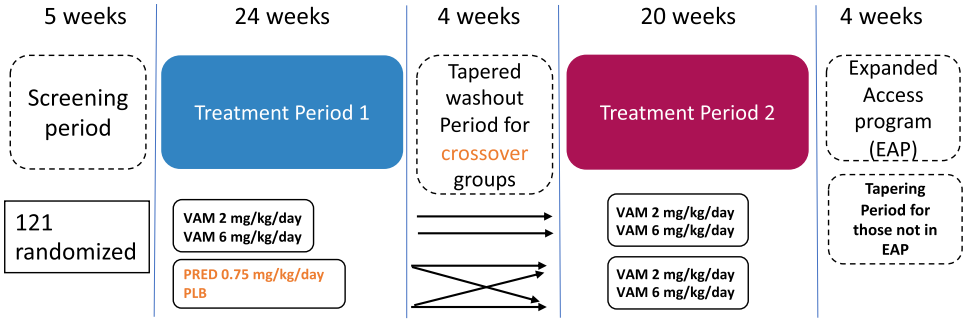

Figure 1. Schematic design of the placebo- and prednisone-controlled randomized, double-blinded trial of vamorolone 2 and 6 mg/kg/day study (VBP15-004). Participants assigned to nonvamorolone groups in period 1 crossed over to 1 of 2 vamorolone doses in period 2.

Abbreviations: EAP, expanded access program; PLB, placebo; PRED, prednisone; VAM, vamorolone.

and 48 and by an ACTH stimulation test at screening, weeks 24 and 48, according to the following methodological approaches.

Unstimulated morning cortisol samples were drawn in clinic before 10 AM and prior to the daily drug administrations. The ACTH stimulation tests at weeks 24 and 48 were performed 48 hours after the last dose of the study drug (prednisone, vamorolone, or placebo). Ninety-five percent of ACTH stimulation tests were started between 8 AM and 11:48 AM (range between 7:40 AM and 12:30 PM). Subjects were covered for potential adrenal suppression by a single 5 or 10-mg hydrocortisone dose (whichever dose was closest to 8 mg/m^2) 24 hours before testing. Standard-dose ACTH stimulation tests were carried out, as follows. After baseline cortisol sampling, 250 mcg Synacthen^{TM} was administered intravenously, and stimulated cortisol levels were drawn at 30 and 60 minutes. Serum cortisol levels were measured in a central lab, on the cobas e 602 analyzer, using the cortisol Elecsys II (Roche Diagnostics) immunoassay, a second-generation monoclonal (rather than historical polyclonal) antibody assay with greater specificity for serum cortisol levels (RRID: AB_2802131, https://scicrunch.org/ResourceWatch/Search?q=AB_2802131). The assay has a lower limit of detection of 1.5 nmol/L (0.054 ug/dL), and a limit of quantitation of 3.0 nmol/L (0.109 ug/dL). Using the cobas e 602 analyzer, the intra-assay coefficient of variation ranges from 1.5% to 5.4%, and the interassay coefficient of variation ranges from 1.9% to 10.1% (23).

We defined adrenal suppression as a cortisol peak at both 30 and 60 minutes <500 nmol/L (18.1 ng/dL) for our first analysis, based on historical thresholds (24). For our second analysis, we defined adrenal suppression as a cortisol peak at both 30 and 60 minutes <400 nmol/L (14.5 ng/dL). The threshold for our second analysis was based on recent studies of second-generation cortisol assays that propose a threshold of between 350 and 414 nmol/L (12.7-15 ng/dL) due to greater specificity of the monoclonal assays for serum cortisol; 400 nmol/L was chosen as it was within the higher end of the suggested threshold values, providing a more conservative revised threshold. In addition, the use of a threshold at the higher end of the proposed range was supported by previous pediatric studies suggesting that younger children have higher ACTH-stimulated cortisol peaks than adults (20-22, 25-28).

In the study protocol, all participants received education plus emergency medical cards outlining a management plan for adrenal suppression, which outlined glucocorticoid stress dosing during times of moderate illness, injury, or surgery. The protocol also included daily glucocorticoid replacement after discontinuation of the study medication at week 48 until results of first morning and ACTH stimulation testing were available to determine the status of the HPA axis. Ongoing glucocorticoid replacement for adrenal suppression was directed at the discretion of the managing physician at each local site. Serum albumin and total protein levels were analyzed for all participants.

Statistical Analyses

The statistical analysis plan for the parent study was published previously (16). The data for this post hoc subanalysis were made available from the clinical trial for which the primary results have previously been reported (16, 17). The study was powered for sample sizes based on motor outcomes (measures of efficacy) at 24 weeks of treatment. The cortisol measures studied here were considered safety outcomes.

Analyses for the current substudy were conducted in SAS and R using ggplot2, and mixed model for repeated measures (MMRM) packages (29-31). A safety population, ie, all participants who received at least 1 dose of study medication during a period (1 or 2, depending on endpoint) was used for analysis. All longitudinal analyses were conducted via restricted maximum likelihood-based MMRM on change from baseline [and least square means (LSM) obtained] in outcome at follow-up visits, adjusting for baseline age, baseline outcome, visit week, treatment arm, and treatment-by-week interaction with an unstructured covariance structure and the Kenward-Roger approximation for denominator degrees of freedom. Fisher's exact tests were used to compare the proportion of boys classified as having adrenal suppression or not in the different treatment groups using the classic (500 nmol/L [18.1 ng/dL]) and revised (400 nmol/L [14.5 ng/dL]) thresholds.

