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Göttingen Minipigs
MAGAZINE

ELLEGAARD
GÖTTINGEN MINIPIGS

Dear reader

This issue of the Göttingen Minipigs Magazine features new scientific articles on recent studies and presents exciting updates and news:

On page 4 you can read about the **EU-funded NHPig project**, which aims at leveraging the translational relevance of mini- and micropig models as alternatives to NHPs. Supported by 27 international organisations, the project is developing a comprehensive database on Göttingen Minipigs for safety testing.

I am excited to share that, in response to the increasing demand of genetically modified Göttingen Minipigs, particularly the

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IgG 1/4 Humanized Göttingen Minipigs, we are expanding our research facility. You can read more about this on page 31.

Another piece of exciting news is that we have introduced an **AI-powered chatbot** designed to assist researchers by providing answers based on validated scientific sources, namely PubMed and minipigs.dk. You can get more information on page 25 or try it out yourself on our website.

2025 presents multiple opportunities to learn more about Göttingen Minipigs in biomedical research:

- **The Minipig Research Forum 2025** takes place April 9-11 in Amsterdam, The Netherlands. This recurring meeting is a key event for knowledge exchange, featuring scientific sessions, poster presentations, and interactive discussions this year on species selection, metabolism research, and dosing challenges and solutions.
- **The Göttingen Minipigs Symposium** returns May 8 in Boston, MA, USA. This full-day event offers presentations and networking focused on Göttingen Minipigs as a relevant species in biomedical research.
- **The Göttingen Minipigs Academy** offers specialised training courses and provides hands-on learning tailored to research and animal care professionals.

I hope you will find valuable insights and resources to support your research and application of Göttingen Minipigs in this edition.

Martin Windfeld Velin, CEO
Ellegaard Göttingen Minipigs A/S

CONTENTS

EU Project NHPig: Replacing NHPs with mini- and micropig models

The landscape of biomedical research is evolving, driven by a pressing need to refine, reduce, and replace the use of animals in non-clinical safety assessment. In this transformative era, the EU-funded NHPig project stands at the forefront of innovation, offering a visionary approach to non-clinical safety testing by leveraging mini- and micropig models as viable alternatives to non-human primates (NHPs). With a substantial €17.5 million backing from the Innovative Health Initiative Joint Undertaking and industry partners, this five-year endeavor signals a paradigm shift in the way we approach drug development and regulatory science.

A Unified Effort for Ethical and Effective Research

NHPig is not just a research project; it is a collaborative powerhouse uniting 27 leading international organizations under the leadership of Ludwig-Maximilians-Universität München (Germany) and Novo Nordisk A/S (Denmark). By bringing together expertise across academia, industry, and regulatory bodies, the project is poised to generate robust scientific insights that will enhance the translational relevance of mini- and micropig models.

At the heart of NHPig's mission is the commitment to the 3R principles: Replacement, Reduction, and Refinement. These guiding tenets underpin every initiative within the project, ensuring that the use of animals in research is not only

The mission of NHPig

NHPig is an EU-funded research project aimed at transforming non-clinical safety assessment by use of mini- and micropig models. The goal is to expand and share biological knowledge on mini- and micropig models and improve the translational understanding between pig models and non-human primates (NHPs) and humans with the possibility to use these pig models as viable alternatives to NHPs in the nonclinical safety testing.

Learn more on the official website
nhpig.eu/

minimized but also optimized to yield the highest scientific and ethical standards. Ellegaard Göttingen Minipigs A/S is one of the 27 organisations in the project, and about their role CSO, Lars Siim Madsen, says: "The Göttingen Minipig has long been recognized for its translational relevance in biomedical research. Through NHPig, we now have the opportunity to further refine its application, ensuring that it serves as a scientifically robust and ethically sound alternative to non-human primates in non-clinical safety assessments."

Building a Comprehensive Knowledge Base

A cornerstone of NHPig's work is the development of a database and IT platform that facilitates cross-species comparison of mini- and micropig biological data. This initiative will bridge the translational gap between pigs, NHPs, and humans, equipping researchers with an invaluable tool for data-driven decision-making in preclinical safety testing. "By participating in NHPig we wish to contribute to the development of cutting-edge models and data-driven insights. Ellegaard Göttingen Minipigs A/S has always invested ressources in knowledge-sharing, and in this forum we get the opportunity to join in the discussion about minipigs in contemporary and future research" Lars Siim Madsen explains.

