Author’s response to reviews

Title: Does Pentaerythritoltetranitrate reduce fetal growth restriction in pregnancies complicated by uterine mal-perfusion? Study protocol of the PETN-study: a randomized controlled multicenter-trial

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Author’s response to reviews:

Dear Editor,

I would like to thank you for your comments and the chance to again improve the manuscript. Please find the detailed list of changes in response to your comments below.

1. In your 'Inclusion criteria' section, please amend the following sentence by replacing '3rd' with 'third'

'Accordingly, at least one 3rd of the pregnant women diagnosed with impaired perfusion of the uterine arteries at mid gestation will develop FGR and associated adverse pregnancy outcomes.'

In the “inclusion criteria” section on page 5 line 34 3rd was replaced by third.

2. In your 'Inclusion criteria' section, please specify that the participants of your study are over 18 years old.

On page 5 line 2 The sentence “… singleton pregnancy at 190 to 226 weeks of gestation who provide informed consent.” was changed to “… singleton pregnancy at 190 to 226 weeks of gestation who are over 18 years old and provide informed consent”.


3. In your Background section, you are missing a reference in line 53. Please amend this.

The missing reference was included at page 3 line 36.

4. We note that the current submission contains some textual overlap with other previously published works, in particular:


Overlap exists in the 1st and 3rd paragraphs of your Introduction.

and


Overlap exists in the 4th, 6th, 7th and 8th paragraph of your Discussion.

While we understand that you may wish to express some of the same ideas contained in these publications, please be aware that we cannot condone the use of text from previously published work. Please rephrase these sections to minimise overlap.

In the first paragraph of the introduction:

“Affecting approximately 10% of pregnancies, fetal growth restriction (FGR), is the most important cause of perinatal mortality and morbidity, affecting short term and long term outcome of the newborns with lifelong consequences as developmental origin of adult diseases [1]. When compared to normally grown fetuses, mortality rates in FGR alone are increased and preterm delivery combined with FGR is reported to be associated with higher rates of cerebral palsy, sensory deficits, learning disabilities, and respiratory illnesses [2]. Impaired placental function determined by insufficient transformation of the uterine arteries and mal-perfusion of the placenta is the leading cause of FGR [3]. Consequently, screening for placental insufficiency based on uterine artery Doppler measurement is well established [4, 5].“

We changed the text to:

Affecting approximately 10% of pregnancies, fetal growth restriction (FGR), is the most important cause of perinatal mortality and morbidity. Mortality rates are increased in FGR at any
gestational age, and in combination with preterm delivery FGR is associated with higher rates of cerebral palsy, sensory deficits, learning disabilities, and respiratory illnesses [2]. Additionally, individuals born growth restricted are facing lifelong consequences as FGR accounts as developmental origin of adult diseases [1]. The leading cause of FGR is mal-perfusion of the placenta based on insufficient transformation of the uterine arteries and impaired placental function [3]. Uterine artery Doppler measurement is used to determine utero-placental perfusion during pregnancy and thus is established as Screening for placental insufficiency [4, 5].

The third paragraph of the introduction:

“The organic nitrate pentaerithrityl tetranitrate (PETN) is widely used for the treatment of angina pectoris, improving perfusion of and oxygen supply to the myocardium. In disparity to other NO-donors, prolonged intake of PETN is not associated with development of tolerance and, aside from vasodilation, also possesses potent endothelium protective qualities by enhancing the expression of antioxidant genes, like heme oxygenase-1 (HO-1), in human endothelial cells [10]. The described biological effects and its well-documented biological safety unveiled PETN to possibly be effective and safe for secondary prevention in pregnancies complicated by and at risk of FGR.”

Was changed to

The organic nitrate pentaerithrityl tetranitrate (PETN) is commonly used in the treatment of angina pectoris. As a No-donor PETN improves perfusion and oxygen supply to the myocardium. In disparity to other NO-donors, aside from vasodilation, PETN also possesses potent endothelium protective qualities by enhancing the expression of antioxidant genes, like heme oxygenase-1 (HO-1), in human endothelial cells [10]. The described biological effects and its well-documented safety unveiled PETN to possibly be effective and safe for secondary prevention in pregnancies complicated by and at risk of FGR.

Regarding the discussion the fourth paragraph, starting on page 4 line 31 was:

“Low-dose aspirin and other antiplatelet agents have been widely studied for secondary and primary prevention of FGR and preeclampsia. Askie and colleagues published a meta-analysis in 2007 of individual patient data of more than 32,000 women in 31 randomized trials [20]. Their data-analysis showed a moderate, but consistent reduction in the relative risk of birth before 34 weeks’ gestation and of preeclampsia, but not of perinatal death or having a small of gestational age infant. Recently data from the ASPRE trial where the effect of 150 mg aspirin to prevent preeclampsia in high risk women was investigated also showed a reduction of FGR cases in women developing preeclampsia [21, 22] when started before 16 weeks of gestation. However,
this beneficial effect seems to be related only to primary, not secondary prophylaxis. Bujold and colleagues published a series of meta-analyses clearly showing no reduction of PE or FGR by LDA when started after 16 weeks [23].”