Concentrations of cortisol in nmol/L were rounded to whole numbers while concentrations in mcg/dL were rounded to 1 decimal before analysis (16). When the measured value was below the lower limit of detection, eg, <8 nmol/L (0.3 mcg/dL), <4 nmol/L (0.15 mcg/dL) was imputed, as per the prespecified plan for the VBP15-004 trial itself; for our longitudinal analyses, this impacted the morning cortisol values in 26 boys, all on vamorolone 6 mg/kg/day (including from crossover groups). It should be noted that this trial was conducted during the COVID pandemic, which resulted in some assessments being carried out remotely. Since cortisol measures were not included in remote assessments, this led to

Table 1. Baseline patient characteristics and cortisol values

Number of participants	Overall n = 118	PLB n = 29	PRED n = 31	VAM2 n = 30	VAM6 n = 28
Age, mean (SD)	5.41 (0.86)	5.38 (0.83)	5.54 (0.86)	5.32 (0.91)	5.42 (0.88)
Height z-score, mean (SD)	−0.70 (1.11)	−0.58 (1.21)	−0.44 (1.03)	−0.77 (1.10)	−1.04 (1.05)
BMI z-score, mean (SD)	0.63 (0.82)	0.53 (0.79)	0.87 (0.77)	0.44 (0.89)	0.67 (0.82)
Unstimulated morning cortisol (nmol/L), mean (SD) [n]	221.29 (70.65) [109]	198.96 (61.52) [26]	212.11 (65.73) [28]	237.96 (82.67) [28]	235.04 (66.52) [27]
Peak ACTH-stimulated cortisol, mean (SD) [n]	638.12 (99.94) [106]	635.44 (102.43) [27]	612.61 (97.50) [28]	648.32 (93.57) [25]	658.58 (105.31) [26]
Peak ACTH-stimulated cortisol, % of patients <400 nmol/L [n]	0, [0]	0, [0]	0, [0]	0, [0]	0, [0]
Peak ACTH-stimulated cortisol, % of patients <500 nmol/L [n]	8.1, [9]	10.7, [3]	14.3, [4]	7.4, [2]	0, [0]

Patient (period 1 safety population) characteristics, morning, and stimulated cortisol concentrations pretreatment. Cortisol samples were taken at 0, 30, and 60 minutes after a 250 mcg ACTH IV injection. The peak concentration was the highest value of the 30- and 60-minute sample measurements (see Methods section for more details). The overall number of participants in each treatment group is provided. The number of patients (n) is also provided when describing the means (SD) and proportions of patients. When providing proportions, n, the number below the relevant threshold is also provided. The individual 30- and 60-minute value summaries are in Supplement 3: Table.
Abbreviations: ACTH, adrenocorticotropic hormone; BMI, body mass index; PLB, placebo; PRED, prednisone 0.75 mg/kg/day; VAM2, vamorolone 2 mg/kg/day; VAM6, vamorolone 6 mg/kg/day; SD, standard deviation.

a rate of missing data of about 12% to 15%. No imputation was conducted for missing cortisol observations. All tests were 2-sided with $P < .05$ considered significant. No multiple testing correction was carried out in this substudy given the prespecified nature of the outcomes being tested (safety).

The peak stimulated cortisol level was considered the highest value of the 2 cortisol concentrations measured at 30 *and* 60 minutes during the ACTH stimulation test. A data-informed approach was used to deal with missing values and is described in Supplement 1 (19).

Spearman correlations between 30- and 60-minute ACTH-stimulated cortisol levels were calculated for baseline, week 24, and week 48. We also evaluated whether important prognostic variables for DMD (including age, 6-minute walk distance, North Star Ambulatory Assessment score, and body mass index z-score) were associated with cortisol concentrations (morning and stimulated peak) using Spearman correlations.

Results

Pretreatment/baseline characteristics and cortisol values are outlined in Table 1.

Unstimulated Morning Cortisol Values

Unstimulated morning cortisol levels were similar at baseline in all groups (Table 1). Mean first-morning cortisol values remained stable over 24 weeks in the placebo arm (Fig. 2). All other treatment groups showed significant reductions in morning cortisol levels from baseline at both 12-week and 24-week MMRM assessments [vs placebo: all comparisons $P < .0001$; Fig. 2; Supplement 5 (Table) (19)]. At week 24, the vamorolone 2 mg/kg/day group showed less of a decrease in first morning cortisol when compared with prednisone [$P = .002$; LSM = 52.8 nmol/L, 95% confidence interval (CI) = 19.7 to 86 nmol/L], whereas the vamorolone 6 mg/kg/day group demonstrated a greater decrease in first-morning cortisol when compared with prednisone ($P = .026$; LSM = −35.4 nmol/L, 95% CI = −66.4 to −4.4 nmol/L).

At week 28, 4 weeks after the start of tapering, the prednisone group demonstrated rapid improvement in morning cortisol [$P < .0001$; LSM difference week 28 vs 24 = 121 nmol/L, 95% CI = 77.4 to 164 nmol/L; Fig. 2; Supplement 5 (Table) (19)]. The morning cortisol levels at week 48 were not significantly different from the 24-week levels in both vamorolone groups (Fig. 2).

Serum total protein and albumin levels were within normal limits for all participants.

ACTH Stimulation Test Results

At the screening (pretreatment) visit, 9/111 (8.1%) of participants had a cortisol peak at either 30 or 60 minutes of <500 nmol/L (<18.1 ng/dL), but 0% (0/112) had a cortisol peak <400 nmol/L (14.5 ng/dL) (Table 1), with peak cortisol ranging from 444 to 919 nmol/L (16.1-33.3 ng/dL). At week 24, the percent (and number) of abnormal peak cortisol values in each group using thresholds of <500 nmol/L (18.1 ng/dL) and <400 nmol/L (14.5 ng/dL), respectively, were 20.0% (4/20), 0% (0/20) of the placebo group; 100% (25/25), 92.0% (23/25) of the prednisone group; 84.2% (16/19), 47.4% (9/19) of the vamorolone 2 mg/kg/day group; and 95.2% (20/21), 90.5% (19/21) of the vamorolone 6 mg/kg/day group (Table 2 and Fig. 3).

At 24 weeks, there were fewer participants classified as having adrenal suppression in the vamorolone 2 mg/kg/day group when compared to the prednisone group using both the 400 nmol/L (14.5 ng/dL) ($P = .002$) and 500 nmol/L (18.1 ng/dL) ($P = .073$) thresholds and when compared to the vamorolone 6 mg/kg/day group using the 400 nmol/L (14.5 ng/dL) ($P = .005$) and 500 nmol/L (18.1 ng/dL) ($P = .33$) thresholds. There was no difference in the number of participants classified as having adrenal suppression in the prednisone vs vamorolone 6 mg/kg/day group using either cortisol threshold ($P = .46$ and $P = 1$ for the 500 nmol/L and 400 nmol/L, respectively).