The project is also characterizing humanized IgG1/4 minipigs and micropigs to evaluate their suitability for testing human therapeutic antibodies. By delving into the immunological responses of these models, NHPig aims to establish pigs as reliable and ethical alternatives for assessing drug safety and efficacy.

Harnessing Technology and AI for Advanced Safety Testing

A defining feature of NHPig's approach is its embrace of cutting-edge technology. The project is establishing biobanks of wildtype and genetically modified minipigs and micropigs, enabling researchers to collect and analyze multi-omics data. These comprehensive datasets will fuel biomarker discovery and improve the predictive accuracy of preclinical models.

Artificial intelligence (AI) is also playing a crucial role in NHPig's objectives. The project is developing AI-integrated digital solutions that enhance preclinical toxicity studies by objectively measuring clinically relevant biomarkers. These innovations will not only improve data accuracy and reliability but also ensure compliance with stringent regulatory requirements.

Regulatory and Ethical Excellence

Ensuring the highest standards of ethical research and regulatory alignment is paramount for NHPig. To this end, the project has established both a Regulatory Advisory Board and an Ethics and Animal Welfare Advisory Board. These bodies will guide the scientific community toward best practices in the responsible use of animals in biomedical research while fostering transparent regulatory interactions.

Shaping the Future of Preclinical Research

The initiation of the NHPig project marks a significant milestone for biomedical research in the EU. Lars Siim Madsen elaborates: "NHPig is a game-changer for preclinical research. By deepening our understanding of mini- and micropig models, we are creating new opportunities for safer and more ethical drug development. This project will provide researchers and regulators with the knowledge needed to make informed decisions that benefit both science and animal welfare."

By expanding understanding of mini- and micropig models, NHPig has set out to find a reliable and translationally relevant alternative to NHPs in non-clinical safety testing. With continued collaboration and innovation, the project is set to leave a lasting impact on biomedical research, helping to shape future regulatory frameworks and improve drug development processes.

Can Göttingen Minipigs Claim a Spot in CNS Drug Research & Development?

By Kristine Langthaler[1], Christopher R. Jones[2], and Christoffer Bundgaard[1]

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This study evaluates the Göttingen minipig as a pharmacokinetic (PK) model for central nervous system (CNS) Research & Development (R&D). It aimed to address the limited knowledge on brain penetration and prediction of systemic clearance, based on in vitro to in vivo extrapolation (IVIVE) techniques, in minipig. Presented are in vivo data on brain drug penetration and PK. Despite a potential higher P-glycoprotein expression in the porcine blood-brain barrier (BBB), in vivo minipig drug brain penetration was comparable to other non-rodent species and human, supporting its suitability for brain drug research. The minipig also appeared to be a suitable PK model and is considered a useful addition to the species used to assess performance of human clearance prediction by IVIVE. This work was performed as part of the PhD project by Kristine Langthaler in a joint collaboration between School of Pharmaceutical Sciences at University of Copenhagen and H. Lundbeck A/S.

1. Introduction

In the field of pharmaceutical CNS R&D, animal models play a crucial role in predicting drug safety and PK behavior of drugs in the human body. One such species gaining popularity is the Göttingen minipig due to its size, ease of handling, anatomical and physiological resemblances to human etc. (Achour et al. 2011; Swindle et al. 2012).

CNS disorders are a global burden, and their increasing prevalence over the last few decades has not been accompanied by a similar increase in new medicines. In most cases, to effectively treat a CNS disease it is essential the drug penetrates into the brain. To predict this with confidence necessitates preclinical testing in appropriate animal models. Knowledge of brain penetration of drugs in minipigs is limited compared to other species such as rats (Friden et al. 2009; Summerfield. et al. 2016). Before reaching the BBB a drug has to be absorbed into the systemic circulation. For oral drugs it is therefore important to minimize clearance (CL) in order to maximize circulating concentration available to penetrate the BBB; given CL influences a drugs systemic half-life as well as bioavailability (fraction of dose reaching the systemic circulation). Animal PK models that can support accurate prediction of human systemic PK, particularly clearance (CL), are therefore very important. Prediction of CL from in vitro metabolic stability data is a well-established approach for prediction of human CL (Obach 1999; Sohlenius-Sternbeck et al. 2010; Lignet et al. 2016) and animal PK models are commonly used to verify performance of the IVIVE technique applied to the human data (Obach 1999; Ito and Houston 2004; Jones et al. 2022). Hence, to establish Göttingen minipigs as a non-rodent PK species for CNS research, it requires the animal to be characterized, using reference compounds that have been tested in other species, in terms of its systemic disposition, brain penetration as well as various absorption-metabolism-distribution-excretion (ADME) parameters (Figure 1).

