Neuromuscular Disorders

Results of an open label feasibility study of Sodium Valproate in people with McArdle disease

--Manuscript Draft--

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Abstract:	McArdle disease results from a lack of muscle glycogen phosphorylase in skeletal muscle tissue. Regenerating skeletal muscle fibres can express the brain glycogen phosphorylase isoenzyme. Stimulating expression of this enzyme could be a therapeutic strategy. Animal model studies indicate that sodium valproate (VPA) can increase expression of phosphorylase in skeletal muscle affected with McArdle disease. This study was designed to assess whether VPA can modify expression of brain phosphorylase isoenzyme in people with McArdle disease. This phase II, open label, feasibility pilot study to assess efficacy of six months treatment with VPA (20 mg/kg/day) included 16 people with McArdle disease. Primary outcome assessed changes in VO 2 peak during an incremental cycle test. Secondary outcomes included: phosphorylase enzyme expression in post-treatment muscle biopsy, total distance walked in 12 minutes, plasma lactate change (forearm exercise test) and quality of life (SF36). Safety parameters. 14 participants completed the trial, VPA treatment was well tolerated; weight gain was the most frequently reported drug-related adverse event. There was no clinically meaningful change in any of the primary or secondary outcome measures including: VO 2 peak, 12 minute walk test and muscle biopsy to look for a change in the number of phosphorylase positive fibres between baseline and 6 months of treatment. Although this was a small open label feasibility study, it suggests that a larger randomised controlled study of VPA, may not be worthwhile.
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	Professor, Universidad Europea de Madrid alejandro.lucia@universidadeuropea.es experienced in designing and running trials in GSDs and using exercise tests
	Nicol Voermans Radboud Universiteit Nicol.Voermans@radboudumc.nl Experienced in GSDs and clinical trials
	Tomas Pinos Vall d'Hebron Institut de Recerca tomas.pinos@vhir.org Published the animal model studies of valproate in GSDV, which inspired this clinical trial
Response to Reviewers:	

20th April 2020

Dear Victor

Re: Results of an open label feasibility study of sodium Valproate in people with McArdle disease

Thank you very much for giving us an opportunity to re-submit a revised manuscript. We are very grateful to your comments and those of the peer reviewers

With best wishes

Ros

SUBMISSION CHECKLIST for Neuromuscular Disorders

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It is the corresponding author's responsibility to ensure all co-authors have read and agreed the contents of the paper (on initial submission and on any revisions or subsequent resubmissions). Please prepare a statement confirming that all authors have agreed the contents of the submission. Please include the title and date, and signature. The corresponding author may sign on behalf of all co-authors.

16th April 2020

Dear Victor,

Re: Results of an open label feasibility study of sodium Valproate in people with McArdle Disease

Thank you very much for giving us an opportunity to submit a revised manuscript following peer review. We are very grateful to yourself and the peer reviewers for their helpful comments. I have re-submitted a revised manuscript with track changes. Our responses to the specific comments are also highlighted below in red.

With best wishes

Ros

Editor's comment

This phase II, open label, feasibility pilot study to assess efficacy of six months treatment with VPA (20 mg/kg/day) included 16 people with McArdle disease….. showed no benefit.
Guess it is a matter of semantics, but I always looked upon open label preliminary studies as preliminary studies from which one could not draw definitive conclusions. But perhaps an open label study that is negative is enough to decide not to proceed further…….
Thanks for this comment, we have removed any statement indicating a definitive outcome and have amended the text in the conclusion to read as follows: 'This feasibility study to assess efficacy of 20 mg/kg/day VPA in McArdle disease was planned to power a larger placebo-controlled trial of VPA in this patient population. Our results demonstrated that VPA was well-tolerated but there was no clinically meaningful benefit after 6 months of treatment.'

Reviewer #1:

Results of an open label feasibility study of Sodium Valproate in people with McArdle disease

General comment
* Well written

Abstract
* Clear and concise
* Could a few comments about the secondary outcome measures be included?