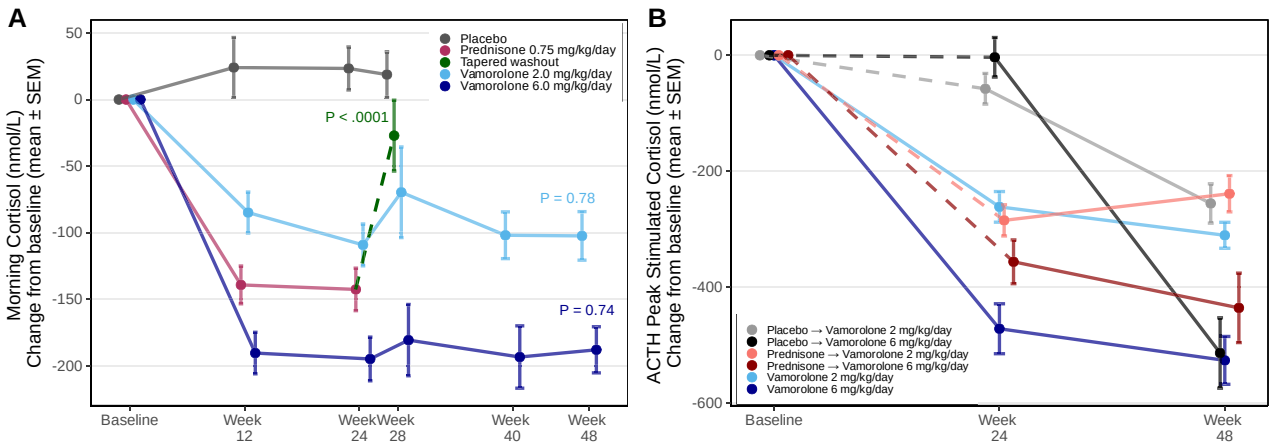

Figure 2. Change in morning and ACTH peak stimulated cortisol levels over 48 weeks of treatment. (A) The merged treatment groups for period 1 are provided over the first 24 weeks, along with the tapered washout for the prednisone group and period 2 for the vamorolone 0- to 48-week groups. The *P*-value in green is for the change from week 24 to week 28 in the prednisone group after starting tapering. The *P*-value in light blue represents the change from week 24 to week 48 in the vamorolone 2 group; similarly, in dark blue, the *P*-value is for change from week 24 to week 48 in the vamorolone 6 group. (B) Change in peak cortisol after ACTH stimulation was plotted for each of the 6 randomized treatment groups. The crossover groups start as dashed (period 1 assignment) before crossover to vamorolone dose groups (solid).

Table 2. Peak cortisol values on standard dose ACTH stimulation testing at 24 and 48 weeks based on historical (<500 nmol/L) and revised (<400 nmol/L) thresholds

Treatment group	Week 24 peak cortisol mean (range) nmol/L [n]	Week 24 peak cortisol % < 500 nmol/L [n]	Week 24 peak cortisol % < 400 nmol/L [n]
Placebo	589.9 (458, 750) [19]	20.0% [4/20]	0% [0/20]
Prednisone 0.75 mg/kg/day	277.6 (138, 436) [25]	100% [25/25]	92.0% [23/25]
Vamorolone 2 mg/kg/day	392.8 (193, 618) [19]	84.2% [16/19]	47.4% [9/19]
Vamorolone 6 mg/kg/day	180.9 (11, 665) [21]	95.2% [20/21]	90.5% [19/21]
Treatment group	**Week 48 peak cortisol mean (range) nmol/L [n]**	**Week 48 peak cortisol < 500 nmol/L [n]**	**Week 48 peak cortisol % < 400 nmol/L [n]**
Vamorolone 2 mg/kg/day	359.4 (154, 621) [20]	90.0% [18/20]	52.4% [11/21]
Vamorolone 6 mg/kg/day	142.1 (8, 397) [18]	100% [18/18]	94.7% [18/19]
Prednisone 0.75 mg/kg/day to Vamorolone 2 mg/kg/day	356.5 (232, 593) [11]	90.9% [10/11]	81.8% [9/11]
Prednisone 0.75 mg/kg/day to Vamorolone 6 mg/kg/day	187.6 (8, 472) [12]	100% [12/12]	91.7% [11/12]
Placebo to Vamorolone 2 mg/kg/day	346.7 (174, 494) [11]	100% [11/11]	63.6% [7/11]
Placebo to Vamorolone 6 mg/kg/day	191.5 (28, 395) [10]	100% [10/10]	100% [10/10]

Characteristics related to stimulated cortisol at the 24- and 48-week visits. Peak concentrations were the maximal values of the 30- and 60-minute samples (see Methods section for more details). When providing means, n, the number of participants with the measurement is provided. When providing proportions, the fraction of participants below the relevant threshold to the number of participants with the measurement is provided. For period 2 (week 48) summaries, the row names also include underlined name of the treatment at week 48.

In addition to a binary threshold-based analysis at week 24, we carried out comparisons using the measured numerical values using MMRM across the duration of the study. Stimulated peak cortisol values in the placebo group were higher than in all the active treatment groups at 24 weeks [all comparisons $P < .0001$; Fig. 3, Table 2, Supplement 5 (19)]. There was a nonsignificant difference in stimulated peak cortisol values with prednisone values trending lower compared with the

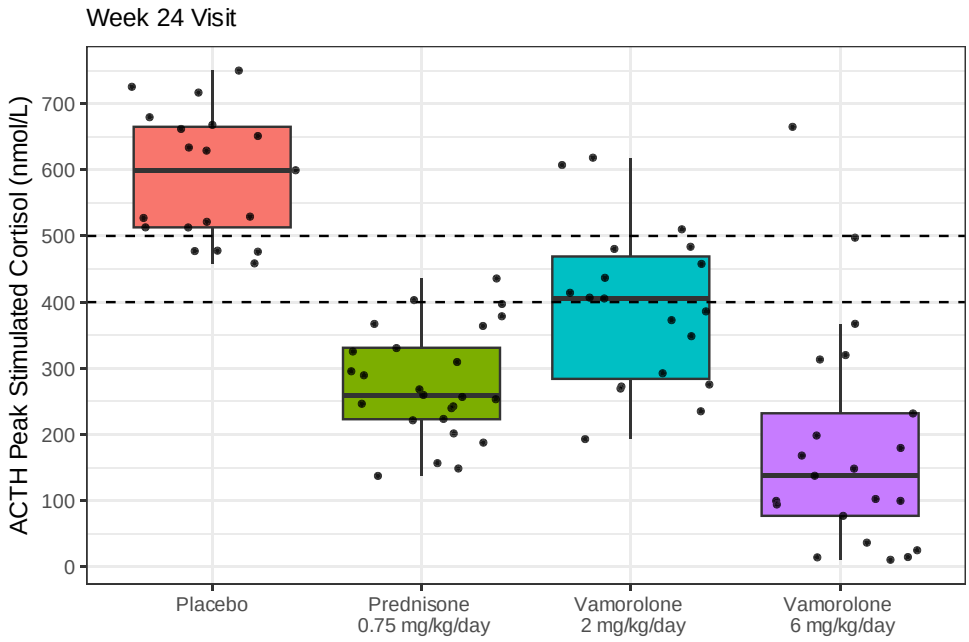

Figure 3. Peak cortisol on the 250 mcg ACTH stimulation test after 24 weeks on study drug (24-week visit). Peak concentrations were the maximum concentration between the 30- and 60-minute samples (see Methods section for more details). For each treatment group, cortisol concentrations for each participant (black points) are overlaid over boxplots. Dashed black lines show thresholds of 400 (revised) and 500 (traditional) nmol/L (14.5 and 18.1 ng/dL).