A concern regarding utility of minipig as a model of brain penetration for translation to humans is the relatively high expression levels of key efflux transporters, such as P-glycoprotein, in the porcine BBB. This transporter expels harmful substances from the brain but can also restrict entry of therapeutic drugs intended to treat brain diseases. Notably, P-glycoprotein is expressed at 4-times higher levels in pigs than in humans (Figure 2) (Zhang et al., 2017), raising concerns of potentially lower brain penetration in the Göttingen minipig (calculated as the unbound brain concentration to unbound plasma concentration ratio, Kp,uu,brain). This could impact its utility in CNS R&D as a non-rodent toxicology/safety pharmacology species or for translation of human brain PK (extent of brain penetration). Therefore, our study aimed to address this knowledge gap and evaluate the suitability of the Göttingen minipig for brain drug research and as an additional PK species for evaluating performance of IVIVE techniques supporting prospective human CL predictions in drug research.

Notice

This article is extracts from two peer-reviewed publications. The full papers are available as open access publications:

Assessing extent of brain penetration in vivo (Kp,uu,brain) in Göttingen minipig using a diverse set of reference drugs
European Journal of Pharmaceutical Sciences
(2023 Nov 1:190:106554)
DOI: 10.1016/j.ejps.2023.106554

Characterization of intravenous pharmacokinetics in Göttingen minipig and clearance prediction using established in vitro to in vivo extrapolation methodologies
Xenobiotica
(2022 Jun;52(6):591-607)
DOI: 10.1080/00498254.2022.2115425

2. Materials and methods

A total of 20 compounds with diverse physiochemical properties and rat Kp,uu,brain values were selected. Of these, 17 were used in minipig brain penetration studies and 18 in protein binding studies. Compounds were combined (up to 4 compounds per cassette) then administered at low doses, to increase throughput and reduce animal usage.