We have added some comments about secondary outcomes in the abstract

Introduction:

* Well written

Results

* 17 recruited (11 men and 5 women) is not correct (11 = 5 = 16, not 17)

Thank you for spotting this typo, the number of participants has been corrected: 14 completed, 2 dropped out, one withdrawn

Discussion:

* It would be better to start with a summary of the main findings and subsequently discuss these findings in light of the studies in animals

We have added a paragraph regarding the main findings

* It would be nice if the authors could discuss the reasons why they think that VPA did not exert the expected effect in some more detail

We do not know why VPA did not have the expected positive outcome, we have added a paragraph describing other conditions SMA and ALS where a similar effect was found i.e. positive animal studies but negative clinical trials. We have also made the point in our discussion that VPA was administered intramuscularly in one animal study which could have resulted in muscle damage and then phosphorylase re-expression

* Could the authors add which alternative HDAC agents could be used in future trials?

We have added the following paragraph 'VPA and phenylbutyrate are classed as Aliphatic HDAC inhibitors, it's possible that this group of drugs have less of an effect on skeletal muscle or anterior horn cell in humans. There are other classes of HDAC inhibitors, although they have greater toxicity and are usually used for cancer treatment for example Vorinostat, Romidepsin, Panobinostat, and Belinostat. The unwanted adverse effects may mean that these drugs are less likely to be suitable for people with neuromuscular disease.'

* Did the results in this study lead to any other new insights?

We have added a sentence explaining that VPA was tolerated in McArdle disease, therefore it can be used safely for other indications such as epilepsy, migraine and bipolar disorder in this population.

* Was the weight gain more than reported in cohorts of patients with epilepsy treated with VPA? Were patients warned for this?

No it was not worse than expected, we have added a sentence to this effect

Reviewer #2:

This is a necessary and important work that properly analyzes the effects of VPA in McArdle disease patients. The manuscript is well written, properly structured and organised and results are clear. However, I feel that previous its publication some minor issues should be addressed:

1) In the introduction section it is stated that "In mature skeletal muscle fibres BGP expression is suppressed due to methylation of the BGP gene promoters as part of a post-natal downregulation of this foetal enzyme (references 13-15)". In this regard, as far as this reviewer is concerned, it has never been experimentally proven that Pygb and/or Pygl gene promoters are methylated, and that this methylation is causing the down regulation of Pygb/Pygl expression postnatally. It is true that different genome browsers, such as UCSC genome browser, shows that Pygb and Pygl promoters

present Cpg islands, but whether these are methylated or not, and whether these methylations cause a downregulation of Pygb and/or Pygl gene expression postnatally has not yet been proven. It is very likely that methylation of these promoters occur, but it can not be stated in the introduction as a proven fact. Besides, references 13 to 15 do not support the statement.

This was a hypothesis, we have removed the statement

2) In the highlights the abreviation for sodium valproate is VS while throughout the manuscript is VPA. In keywords the term valproic acid is used, while in the manuscript sodium valproate is used instead.

We have changed to VPA throughout

3) A little explanation of why the VPA dose of 20 mg/kg/day and also the length of the treatment were chosen would be useful.

We have added the following statement in the methods section 'This dose was chosen as it is the lowest recommended dose for treating epilepsy and we wanted to minimise known drug-related side-effects such as weight gain, drowsiness and thrombocytopenia that might outweigh any potential benefit. The daily dose was rounded up to the nearest available tablet strength and the maximum dose was 2g/day. Treatment for six months was chosen as a cut-off point for the end of the trial, the ovine clinical trial was conducted over 15 weeks and showed the number of phosphorylase positive fibres increased over time[17]. We, therefore, decided to treat our patients for a longer time period to maximise any potential positive impact.'

4) Although the authors find in McA patient biopsies some percentage of regenerating fibres, these are not positive for glycogen phosphorylase. In the introduction is stated that regenerating fibres express glycogen phosphorylase (potentially Pygb or Pygl isoforms). Isn't it surprising? Any possible explanation? Any positive control for the technique was used?

A positive control was used and the stain was positive on smooth muscle fibres of vessels, it is possible that the fibres that were positive for neonatal myosin were not regenerating fibres. We have added a statement and very recent reference to that effect.

5) If the hypothesis is that VPA enhances the expression of the Pygb and/or Pygl isoforms, did the authors ever considered the possibility to analyse Pygb/Pygl mRNA by qPCR or protein levels by western blot?. Please, develop why these techniques were not used.

We have made some edits to the text and added a reference

'The histochemical method used identifies all isoforms of phosphorylase, given that the mutations in PYGM result in no phosphorylase expression, it was not considered necessary to include other methods to identify specific phosphorylase isoforms such as PCR.'