vamorolone 2 mg/kg/day group ($P = .063$; LSM difference = 77.4 nmol/L, 95% CI = −4.2 to 159 nmol/L). Stimulated peak cortisol values were lower in the vamorolone 6 mg/kg/day group compared with the vamorolone 2 mg/kg/day group ($P < .0001$; LSM difference = 191 nmol/L, 95% CI = 109 to 273 nmol/L) and with the prednisone group ($P = .003$; LSM difference = 113 nmol/L, 95% CI = 38.8 to 188 nmol/L).

At 48 weeks, there was no further significant decrease in cortisol peaks compared to 24 weeks for the vamorolone 6 mg/kg/day ($P = .30$) and vamorolone 2 mg/kg/day ($P = .26$) groups. There was no significant difference in peak cortisol values for participants who switched from prednisone 0.75 mg/kg/day to vamorolone 2 mg/kg/day ($P = .218$) or for participants switching from prednisone 0.75 mg/kg/day to vamorolone 6 mg/kg/day ($P = .099$). As expected, based on period 1 results, the placebo to vamorolone 2 mg/kg/day and 6 mg/kg/day groups both demonstrated a significant decrease in cortisol peak at week 48 [$P < .0001$; Fig. 2 and Supplement 5 (19)].

Testing Outcomes in Glucocorticoid-naïve Boys With DMD—Comparison of Cortisol Thresholds

Data from stimulated cortisol samples in glucocorticoid-naïve boys with DMD, including both baseline visit values from boys in all treatment groups and 24-week data for boys in the placebo group, were analyzed to evaluate cortisol thresholds. These data included 131 ACTH stimulation tests. None of the glucocorticoid-naïve boys had a stimulated cortisol peak <400 nmol/L (14.5 ng/dL), but 13 (9.9%) ACTH stimulation tests in the glucocorticoid-naïve boys had a cortisol peak <500 nmol/L (18.1 ng/dL). The lowest peak stimulated cortisol value among the tests in glucocorticoid naïve boys was 444 nmol/L (16.1 ng/dL).

Associations With Cortisol Values

There was little association (Spearman correlations range: −0.2-0.21) between possible prognostic variables (age, 6-minute walk distance, North Star Ambulatory Assessment score, body mass index z-score) and cortisol measurements (morning and ACTH stimulated peak values) at the baseline, week 24, and week 48 visits.

ACTH Stimulation Testing—Timing of Peak Cortisol

There was a total of 262 ACTH stimulation tests performed during the study with samples drawn at both 30 and 60 minutes (baseline = 104 tests; week 24 = 83 tests; week 48 = 75 tests). One outlier was identified; this was a 30-minute stimulated cortisol value that was decreased substantially from the unstimulated cortisol value taken at 0 minutes and with a large rebound at 60 minutes; this may have been a measurement error (due to sample handling or recording issues) and was removed from all analyses. Peak cortisol values overwhelmingly occurred at 60 minutes in 252/261 (96.6%) of the paired 30-minute and 60-minute measurements.

It is noteworthy that if only a 30-minute timepoint ACTH stimulation test measurement had been taken, many participants would have been characterized as having adrenal suppression. Using the 500 nmol/L (18.1 ng/dL) and 400 nmol/L (14.5 ng/dL) thresholds, 27/115 (23.5%) and 24/151 (15.9%) observations were below the threshold at 30 minutes but above the threshold at 60 minutes, respectively. See Supplement 2, 3, and 4 (Tables) (19) for 30- and 60-minute stimulated cortisol values.

Morning and peak stimulated cortisol values had little association at baseline (Spearman correlation = 0.20) but were correlated among prednisone- and vamorolone-treated participants at week 24 (Spearman correlation = 0.87) and vamorolone-treated participants at week 48 (Spearman

correlation = 0.83). Spearman correlations between 30- and 60-minute cortisol concentrations were stronger among the steroid-treated participants (week 24 Spearman correlation 0.98, week 48 Spearman correlation 0.99) compared to steroid-naïve participants (baseline Spearman correlation 0.87). The following linear model was created with 30-minute cortisol values to predict the 60-minute serum cortisol response using data at the 48-week visit (vamorolone-treated boys with DMD ~ 5 to 8 years of age): $y = -4.06664 + 1.16240x$ [intercept standard error = 3.9652, slope standard error = 0.0146]. This model had an $R^2 = 0.9886$; this corresponds to an extremely strong model fit (an R^2 of 1 corresponds to a perfect linear fit, with the regression line passing through each datapoint). Modeling with data at the 24-week visit yielded a similar (in terms of coefficients) equation.

Adverse Events Related to Adrenal Insufficiency

There were no adverse event reports of symptomatic adrenal suppression or adrenal crisis during the study period. In addition, the clinical database was searched for symptoms or signs that can be associated with symptomatic adrenal suppression (1) including, "decreased appetite", "weight loss", "malaise", "asthenia", "fatigue", "myalgia", "arthralgia", "pain in extremity", "muscular weakness", "lethargy", "hypotension", "presyncope", "syncope", "peripheral coldness", "dizziness", "hypoglycemia", "pallor", "seizure", and "headache". The database search identified reports of some of these adverse events, but they were isolated occurrences devoid of temporal association with any dose interruptions or dose reductions that could precipitate symptomatic adrenal suppression. The cumulative review demonstrated that these adverse events were standalone occurrences and were not representative of symptomatic adrenal suppression or adrenal crisis. In addition, the indications for concomitant systemic glucocorticoid administration (ie, steroid stress dosing) were analyzed, and symptomatic adrenal insufficiency was not one of them. Overall, the number of boys in each group who received hydrocortisone as part of preventative steroid stress dosing for various health events were as follows: vamorolone 2 mg/kg/day = 7 boys; vamorolone 6 mg/kg/day = 5 boys; placebo-to-vamorolone 2 mg/kg/day = 2 boys; all other treatment groups = 0 boys.