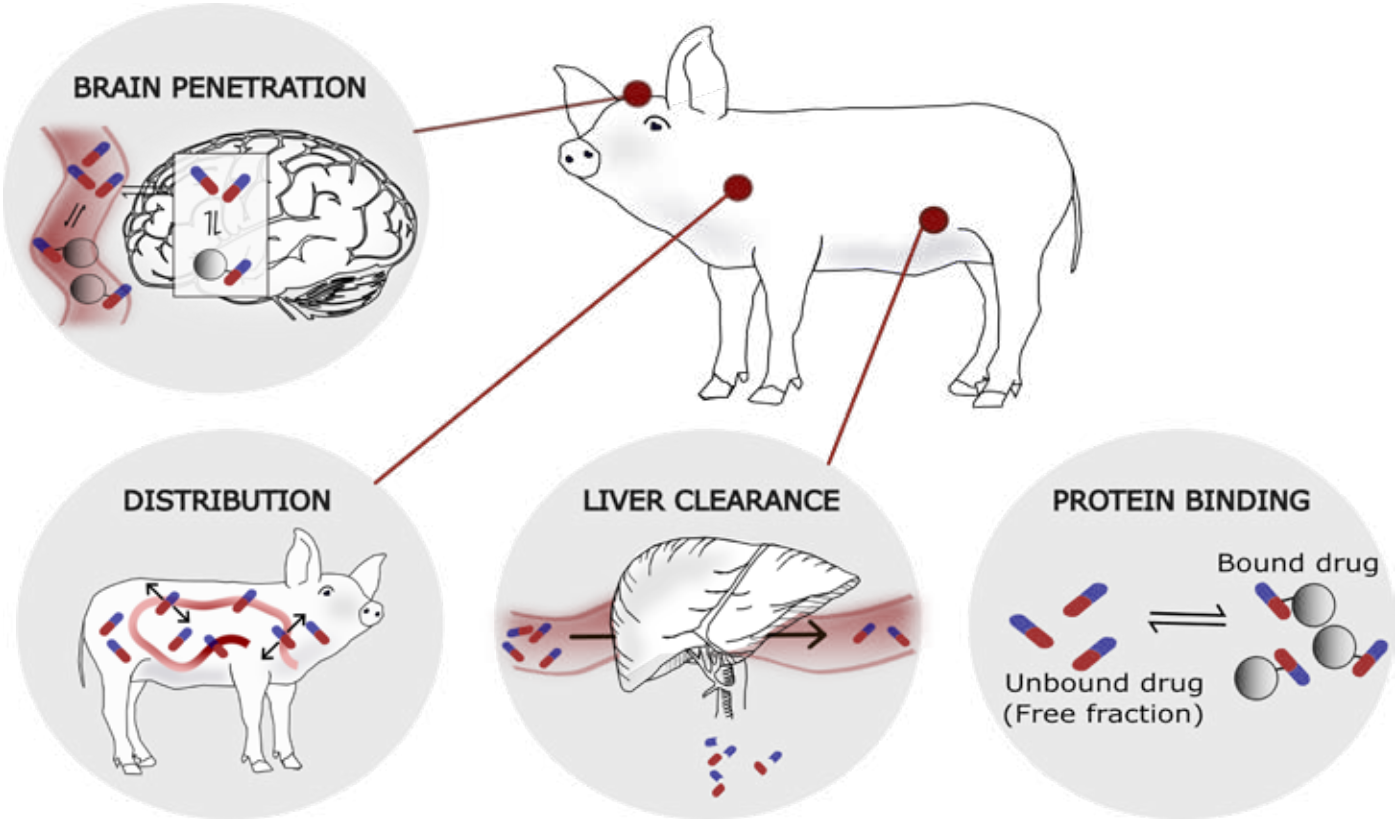

Figure 1
Key areas investigated. Brain penetration, protein binding, and crucial PK parameters (volume of distribution (Vss) and liver clearance (CL)).

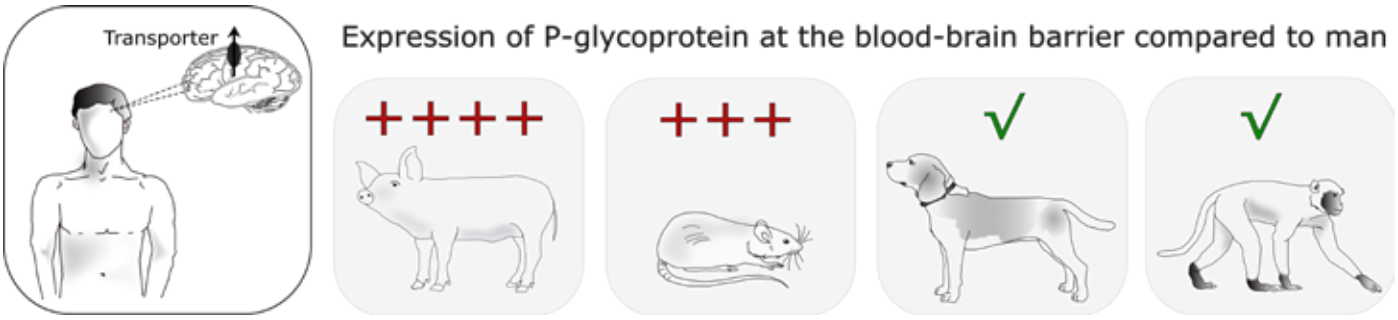

Figure 2
Fold difference in P-glycoprotein expression at the blood-brain barrier relative to human. Dog and non-human primate (NHP) have similar levels, while rats and pigs (excluding Göttingen minipigs) show 3- and 4-fold higher expression, respectively.

Binding to human, rat and Göttingen minipig whole plasma and rat or minipig brain homogenate was quantitatively assessed by equilibrium dialysis. The free fractions in these matrix were used to correct the total plasma and brain compound concentrations to unbound concentrations for calculation of the in vivo unbound brain to plasma drug partition coefficient (Kp,uu,brain). This coefficient is recognized as the key parameter for evaluating the extent of brain penetration (Loryan et al. 2022) and is calculated according to equation 1.

Compounds with Kp,uu,brain values < 0.3 are considered to have restricted brain penetration, whilst those approximating a value of 1 are considered to have unrestricted brain penetration.