'Although neonatal myosin is often used as a marker for fibre regeneration there is evidence it can be up-regulated[20]. Muscle biopsies from a variety of neuromuscular conditions often show fibres with neonatal myosin for unknown reasons[14]. In our study the presence of a few fibres that showed neonatal myosin but no phosphorylase suggests that these were not regenerating fibres but rather that neonatal myosin had been upregulated. There are currently no antibodies to the brain isoform that reliably work on human muscle biopsies, however, the histochemical stain for phosphorylase detects all three isoforms of phosphorylase, we are therefore confident that VPA treatment did not up-regulate either the brain or liver isoform in our participants. In the sheep study[17], it was not possible to completely exclude a local toxic effect of intramuscular VPA, which

could have triggered muscle regeneration and thus the expression of foetal isozyme in injected muscles. Saline injected sheep showed a few fibres with neonatal myosin suggesting that mild muscle damage may have resulted from injection. In addition, there was a mild inflammatory response which was not seen in the muscle biopsies of our participants treated with oral VPA. An *in vitro* study analysed muscle cultures from KI mice exposed to VPA for 72 hours at 1, 2 and 5 mM and showed a dose-dependent increase in BGP was shown together with a reduction in intracellular glycogen content[18]

Reference: V Dubowitz, C Sewry, A Oldfors. Metabolic Myopathies 1: Glycogenoses and Lysosomal Myopathies. In: Muscle Biopsy: A Practical Approach. Fifth Edition 2020, Elsevier Oxford

Highlights

- Sodium Valproate (VS) stimulates expression of BGP in GSDV animal models
- An open label study of VS in GSDV showed no benefit in people with GSDV
- VS did not stimulate the expression of BGP in people with GSDV

Results of an open label feasibility study of Sodium Valproate in people with McArdle disease

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The corresponding author ensures all co-authors have read and agreed the contents of the paper

ABSTRACT

McArdle disease results from a lack of mMuscle gGlycogen phosphorylase in skeletal muscle tissue. Regenerating skeletal muscle fibres can express the brain glycogen phosphorylase isoenzyme. Stimulating expression of this enzyme could be a therapeutic strategy. Animal model studies indicate that sodium valproate (VPA) can increase expression of phosphorylase in skeletal muscle affected with McArdle disease. This study was designed to assess whether VPA can modify expression of brain phosphorylase isoenzyme in people with McArdle disease. This phase II, open label, feasibility pilot study to assess efficacy of six months treatment with VPA (20 mg/kg/day) included 16 people with McArdle disease. Primary outcome assessed changes in VO_2peak during an incremental cycle test. Secondary outcomes included: phosphorylase enzyme expression in post-treatment muscle biopsy, total walked distance walked in 12 minutes, plasma lactate change (forearm exercise test) and quality of life (SF36). Safety parameters were collected. 14 participants completed the trial, VPA treatment was well tolerated; weight gain was the most frequently reported drug-related adverse event. There was no clinically meaningful change in any ofeither the primary or secondary outcome measures including: VO_2peak, 12 minute walk test and muscle biopsy to look for a change in the number of phosphorylase positive fibres between baseline and 6 months of treatment.

Although this was a small open label feasibility study, it suggests that a larger randomised controlled study of VPA, may not be worthwhile. ~~or any other secondary outcomes. Treatment with VPA does not benefit people with McArdle disease~~study participants.

KEYWORDS:

Glycogen Storage Disease type V, Sodium Valproate (VPA)~~valproic acid~~, VO_2peak, 12-minute walking test, outcome measures

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(I suggest to amend the highlights to match VPA, and to keep the key words and manuscript the way it is):

In the highlights the abreviation for sodium valproate is VS while throughout the manuscript is VPA. In keywords the term valproic acid is used, while in the manuscript sodium valproate is used instead.