Discussion

In this post hoc analyses, we expand on the data presented in a randomized controlled trial of vamorolone (2 doses) compared to placebo and prednisone, where morning cortisol was reported at baseline, 12, and 24 weeks and the cortisol response to standard-dose ACTH was reported at baseline and after 24 weeks of therapy (16). In the original protocol, the definition of adrenal insufficiency was prespecified at <500 nmol/L, which is the classic threshold to define adrenal insufficiency based on the historical use of polyclonal antibody cortisol assays. However, in the study, a monoclonal antibody assay was used to determine serum cortisol values, providing a rationale to reexamine these data using a lower post hoc cortisol threshold of <400 nmol/L to define adrenal insufficiency. Consideration for the use of a revised threshold was prompted by the fact that monoclonal antibody assays are more specific for serum cortisol. Recent studies have shown that a lower cortisol threshold than historically implemented may be clinically appropriate (20-22, 25, 26). Additional post hoc analyses included evaluation of the cortisol levels in response to discontinuation of prednisone and assessment of the timing of peak cortisol on standard-dose ACTH stimulation testing (30 vs 60 minutes).

In the parent study (16, 17), there were no adverse events reported that were related to adrenal insufficiency; however, in our study, asymptomatic adrenal suppression was evident in both the prednisone- and vamorolone-treated participants, based on both first-morning cortisol values at 12, 24, and 48 weeks and on standard-dose ACTH stimulation testing at 24 and 48 weeks. These new data expand on the period 1 (first 24 weeks) of the randomized trial by recapitulating dose-dependent adrenal suppression after 48 weeks of vamorolone 2 and 6 mg/kg and following the switch from placebo and prednisone to these 2 doses of vamorolone.

Classic exogenous glucocorticoids exert negative feedback on the HPA axis using the same mechanism as endogenous cortisol in the regulation of cortisol synthesis and release. Cortisol suppresses the HPA axis by decreasing corticotrophin-releasing hormone (CRH) and ACTH synthesis and secretion. The suppression of CRH is proposed to be mediated through transrepression by direct binding of the liganded glucocorticoid receptor to a negative glucocorticoid regulatory element and via tethering to the cAMP response element-binding protein, preventing its interaction with the cAMP response element in the CRH promotor. The glucocorticoid receptor complex also directly inhibits transcription of the *POMC* gene in the corticotroph cells of the pituitary, again via binding to a negative glucocorticoid regulatory element and by tethering to Nur77 and interfering with NeuroD1-stimulated POMC transcription (32). Given that the vamorolone-bound glucocorticoid receptor retains its transrepression activity (9), it is unsurprising that vamorolone suppresses the HPA axis.

Evidence suggests that prolonged glucocorticoid exposure can put some individuals at risk of persistent adrenal suppression after cessation of steroid treatment, lasting up to 2 years in some (1), though with significant interpatient variability. In the current study, we showed that 24 weeks of daily prednisone (0.75 mg/kg/day) resulted in profound adrenal suppression followed by significant improvement in morning cortisol for most of the participants after a 4-week washout period [Fig. 2 and Supplement 4 (19)]; however, ACTH-stimulated cortisol levels were not performed to evaluate for recovery of the stress response by this more accurate method. Vamorolone-treated participants did not undergo a washout period, so we were also unable to determine the pattern of cortisol recovery from vamorolone-induced adrenal suppression.

In this trial, we did not find episodes of adrenal crisis among the boys with DMD. It remains theoretically possible that our rigorous approach to the risk of adrenal suppression, including stress dosing education and provision of hydrocortisone coverage, may have prevented overt adrenal insufficiency (16, 17). As a standard of care, it is imperative that all patients and families are aware of the adrenal suppression risk when either classic glucocorticoids (prednisone or deflazacort) or vamorolone therapy are initiated and that empiric "steroid stress dosing" is given during times of moderate to severe illness, surgery, or injury. A specific protocol for treatment and prevention of adrenal crises associated with chronic prednisone and deflazacort treatment in DMD has been published (The PJ Nicholoff Steroid Protocol for Duchenne and Becker

Muscular Dystrophy and Adrenal Suppression) (2); plans are underway for this protocol to be updated to include guidelines for adrenal suppression management in patients with DMD on vamorolone.

Given the differences in mechanisms of action between vamorolone and classic glucocorticoids (including mineralocorticoid receptor antagonism) and the lack of experience with vamorolone at doses higher than 6 mg/kg/day, we recommend steroid stress dosing using hydrocortisone in patients receiving vamorolone as per standard preventative or symptomatic adrenal suppression management, as set out in the Endocrine Society clinical practice guidelines (24). Since vamorolone 2 mg/kg/day clearly showed less glucocorticoid-like activity (assessed as adrenal suppression) than the standard dose of prednisone (0.75 mg/kg/day), it is further recommended that patients who transition from any therapeutic dose of classic glucocorticoids to vamorolone are prescribed vamorolone 6 mg/kg/day rather than 2 mg/kg/day, in order to avoid symptomatic adrenal insufficiency during the switching period (33, 34). The vamorolone dose can then be progressively tapered according to clinical indication, with monitoring for signs and/or symptoms of adrenal insufficiency in the event of downward dose titration (33, 34). If the decision to discontinue vamorolone is made, the vamorolone dose must also be progressively tapered, with monitoring for signs and/or symptoms of steroid withdrawal or adrenal insufficiency during this process (33, 34).

In addition to our main objective of determining the impact of vamorolone and prednisone on the HPA axis up to 48 weeks in the controlled DMD study, our results provide important insights into morning cortisol and ACTH stimulation testing in children. The diagnostic cortisol threshold of <500 nmol/L (18.1 µg/dL) or higher has been used historically for the diagnosis of adrenal insufficiency (24, 35, 36). More recently, there has been a significant shift in cortisol testing methodology, with polyclonal antibodies that were used in the first-generation assays being replaced with monoclonal antibodies that have less cross-reactivity with other steroids in the second-generation assays; this includes the new Roche Elecsys Cortisol II immunoassay that was used in the present study (20).