PK parameters were calculated using non-compartmental analysis of plasma concentration-time profiles using Phoenix WinNonlin software. The plasma elimination half-life, volume of distribution (Vss), and total clearance were determined, using equations 2-4.

$$K_{p,uu,brain} = \frac{Total\ concentration\ in\ brain \cdot free\ fraction\ in\ brain\ homogenate}{Total\ concentration\ in\ plasma \cdot free\ fraction\ in\ plasma} \quad (1)$$

$$Half\ life = \frac{0.693 \cdot V_{ss}}{Total\ clearance} \quad (2)$$

$$V_{SS} = MRT \cdot Total\ clearance = \frac{Dose \cdot AUMC_{0-\infty}}{[AUC_{0-\infty}]^2} \quad (3)$$

$$Total\ clearance = \frac{Dose}{AUC_{0-\infty}} \quad (4)$$

V_{ss} is the volume of distribution, MRT is the mean residence time; $AUC_{0-\infty}$ is the area under the curve to infinity; $AUMC_{0-\infty}$ is the area under the first moment curve to infinity.

3. Results

Binding of compounds to Göttingen minipig plasma proteins and brain homogenate.

To evaluate brain penetration, firstly the compound free fraction in whole plasma and brain homogenate was determined by equilibrium dialysis. A 300-fold range in binding affinity to brain homogenate and a 125-fold range in binding affinity to plasma proteins was observed for the reference set. Comparison of rat and Göttingen minipig brain tissue homogenate, as well as human and Göttingen minipig plasma, revealed a strong level of agreement across the reference set (with majority of individual compound data points falling close to the line of unity, as depicted in Figure 3).

Compound brain penetration using Göttingen minipig.

Kp,uu,brain was calculated from the unbound fractions and the in vivo brain to plasma ratios and provided an overview of the distribution into the brain for the reference set. Values ranged from 0.02 for cimetidine to 2.40 for fluoxetine (Figure 4A), demonstrating a high dynamic range covering restricted, partially restricted and unrestricted brain penetration as well

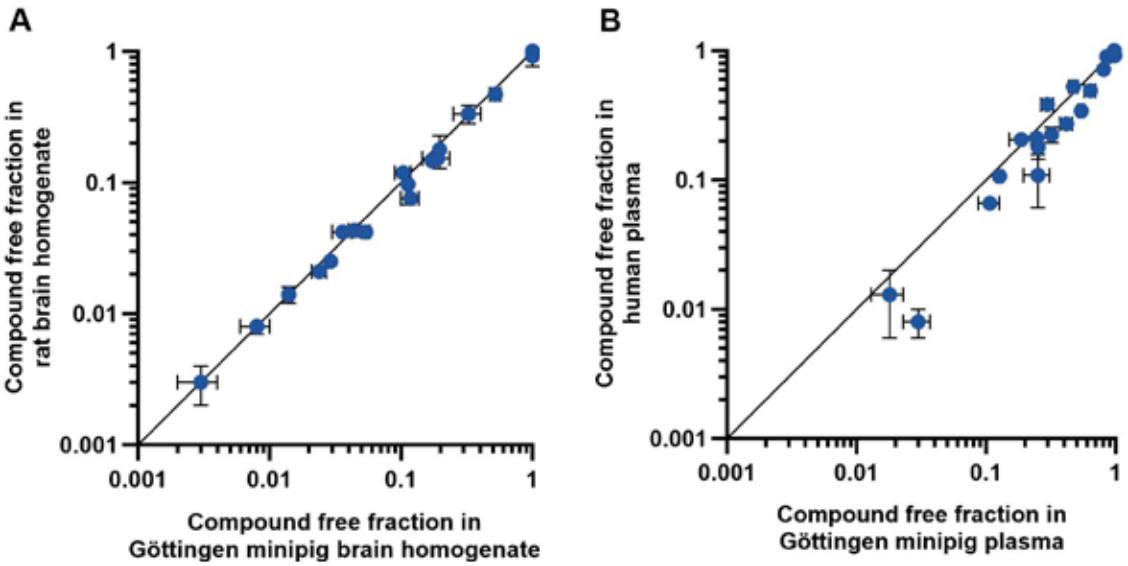

Figure 3A-B
Comparison of compound free fractions in Göttingen minipig brain homogenate and plasma versus rat brain homogenate and human plasma. The solid line indicates unity. Data are mean ± standard deviation (n=18, measured in triplicate, on a single occasion).