~~1.~~ INTRODUCTION:

McArdle disease is an autosomal recessive condition caused by mutations in the muscle glycogen phosphorylase gene (*PYGM*). Affected patients lack the enzyme, muscle glycogen phosphorylase (MGP), which is essential for glycogen breakdown in skeletal muscles,[1-4] which~~This~~ results in severe impairment of physical activity, especially when the onset of exercise is abrupt, high intensity or isometric in nature.[5, 6~~5-8~~] Currently, there is no satisfactory drug treatment for McArdle disease. Identifying new therapeutic strategies are therefore warranted.[9~~7~~]

Mammals have three glycogen phosphorylase isoforms encoded by different genes that are tissue specific: muscle (MGP), liver (LGP) and brain (BGP).[8-10~~10-12~~] MGP, the exclusive form expressed in mature skeletal muscle fibres, is absent in people with McArdle disease due to recessively inherited mutations in the corresponding gene.[9~~10~~] BGP is encoded by *PYGB* and is expressed in developing~~foetal~~ muscle tissue both *in vivo* and *in vitro*, and~~it~~ is thus transiently~~may be temporarily~~ expressed in regenerating skeletal muscle fibres.[13-15~~11-14~~] ~~In mature~~

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~~skeletal muscle fibres BGP expression is suppressed due to methylation of the BGP gene promoters as part of a post-natal downregulation of this foetal enzyme[13-15].~~

In-vitro studies on human primary skeletal muscle cell cultures derived from people with McArdle disease showed expression of BGP.[13] Such findings combined with knowledge of the normal physiological response to muscle damage (muscle regeneration) suggest that pharmaceutical reactivation of BGP in mature skeletal muscle fibres may be a therapeutic strategy for McArdle disease.[13]

Sodium valproate (Valproic Acid, VPA) belongs to a group of drugs known as 'histone deacetylase (HDAC) inhibitors' that can activate the expression of methylated genes by increasing the accessibility of the demethylase enzyme to the DNA.[~~16, 17~~15,16] A trial of VPA treatment in an ovine model of McArdle disease resulted in an increased number of glycogen phosphorylase positive skeletal muscle fibres, suggesting activation of BGP.[~~18~~ 17] Encouraging results were also obtained in an *in vitro~~e~~* knock-in (KI) mouse model of McArdle disease carrying the p.R50X mutation. Following VPA exposure, cultured myotubes from the mouse model expressed BGP in association with a dose-dependent decrease in muscle glycogen accumulation.[~~17~~18]

Based on this preclinical research, VPA could be considered as a potential therapeutic target for McArdle disease. This study was designed as a feasibility/pilot study to: a) determine whether or not VPA has an effect on BGP expression and b) to power a future randomised, placebo-controlled study (RCT).

~~2.~~ METHODS:

The study was conducted at two sites: UCL Institute of Neurology, London, UK and Rigshospitalet, Copenhagen, Denmark. Protocol and study documents were

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approved by ethical committees and regulatory bodies for each site. Informed consent was obtained from all participants prior to any study procedures and the study was conducted in line with good clinical practice as determined by the Declaration of Helsinki. The trial was registered at ClinicalTrials.gov (NCT03112889).

~~2.1.~~ Study design

A phase II, open label, multi-centre feasibility study.

~~2.2.~~ Participants

~~2.2.1.~~ Based upon previous research in McArdle disease, it was anticipated that data from 16 participants would be adequate to provide a good estimate of the standard deviation of the change in the VO_2peak to inform the sample size calculation for a future RCT.[19]

~~All participants had a genetically confirmed diagnosis of McArdle disease and were over 18 years of age.~~

~~Based upon previous research in McArdle disease, it was anticipated that data from 16 participants would be adequate to provide a good estimate of the standard deviation of the change in the VO_2peak to inform the sample size calculation for a future RCT.[19]~~

Inclusion criteria:

All participants were over 18 years of age and diagnosed with GSDV (confirmed by DNA analysis for recessive mutations in *PYGM* and/or muscle biopsy showing subsarcolemmal blebs of glycogen and absence of skeletal muscle glycogen phosphorylase on histochemical stain. All participants (male and female) had to use contraception throughout the study unless they were post-menopausal or infertile.

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~~2.2.2.~~

~~All participants were required to use contraceptives for the duration of the study unless they were clinically confirmed infertile or post-menopausal women. Participants had to have a normal acyl-carnitine profile at screening and had to be able to perform the exercise assessments.~~

Exclusion criteria:

~~2.2.3.~~ The following were exclusion criteria: pregnancy, diabetes, inflammatory disorders e.g.systemic lupus erythematosus, sensitivity/allergy to VPA, treatment with VPA within 12 months prior to recruitment, pre-existing liver disease or a family history of severe liver disease affecting a first degree relative, anti-convulsant medication or any other medication known to interact with VPA, sensitivity to local anaesthetics that would prevent muscle biopsy, any co-morbid illness or disability which would prevent an exercise assessment. Other metabolic condition affecting either the patient or a first-degree relative such as porphyria, mitochondrial disease, abnormal acyl carnitine profle or low serum carnitine