Even prior to the studies evaluating revision of the cortisol threshold using monoclonal assays, there were limited data supporting a specific cortisol threshold in pediatrics. Studies using first-generation assays suggested that younger children may have slightly higher cortisol peaks on ACTH stimulation testing compared to adolescents and adults (27, 28), though in clinical practice and within guidelines, a cortisol threshold between 440 and 600 nmol/L has been cited for use in both children and adolescents (1, 24). There are a paucity of studies evaluating the differences in cortisol values on first- and second-generation assays specifically within the pediatric population, and to date, there is no clear guideline on which threshold to use in children when implementing monoclonal assays.

Here, we carried out a post hoc evaluation of the stimulated cortisol values using a revised cortisol threshold of 400 nmol/L. We chose a threshold at the upper end of the suggested revised range, to be cautious and in the context of the pediatric data suggesting that younger children have higher peaks (27, 28). In the glucocorticoid-naïve group, using the historical stimulated cortisol threshold of <500 nmol/L (18.1 µg/dL) to define adrenal insufficiency, we have shown that 9.9% of glucocorticoid-naïve boys' measurements fell within the adrenal insufficiency category. This observation raised the question of an association between endogenous adrenal insufficiency and the underlying disease, a relevant consideration given that boys with DMD experience weakness and fatigue that are improved with glucocorticoid replacement. However, with the revised threshold of 400 nmol/L (14.5 µg/dL), none of the glucocorticoid-naïve boys would be classified as meeting the revised criteria for adrenal insufficiency. Independent of meeting criteria for adrenal insufficiency, a subset of the steroid-naïve DMD participants in the VBP15-004 study showed lower ACTH-stimulated cortisol than expected (16, 17). Further research is needed to determine if abnormally low ACTH-stimulated cortisol is an aspect of the DMD phenotype in the absence of steroid treatment.

It is interesting to note that there have been 3 case reports of boys with Xp21 contiguous gene deletion syndrome presenting with a co-occurrence of DMD, congenital adrenal hypoplasia, and glycerol kinase deficiency (37-39); this unique, albeit rare, situation reminds the clinician to consider adrenal insufficiency in the event of relevant clinical signs and symptoms.

A recently published study of cortisol thresholds on low-dose ACTH stimulation testing in 36 pediatric patients also supports the need to decrease cortisol thresholds in children and suggests an even more significant adjustment with monoclonal antibody immunoassays from 18.1 µg/dL (500 nmol/L) to 12.5 µg/dL (350 nmol/L) in the context of low-dose ACTH testing (40). These findings highlight the critical importance of establishing a valid cortisol threshold for children in the context of second-generation assays given the significant implications of over- or underdiagnosis of adrenal insufficiency. Our data provide some insight into the effects of a revised threshold on data interpretation in DMD patients; however, more research is needed before we can extrapolate any thresholds derived from a population of rare genetic disease patients to the general pediatric population.

A second consideration related to ACTH stimulation was the timing of cortisol sampling. Current guidelines and clinical resources suggest testing cortisol at 30- and/or 60-minute time intervals with standard-dose ACTH stimulation testing (24, 41). We measured cortisol at both 30 and 60 minutes and considered a test to be abnormal if the maximum peak cortisol was below the threshold. Most, but not all, of the ACTH stimulation tests in our study demonstrated maximum peak cortisol levels at 60 minutes. Furthermore, analysis of outcomes demonstrated that several participants would have been classified as having adrenal suppression if cortisol was only measured at 30 minutes. This aligns with previous studies that have demonstrated that peak cortisol typically occurs at 60 minutes (42, 43) and highlights the importance of considering the timing of cortisol sampling when determining a cortisol threshold, a message that was also highlighted in a recent article by Husni et al (42). To avoid the risk of false positives, testing at both 30 and 60 minutes is proposed. However, for boys treated with vamorolone, we demonstrated that the 30-minute cortisol value was a good predictor of 60-minute cortisol levels, and while we believe that testing at both 30 and 60-minutes is the preferred practice, we have provided an equation (specific to age range herein and vamorolone treatment) that may allow centers that test only at 30 minutes to extrapolate the 60 minutes values in standard dose testing in boys with DMD.

The strengths of this study are the relatively large number of pediatric clinical trial participants with ACTH stimulation

tests pre- and posttreatment on prednisone and vamorolone but also on placebo. As such, this study allowed us to assess the impact of vamorolone on the HPA axis relative to classic glucocorticoid therapy and also provided a serendipitous opportunity to shed light on the nuances related to a revised cortisol threshold in a steroid-naïve pediatric population when using a second-generation assay to evaluate the HPA axis.

Limitations of our study included the small size of the crossover groups and a protocol that allowed for the ascertainment of unstimulated, morning cortisol levels up to 10 AM in the morning, which is within the timing associated with the thresholds provided by the assay's label but is later in the day than 9:00 AM, which has been demonstrated in previous studies to have high specificity in the prediction of stimulated cortisol levels (44, 45); this may have led to lower than anticipated morning cortisol values. That said, the morning cortisol levels were found to be highly correlated with the diagnostic ACTH stimulation test results of the same glucocorticoid-treated participants. Further studies are needed to evaluate the duration of adrenal suppression following withdrawal of vamorolone, which may be different from classic glucocorticoid therapy given the alternative mechanisms of action of this novel drug. Additional studies are also needed to define optimal cortisol thresholds for the evaluation of adrenal insufficiency in children without DMD and in other age groups when using second-generation immunoassays.

Conclusions

Vamorolone causes adrenal suppression, and all patients being treated with vamorolone should be managed for the risk of adrenal insufficiency with medical alert cards, adrenal insufficiency education, and hydrocortisone stress dosing guidelines in times of physiological stress. We also compared the effect on data interpretation using the traditional <500 nmol/L cut-off vs a revised <400 nmol/L cut-off for peak standard-dose ACTH-stimulated cortisol levels and demonstrate the importance of establishing a revised cortisol threshold to be used with monoclonal antibody cortisol testing in children; more studies are needed in this regard. Finally, while the peak cortisol response occurred after 60 minutes in most of the participants in our study, we affirm that testing levels at *both* 30 and 60 minutes in response to standard-dose ACTH is the most effective strategy for capturing the peak stimulated cortisol value.

Acknowledgments

The authors thank the children and families who participated in the study and the research personnel who contributed to the study operations. L.M.W. is supported by a Tier 1 (Senior) Research Chair Award from the University of Ottawa and the Children's Hospital of Eastern Ontario Research Institute.