We discarded the last sentence, which is identical to one in Schleußner 2014.

The sixth paragraph was:

“The clinical benefit of using NO donors as a prophylactic drug for pregnant women at risk was demonstrated for the first time by Lees et al. [11]. In this randomized placebo-controlled blinded trial glycerol trinitrate (GTN) patches increased the likelihood of a complication-free pregnancy. However, this study failed to demonstrate a reduction in FGR, preterm delivery or PE, possibly due to the small sample size of not more than 40 women. Furthermore, the use of GTN is associated with development of tachyphylaxia, i.e. nitrate tolerance, which is initiated within a few days, thus limiting the clinical effect [28]. A recent study provided by Valensise and colleagues showed a significant improvement of maternal hemodynamics in hypertensive pregnancies upon the addition of NO-donors to antihypertensive therapy and plasma volume expansion, enabling a prolongation of pregnancy. The authors conclude that the improvement of hemodynamics by NO donors affect the materno-placental perfusion reducing pregnancy complications associated with impaired perfusion [29]. NO donors can improve impaired uteroplacental perfusion, the major reason of placental insufficiency, without any effects on fetal circulation [30].”

Changes were made starting on page 9 line 7 to:

Lees and co-authors demonstrated the clinical effect of NO-donors in pregnant women at risk before [11]. In a randomized placebo-controlled blinded trial he demonstrated an increased likelihood for complication free pregnancy upon administration of glycerol trinitrate (GTN). Due to the small sample size the study, however, failed to demonstrate significant effects for the primary reduction in FGR, preterm delivery and PE. Furthermore, the development of nitrate tolerance was observed within a few days, could additionally limit the clinical effect [28]. Recently, the group of Valensise showed an improvement of maternal hemodynamics in complicated pregnancies when NO-donors were added to the initiated hypertensive therapy. The authors conclude that, as a consequence of changes in maternal hemodynamics, utero-placental perfusion is ameliorated and thus pregnancy outcome improved [29]. Complementary, it was shown by our group before, that NO-donors improve placental perfusion without affecting fetal circulation [30].
The seventh and eighth paragraph were:

“In contrast to all other organic nitrates, such as nitroglycerin, isosorbide-5-mono-nitrate, and isosorbide dinitrate, PETN is an organic nitrate which does not develop a nitrate tolerance and has recently been discovered to have further features [28, 31]. Using microarray analysis PETN has been shown to enhance the expression of the antioxidant genes HO-1 and ferritin heavychain (FeHc) in human endothelial cells. These differences in HO-1 and FeHc expression potentially explain the diverse effect on mitochondrial ROS production, the inhibition of mitochondrial aldehyde dehydrogenase activity and thus the avoidance of tolerance development [28]. “

We changed it to a new paragraph at page 9 line 25 to 34 as follows:

PETN, however, has notably peculiar features which differentiate it from all other organic nitrates, such as nitroglycerin, isosorbide-5-mono-nitrate, and isosorbide dinitrate. Upon treatment with PETN no develop a nitrate tolerance can be observed, probably due to its specific impact on intracellular signalling pathways [28, 31]. PETN has been shown to increase the expression of the antioxidant genes HO-1 and ferritin heavychain (FeHc) in human endothelial cells. These increase in HO-1 and FeHc expression potentially impact on mitochondrial ROS production and the inhibition of mitochondrial aldehyde dehydrogenase activity and thus the avoidance of tolerance developement [28]. So far it remains speculative, whether reduced ROS production and enhanced HO-1 expression might also serve as a mechanism provoking improved endothelial cell function and thus vascular health.

5. In the Ethics approval and consent to participate section, please provide the full names of all IRBs which approved your study protocol. If the IRBs are too numerous to list in this section, please provide their names in an additional file and list only the main IRB in this section, citing the additional file for the names of all IRBs.

Local IRBs were included in the text:

Ethical approval has been obtained from the ethical committee of the University Hospital of Jena as leading committee (Institutio-nal review board (IRB) Friedrich Schiller University, Jena) (5085-02/17). Each of the 14 German study centers obtained ethical approval of their local committees (IRB Universitäts-Kinderklinik, Kiel, IRB Hannover Medical School, Hannover, IRB Medical School, Rheinischen Friedrich-Wilhelms-University, Bonn, IRB State Office for Health and Social Affairs (LAGeSo) Berlin (two centres), IRB of the state of Saxony-Anhalt, Halle (two centres), IRB Medical School, Leipzig, IRB Technical University Dresden, IRB Medical School, Eberhard-Karls-University, Tübingen, IRB University of Ulm, IRB Ludwig Maximilian University, Munich, IRB national Medical association Bavaria, Munich), which than were referred to the Jena committee before approval was issued. Written informed consent must be
obtained before the performance of any protocol related procedures that are not part of usual subject care.

6. Please remove the cover letter and SPIRIT documents from the end of your manuscript file as these are no longer needed in the publication process.

7. At this stage, please upload your manuscript as a single, clean version that does not contain any tracked changes, comments, highlights, strikethroughs or text in different colours. All relevant tables/figures/additional files should also be clean versions. Figures (and additional files) should remain uploaded as separate files.