~~Participants with a history of diabetes, inflammatory disease, liver disease, porphyria or positive family history of either liver or mitochondrial disease were excluded. A list of inclusion and exclusion criteria can be found at ClinicalTrials.gov (NCT03112889).in Supplement xx.~~

~~2.3. Intervention~~

All participants received VPA extended release tablets (by Sanofi-Aventis) once daily. Participants were warned of the expected VPA related side-effects such as weight gain, fatigue, alterations in blood indices and risk of liver damage. VPA was introduced slowly with an escalating dose regimen with 5mg/kg/day increments each week for three weeks up to the full dose treatment (20mg/kg/day). This dose was

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chosen as it is the lowest recommended dose for treating epilepsy and we wanted to minimise known drug-related side-effects such as weight gain, drowsiness and thrombocytopenia that might outweigh any potential benefit. The daily dose was rounded up to the nearest available tablet strength and the maximum dose was 2g/day. The ovine clinical trial conducted over 15 weeks showed an increase in the number of phosphorylase positive fibres over time[17]. We decided to treat our patients for 6 months, a longer time-period than the ovine trial, to maximise any potential positive impact. After six months on full dose treatment and after the final study visit, VPA dose was reduced by 5mg/kg/day each week for three weeks and then discontinued.

~~Dose selection: The 20 mg/kg/day dose was selected based on the evidence available for VPA use as a treatment option for epilepsy (NICE, 2018). The suggested maintenance dose for epilepsy treatment is 20–30 mg/kg/day (NICE, 2018). Thus the lowest recommended therapeutic dose for epilepsy was chosen. To reduce the risks of having a negative trial due to short treatment duration, a six-month treatment period was selected; slightly longer than the treatment period used in the ovine model study.~~

Commented [SR{4]: **Reviewer Comment:** A little explanation of why the VPA dose of 20 mg/kg/day and also the length of the treatment were chosen would be useful.

Commented [SR{5]: please confirm if this statement is correct

Commented [SR{6]: (Renata to add references once the final amended version is completed)

~~2.4.~~2.1. Study visits

At screening, participants underwent a full medical history and examination. Investigations included ECG, laboratory blood tests for free carnitine, acyl carnitine profile, full blood count, liver and renal function. Participants performed an incremental baseline cycle test to determine exercise capacity.

Following screening, there were three study visits at week 0 (baseline – V1), week 16 (+-7 days – V2) and week 28 (+-7 days – V3). In between visits participants were

telephoned every 4 weeks (+- 7 days) from baseline until week 40 to assess adverse events (AEs) and study compliance.

Assessment of Compliance. Compliance was also assessed at each study visit, and during telephone calls and returned pills were counted on V2 and V3. Compliance >90% was the minimum threshold for participants to continue in the trial.

~~2.5.~~ Outcome measures

Screening visit cycle test: All participants exercised~~cycled~~ on a cycle ergometer. Oxygen consumption was assessed with the Cortex ergospirometry system (Cortex Metalyzer II, Cortex Biophysik GmbH, Leipzig, Germany) in the UK or Quark CPET (Cosmed Srl., Milan, Italy) in DK. An incremental cycle ergometer test was performed (from zero to 20W in the first minute, increased by at least 5W every two minutes) to determine each participant's aerobic capacity (VO_2peak).

Primary outcome:

~~2.5.1.~~ VO_2peak was measured in a constant-to-maximal workload cycle test on V1, V2 and V3. After fasting for four hours, participants cycled for 15 minutes at a constant workload at 65% of the VO_2peak determined in the screening test. After 15min, the power output was increased by at least five watts every minute until maximal volition to determine the VO_2peak.