Funding

This work was funded by the National Institutes of Health National Institute of Neurological Disorders and Stroke, grant R44NS095423 (E.P.H., P.R.C.); National Institute of Child Health and Human Development, grant 5U54H D090254 (E.P.H., L.S.C.); National Institute of Arthritis and Musculoskeletal and Skin Diseases, grant U34AR 068616 (P.R.C.); and European Commission Horizons 2020, grant 667078 (M.G.). L.M.W. is supported by a Senior (Tier 1) Clinical Research Chair in Pediatric Bone Disorders from the University of Ottawa and the Children's Hospital of Eastern Ontario Research Institute.

Disclosures

A.A. declares past honorarium for consultancy to ReveraGen (with funds to Dr. Ahmet's institution). R.T. has no disclosures to declare. U.J.D. has received past consulting fees from ReveraGen and declares grants from the Foundation to Eradicate Duchenne, Department of Defence, and National Institutes of Health. R.R. is a paid consultant for Santhera. M.G. declares honoraria for participating in advisory boards for PTC Therapeutics, Capricor, Pfizer, and NS Pharma (with funding to Dr. Guglieri's institution). She has had research collaborations with ReveraGen, PTC, Sarepta, Duchenne UK, and MDUK through Newcastle University. She is or has been principal investigator for clinical trials with Roche, Italfarmaco, Santhera, ReveraGen, Summit, Pfizer, and PTC Therapeutics. She received speaker honoraria from Italfarmaco, Roche, Novartis, and Sarepta. P.C. served as a consultant for Catalyst Medical Education and Med Learning Group. P.C. has also received research grants from ReveraGen, NS Pharma, Amicus, and Sanofi and Spark; E.P.H. reports receiving salary support for ReveraGen and AGADA Biosciences; grants from the National Institutes of Health and the European Commission; being a board member of TRiNDS outside the submitted work; and having a patent for ES2744879T3 owned by ReveraGen. L.M.W. declares honorarium for consultancy to PTC Therapeutics, Roche, and Santhera (with funds to Dr. Ward's institution) and participation in clinical trials with ReveraGen, Catabasis, and Edgewise (with funds to Dr. Ward's institution).

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Clinical Trial Information

clinicaltrials.gov (NCT03439670).

References

1. Ahmet A, Mokashi A, Goldbloom EB, *et al.* Adrenal suppression from glucocorticoids: preventing an iatrogenic cause of morbidity and mortality in children. *BMJ Paediatr Open*. 2019;3(1):e000569.
2. Kinnett K, Noritz G. The PJ Nicholoff steroid protocol for Duchenne and Becker muscular dystrophy and adrenal suppression. *PLoS Curr*. 2017;9:ecurrents.md.d18deef7dac96ed135e0dc8739917b6e.
3. Weber DR, Hadjiyannakis S, McMillan HJ, Noritz G, Ward LM. Obesity and endocrine management of the patient with Duchenne muscular dystrophy. *Pediatrics*. 2018;142(Suppl 2):S43–S52.
4. Birnkrant DJ, Bushby K, Bann CM, *et al.* Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and neuromuscular, rehabilitation, endocrine, and gastrointestinal and nutritional management. *Lancet Neurol*. 2018;17(3):251-267.
5. Bushby K, Finkel R, Birnkrant DJ, *et al.* Diagnosis and management of Duchenne muscular dystrophy, part 1: diagnosis, and