~~2.5.2.~~ *Secondary outcomes;*

a) *Muscle biopsy to assess number of phosphorylase positive fibres*: V1 and V3. Where available, recent diagnostic muscle biopsies of good quality undertaken prior to screening were used for analysis at baseline. The presence of phosphorylase was assessed using histochemistry and neonatal myosin staining was used to assess the presence of possible regenerating

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fibres. The histochemical method used identifies all isoforms of phosphorylase, given that the mutations in *PYGM* result in no phosphorylase expression, it was not considered necessary to include other methods to identify specific phosphorylase isoforms such as PCR. Muscle biopsy slides were scanned, and the number of glycogen phosphorylase muscle fibres were counted using ImageJ imaging software.

b) *Plasma lactate levels during a non-ischaemic forearm test: V1, V2, V3.* Repetitive maximal handgrip contractions using a hand-held dynamometer were performed every other second for one minute. Plasma lactate and serum ammonia levels were analysed at 0, 2 and 5 minutes.

c) *12-Minute Walk Distance (12MWD)*: V1, V2, V3 after 45 minutes of rest, following the cycle ergometer test. Participants were required to complete as many 10m shuttle walks as possible for 12 minutes on a marked corridor.[20] The total walked distance was analysed.

d) *Quality of life assessment: Short Form 36 (SF36 health survey)* V1, V2, V3 was completed and scored using the QualityMetric Health Outcomes™ Scoring Software. ~~Normative values of the 2009 population graded by age and gender.~~

e) *Safety measures:* All participants completed a symptom diary, which included: concomitant medications use, adverse events, myoglobinuria and significant worsening of McArdle symptoms information. The study team recorded adverse events at each visit and at frequent telephone calls. During V1, V2 and V3 safety blood analyses included: full blood count, CK, LFT, U&E, platelets, coagulation screen (PT, APTT, INR and fibrinogen), lactate, ammonia, glucose and VPA blood level.

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f) Adverse events were assessed.

~~2.6.~~

~~2.7.~~ Statistical analysis

Due to the pilot nature of the study, the study was not powered to show statistically significant differences between treatment groups, and so all analysis was descriptive in nature. Summaries at each time point were produced, in addition to summaries of the changes from baseline to both V2 and V3. Changes from baseline were also calculated as a percentage of the baseline. Continuous variables were summarised by the mean and standard deviation and data range if found to follow a Normal distribution, and by the median and inter-quartile range, and data range if not normally distributed. Categorical variables were summarised by the frequency and percentage of values in each category.

The primary outcome was VO_2peak, measured during exercise on a cycle ergometer at maximum volition. Clinically important increases in VO_2peak and 12-minute walk distance were predefined defined as greater than 10% of the baseline value. The clinical importance of any effects was compared to this fixed value.

We planned to use these data to provide an estimate of standard deviation of the change in each of these factors that would be required for the sample size calculation of a larger RCT in the future. Since exercise capacity in McArdle disease is relatively stable over time it was anticipated that baseline data from this study and pooled data from previously published studies would be able to provide data for the placebo arm of a future RCT.

~~3.~~ RESULTS:

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~~3.1.~~ Participants demographics.

19 participants were screened, and 17 recruited (121~~1~~ men and 5 women), mean age was 46.2 years (range 21 to 67 years). One recruited participant was withdrawn following screening because he failed to attend a pre-treatment muscle biopsy and V1 assessments (baseline). Two participants failed screening as they did not meet inclusion/exclusion criteria. Two participants dropped out between V1 and V2: one was lost to follow up and the second dropped out because of gastrointestinal AEs. In total, 16 participants attended V1 while 14 participants completed all trial visits. Mean drug compliance at V3 was 98.7% ± 1.6 (range: 95 – 100). The mean VPA level at V2 was 72+-27 (range 29-132) and at V3 66 +-23 (range 28-101).
Safety blood analyses did not demonstrate any clinically significant alterations.

~~3.2.~~ Primary outcome:

There was no improvement in VO_2peak from baseline to V3 measured by the cycle test (Figure 1, Table 1).

~~3.3.~~ Secondary Outcomes:

Exercise testing: Results for the secondary outcome measures associated with the cycle test are shown in table 2. There was no clinically meaningful change between baseline and V3.

Muscle biopsy analysis: The median percentage change in the number of~~of~~ phosphorylase positive fibres from~~at~~ baseline and V3 was 0.0~~zero~~ (IQR 0.0, 0.2), while the mean percentage change in the number of~~of~~ neonatal myosin positive~~regenerating~~ fibres was 0.6 +/-2.2~~2.1~~ at V3 (SD: ± 2.9) but these fibres did not ~~indicating no~~ expression~~ of~~ phosphorylase.~~ase enzyme in skeletal muscle after treatment.~~

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Forearm exercise test: The mean +/-SD change in plasma lactate from baseline to V3 was 0.12 +/- 0.34 (range -0.52, 0.78) indicating no clinically meaningful difference.

12-minute walk test: The mean total distance walked was 966m (range 683-1292m) at baseline and 949m (range 606-1690m) at V3.The mean variation in the total walked distance was 31m from baseline to visit 3 was 31m, indicating a 3% change which does not represent a clinically meaningful difference.

Quality of life: Results for the SF36 Quality of life questionnaire are shown in table 3. There were no clinically meaningful changes in the two main SF36 health domain scales from baseline to V3.

Adverse Events

VPA was ~~well~~ tolerated well. None of the participants experienced myoglobinuria during the course of the trial. Table 4 summarises AE data. There were a mean of 10 adverse events per participant (155 in total), most were rated as mild (67%) and unrelated to the study drug (60%). Weight gain was the only definite drug-related AE, the mean weight gain from V1 to V3 was +3.5kg (SD: 4.8; range: -3kg to +17kg) considered to be within the expected range for individuals taking VPA for other reasons. There were 21 AEs (14%) deemed as 'probably' related to VPA. There was one SAE, which was not considered to have been related to the study drug. One participant withdrew from the study due to gastrointestinal symptoms considered to have been related to VPA use.

~~4.~~ DISCUSSION:

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This open label study assessed the use of VPA in people affected by McArdle disease. Several endpoints were used to assess treatment efficacy, including the

primary endpoint: change in VO_2peak, and secondary endpoints: total distance walked on a 12MWT, forearm exercise ~~, increase in lactate production~~ test, histochemical~~following exercise , the~~ expression of phosphorylase enzyme in skeletal muscle and safety blood parameters. There was no clinically meaningful change from baseline to visit three for any of the primary and secondary endpoints. ~~and changes in total walked distances. All assessed endpoints failed to confirm benefits of VPA in the assessed sample.~~

VPA has previously been shown to stimulate the brain isoform of phosphorylase ~~expression~~ in two animal models of McArdle disease [17, 18]. In the sheep model, animals received increasing doses of enteric administration of enteral VPA (20 – 60mg/kg body weight). Muscle biopsies were performed at different times during the treatment phase, and in different muscle groups. In the same study, a group of sheep received intramuscular injections of VPA. An increase in phosphorylase positive fibres was seen in post-treatment muscle biopsies, which increased with higher doses of VPA and over time. However, neonatal myosin staining was not reported to confirm if the phosphorylase activity was~~positive fibres were~~ related to ~~, mature muscle fibres or to~~ regenerating fibres or induced. Although neonatal myosin is often used as a marker for fibre regeneration there is evidence it can be up-regulated[20]. Muscle biopsies from a variety of neuromuscular conditions often show fibres with neonatal myosin for unknown reasons[14]. In our study, the presence of a few fibres that showed neonatal myosin but no phosphorylase suggests that these were not regenerating fibres but rather that neonatal myosin had been upregulated. There are currently no antibodies to the brain isoform that reliably work on human muscle biopsies, however, the histochemical stain for phosphorylase

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detects all three isoforms of phosphorylase, we are therefore confident that VPA treatment did not up-regulate either the brain or liver isoform in our participants.

In the earlier sheep study[17], it wais not possible to completely exclude a local toxic effect of intramuscular ~~IM~~ VPA, which could have triggered muscle regeneration and thus the expression of foetal isozyme in injected muscles. Saline injected sheep showed a few fibres with neonatal myosin suggesting that mild muscle damage may have resulted from injection. In addition, there was a mild inflammatory response, which was not seen in the muscle biopsies of participants in this trial who were treated with oral VPA. However, a~~However, a~~n *in vitro* study analysed muscle cultures from KI mice exposed to VPA for 72 hours at 1, 2 and 5 mM[17] and showed a ~~. A~~ dose-dependent increase in BGP was shown together with a reduction in intracellular glycogen content.[17,18]