pharmacological and psychosocial management. *Lancet Neurol*. 2010;9(1):77-93.
6. Liu D, Ahmet A, Ward L, *et al*. A practical guide to the monitoring and management of the complications of systemic corticosteroid therapy. *Allergy Asthma Clin Immunol*. 2013;9(1):30.
7. Rogliani P, Ritondo BL, Puxeddu E, Pane G, Cazzola M, Calzetta L. Experimental glucocorticoid receptor agonists for the treatment of asthma: a systematic review. *J Exp Pharmacol*. 2020;12:233-254.
8. Vandewalle J, Luypaert A, De Bosscher K, Libert C. Therapeutic mechanisms of glucocorticoids. *Trends Endocrinol Metab*. 2018;29(1):42-54.
9. Liu X, Wang Y, Gutierrez JS, *et al*. Disruption of a key ligand-H-bond network drives dissociative properties in vamorolone for Duchenne muscular dystrophy treatment. *Proc Natl Acad Sci U S A*. 2020;117(39):24285-24293.
10. Hoffman EP, Riddle V, Siegler MA, *et al*. Phase 1 trial of vamorolone, a first-in-class steroid, shows improvements in side effects via biomarkers bridged to clinical outcomes. *Steroids*. 2018;134:43-52.
11. Reeves EKM, Hoffman EP, Nagaraju K, Damsker JM, McCall JM. VBP15: preclinical characterization of a novel anti-inflammatory delta 9,11 steroid. *Bioorg Med Chem*. 2013;21(8):2241–2249.
12. Heier CR, Yu Q, Fiorillo AA, *et al*. Vamorolone targets dual nuclear receptors to treat inflammation and dystrophic cardiomyopathy. *Life Sci Alliance*. 2019;2(1):e201800186.
13. Othonos N, Pofi R, Arvaniti A, *et al*. 11beta-HSD1 inhibition in men mitigates prednisolone-induced adverse effects in a proof-of-concept randomised double-blind placebo-controlled trial. *Nat Commun*. 2023;14(1):1025.
14. Fenton CG, Doig CL, Fareed S, *et al*. 11beta-HSD1 plays a critical role in trabecular bone loss associated with systemic glucocorticoid therapy. *Arthritis Res Ther*. 2019;21(1):188.
15. Morgan SA, McCabe EL, Gathercole LL, *et al*. 11beta-HSD1 is the major regulator of the tissue-specific effects of circulating glucocorticoid excess. *Proc Natl Acad Sci U S A*. 2014;111(24):E2482-E2491.
16. Guglieri M, Clemens PR, Perlman SJ, *et al*. Efficacy and safety of vamorolone vs placebo and prednisone among boys with Duchenne muscular dystrophy: a randomized clinical trial. *JAMA Neurol*. 2022;79(10):1005–1014.
17. Dang UJ, Damsker JM, Guglieri M, *et al*. Efficacy and safety of vamorolone over 48 weeks in boys with Duchenne muscular dystrophy: a randomized controlled trial. *Neurology*. 2024;102(5):e208112.
18. Mah JK, Clemens PR, Guglieri M, *et al*. Efficacy and safety of vamorolone in Duchenne muscular dystrophy: a 30-month nonrandomized controlled open-label extension trial. *JAMA Netw Open*. 2022;5(1):e2144178.
19. Ahmet A, Tobin R, Dang UJ, *et al*. 2024. Dryad: Supplemental Material from: Adrenal suppression from vamorolone and prednisone in Duchenne muscular dystrophy: results from the phase 2b clinical trial. https://doi.org/10.5061/dryad.sbcc2frgb.
20. Javorsky BR, Raff H, Carroll TB, *et al*. New cutoffs for the biochemical diagnosis of adrenal insufficiency after ACTH stimulation using specific cortisol assays. *J Endocr Soc*. 2021;5(4):bvab022.
21. Kline GA, Buse J, Krause RD. Clinical implications for biochemical diagnostic thresholds of adrenal sufficiency using a highly specific cortisol immunoassay. *Clin Biochem*. 2017;50(9):475-480.
22. Raverot V, Richet C, Morel Y, Raverot G, Borson-Chazot F. Establishment of revised diagnostic cut-offs for adrenal laboratory investigation using the new Roche Diagnostics Elecsys((R)) cortisol II assay. *Ann Endocrinol (Paris)*. 2016;77(5):620-622.
23. Elecsys Cortisol II System Information. *Elecysis Cortisol II Assay Insert 2021-07*, V 3.0 (English).
24. Bornstein SR, Allolio B, Arlt W, *et al*. Diagnosis and treatment of primary adrenal insufficiency: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab*. 2016;101(2):364-389.
25. Zha L, Li J, Krishnan SM, *et al*. New diagnostic cutoffs for adrenal insufficiency after cosyntropin stimulation using Abbott architect cortisol immunoassay. *Endocr Pract*. 2022;28(7):684-689.
26. Vogeser M, Kratzsch J, Ju Bae Y, *et al*. Multicenter performance evaluation of a second generation cortisol assay. *Clin Chem Lab Med*. 2017;55(6):826-835.
27. Lashansky G, Saenger P, Fishman K, *et al*. Normative data for adrenal steroidogenesis in a healthy pediatric population: age- and sex-related changes after adrenocorticotropin stimulation. *J Clin Endocrinol Metab*. 1991;73(3):674-686.
28. O'Grady MJ, Hensey C, Fallon M, *et al*. Requirement for age-specific peak cortisol responses to insulin-induced hypoglycaemia in children. *Eur J Endocrinol*. 2013;169(2):139-145.
29. Sabanes Bove D, Li L, Dedic J, *et al*. mmrm: Mixed Models for Repeated Measures. R package version 0.3.11. 2024.
30. R Core Team. R: A Language and Environment for Statistical Computing. Vienna, Austria. https://www.R-project.org/. 2023.
31. Wickham H. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag; 2016.
32. Gjerstad JK, Lightman SL, Spiga F. Role of glucocorticoid negative feedback in the regulation of HPA axis pulsatility. *Stress*. 2018;21(5):403-416.
33. FDA Vamorolone Prescribing Information. Accessed April 24, 2024. https://www.accessdata.fda.gov/drugsatfda_docs/label/2023/215239s000lbl.pdf.
34. European Union Summary of Product Characteristics. Accessed April 24, 2024. https://www.ema.europa.eu/en/documents/product-information/agamree-epar-product-information_en.pdf.
35. Stewart PM, Corrie J, Seckl JR, Edwards CR, Padfield PL. A rational approach for assessing the hypothalamo-pituitary-adrenal axis. *Lancet*. 1988;1(8596):1208-1210.
36. Hurel SJ, Thompson CJ, Watson MJ, Harris MM, Baylis PH, Kendall-Taylor P. The short Synacthen and insulin stress tests in the assessment of the hypothalamic-pituitary-adrenal axis. *Clin Endocrinol (Oxf)*. 1996;44(2):141-146.
37. Bartley JA, Patil S, Davenport S, Goldstein D, Pickens J. Duchenne muscular dystrophy, glycerol kinase deficiency, and adrenal insufficiency associated with Xp21 interstitial deletion. *J Pediatr*. 1986;108(2):189-192.
38. Rathnasiri A, Senarathne U, Arunath V, *et al*. A rare co-occurrence of Duchenne muscular dystrophy, congenital adrenal hypoplasia and glycerol kinase deficiency due to Xp21 contiguous gene deletion syndrome: case report. *BMC Endocr Disord*. 2021;21(1):214.
39. Clarke A, Roberts SH, Thomas NS, Whitfield A, Williams J, Harper PS. Duchenne muscular dystrophy with adrenal insufficiency and glycerol kinase deficiency: high resolution cytogenetic analysis with molecular, biochemical, and clinical studies. *J Med Genet*. 1986;23(6):501-508.
40. Cortez S, Arbelaez AM, Wallendorf M, McNerney K. Peak serum cortisol cutoffs to diagnose adrenal insufficiency across different cortisol assays in children. *J Clin Res Pediatr Endocrinol*. 2023;15(4):375-379.
41. Nieman LK, Raff H, DeSantis A. Diagnosis of adrenal insufficiency in adults. *Up To Date*. Accessed July 4, 2024. https://www.uptodate.com/contents/diagnosis-of-adrenal-insufficiency-in-adults
42. Husni H, Abusamaan MS, Dinparastisaleh R, Sokoll L, Salvatori R, Hamrahian AH. Cortisol values during the standard-dose cosyntropin stimulation test: personal experience with Elecsys cortisol II assay. *Front Endocrinol (Lausanne)*. 2022;13:978238.
43. Munro V, Elnenaei M, Doucette S, Clarke DB, Imran SA. The effect of time of day testing and utility of 30 and 60 minute cortisol values

in the 250 mcg ACTH stimulation test. *Clin Biochem*. 2018;54: 37-41.
44. Maguire AM, Biesheuvel CJ, Ambler GR, Moore B, McLean M, Cowell CT. Evaluation of adrenal function using the human corticotrophin-releasing hormone test, low dose synacthen test and 9am cortisol level in children and adolescents with central adrenal insufficiency. *Clin Endocrinol (Oxf)*. 2008;68(5):683-691.
45. Le Roux CW, Meeran K, Alaghband-Zadeh J. Is a 0900-h serum cortisol useful prior to a short synacthen test in outpatient assessment? *Ann Clin Biochem*. 2002;39(Pt 2):148-150.