VPA is a well-known drug prescribed as a treatment option for epilepsy and migraine[21] ~~(Linde et al., 2013)~~. Its efficacy has also been evaluated for other conditions, including bipolar disorders and schizophrenia.[22,23] ~~(Wang et al., 2016, Cipriani et al., 2013).~~ More recently, studies in the field of neuromuscular disease have explored the role of VPA as a histone deacetylase inhibitor (HDAC). Even though *in vitro* studies have indicated an ~~beneficial~~ effect of VPA in spinal muscular atrophy, similar efficacy was not~~has not been~~ confirmed in clinical trials [24,25,26]~~by clinical trials performed in humans with the condition~~ ~~(Kissel et al., 2014, Krosschell et al., 2018, Kissel et al., 2011)~~. A Phase III study of VPA in amyotrophic lateral sclerosis showed no evidence for slowing~~also failed to demonstrate effectiveness~~ ~~in~~ disease progression or increasing ~~and~~ survival[27]. ~~(Piepers et al., 2009). A recent study suggested that VPA may be of potential value in oculopharyngeal muscular dystrophy, but there has been no clinical trial in humans with the condition (Abu-Baker et al., 2018).~~

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~~It was not possible to predict the effect of VPA in humans with McArdle disease based on previous research into other neuromuscular conditions because they have different pathophysiologies. However, for McArdle disease, both *in vitro* and *in vivo* research in animal models has provided strong evidence that VPA had a beneficial effect, supporting further study of VPA in humans affected by the condition. Therefore, a proof-of-concept study was developed to confirm early VPA efficacy data in humans living with the condition.~~

~~This study confirmed VPA was safe in the assessed patient population. Weight gain, an expected VPA-related AE was also seen in McArdle disease patients. Even though VPA therapy was safe in the assessed patients, Tt~~This study showed weight gain and GI disturbances to be the most significant side–effects from VPA~~e~~, otherwise the drug was well-tolerated, indicating its use would be safe for other indications, such as epilepsy. Migraine and bipolar disorder in the McArdle population. ~~efficacy~~ However, this small open label study failed to show any clinically meaningful therapeutic effect. ~~results of this open label feasibility study did not confirm the findings from animal studies and no clinically meaningful benefit was found. Therefore, we do not recommend any further study of VPA in people with McArdle disease. However, the strategy to upregulate BGP as a possible therapeutic strategy may still be feasible using alternative HDAC agents in future trials.~~

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I would not compare with epilepsy, the background is different (eg some patients have epilepsy and other conditions with cognitive impairment) or use additional anti-epileptic drugs in combination. I would not combine both as theyare completely different populations (epilepsy x NMD field)

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I can generate a paragraph on why VPA failed in other diseases. Please guide me on the strategy and I develop the argument

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~~5.~~ CONCLUSIONS:

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This feasibility study to assess efficacy of 20 mg/kg/day VPA in McArdle disease was planned to power a larger placebo-controlled trial of VPA in this patient population. Our results demonstrated that VPA was well-tolerated but there was no clinically meaningful benefit~~benefit from baseline~~ after 6 months of ~~VPA~~ treatment.

Acknowledgments:

We would like to dedicate this article to the memory of Professor John Howell who pioneered this work in an ovine McArdle disease model.

We would like to thank Muscular Dystrophy UK for funding this study and AGSDUK for their support in recruitment. This research was supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre. RSS received a scholarship from CAPES Foundation Ministry of Education Brazil, and the research study is described in more details in her PhD thesis.

We would also like to acknowledge ~~Professor John Howell,~~ Professor Byron Kakuluss for his~~and Professor Caroline Sewry for their~~ advice and support during the development of the study protocol.

Conflicts of interest:

None of the authors report any conflicts of interest related to the study medication.

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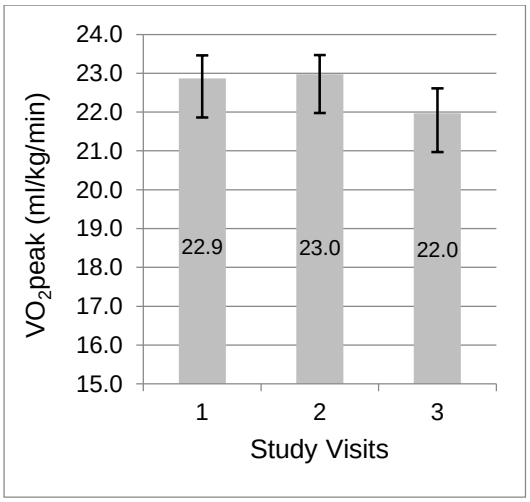
Figure 1



Mean VO_2peak for participants who completed all trial visits (n=14). Values are mean ± standard deviation. On V2: two participants were not included in mean VO_2peak analysis as they did not perform a maximal test.

Table 1

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Table 4

The corresponding author ensures all co-authors have read and agreed the contents of the paper