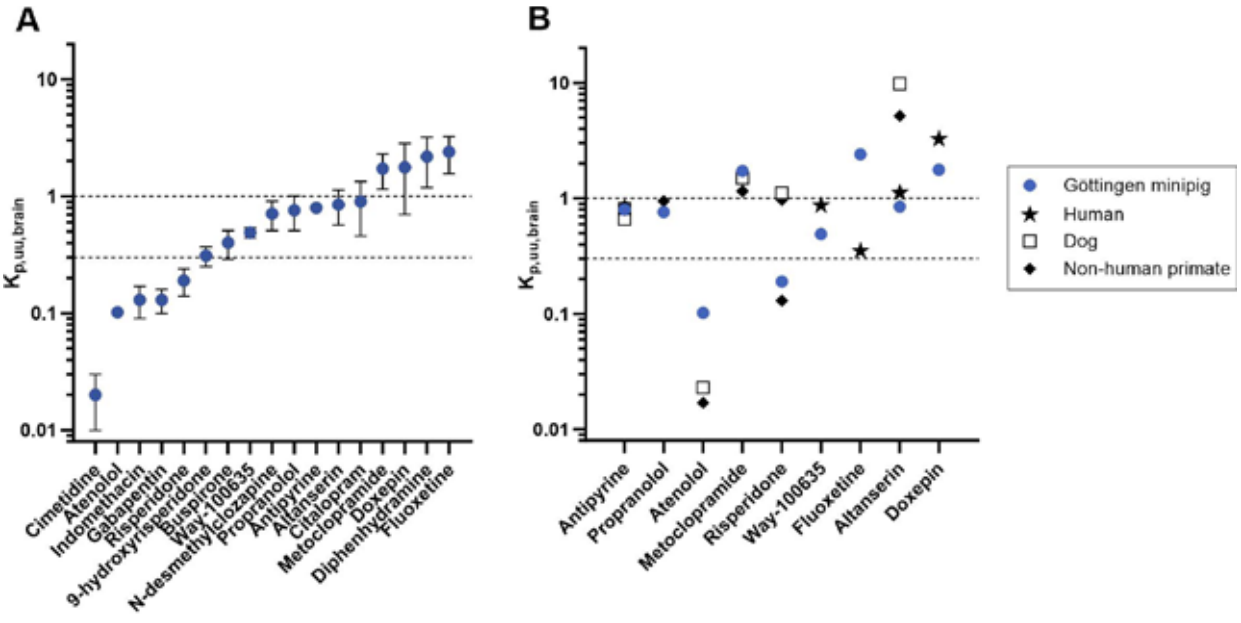

Figure 4
A) Kp,uu,brain for 17 compounds in Göttingen minipigs. B) Comparison of Kp,uu,brain across species for 9 reference compounds: Göttingen minipig (blue circles), human (black stars), non-human primate (black diamonds), and dog (open squares). Data are mean ± standard deviation. For more details, refer to Langthaler et al. 2023.

as possible active uptake in minipigs (e.g. Kp,uu,brain >>1). A subset of 9 reference compounds were compared with published data for other non-rodent species often used in R&D – namely dog and non-human primate (NHP). Acknowledging that human brain exposure data is sparce, nonetheless a comparison was possible for 4 of the reference compounds (Figure 4B).

Systemic PK in Göttingen minipig – determination of volume of distribution and liver clearance for the reference set.

Plasma PK parameters were calculated for the reference set of compounds (n=20) from mean IV plasma concentration-time profiles. The CL and Vss values have been summarized in Table 1 (additional PK-parameters have been included in Langthaler et al. 2022).

The total CL and Vss data found for antipyrine, atenolol, cimetidine, diazepam, and verapamil in female Göttingen minipigs (this study) were in good agreement with previously published female Göttingen minipig data; falling within 2-fold for total CL and 2.5-fold for Vss (Figure 5A-B) (Lignet et al. 2016; Patel et al. 2016; Ding et al. 2021). Indicating that robust PK data can be generated in this species. In previously published studies, reference compounds were dosed discretely (one compound per animal). The good agreement shown between cassette and discretely dosed studies was very encouraging and provided greater confidence that a cassette dosing paradigm can be used to characterize PK; which is particularly attractive from a cost and 3R's perspective in non-rodent species.

Ion Class	Compound	Dose	Plasma Half-life	Plasma, Volume of distribution (V_{ss})	Plasma, total clearance (CL)
		(mg/kg)	(h) Mean ± SD	(L/kg) Mean ± SD	(mL/min/kg) Mean ± SD
Neutral	Antipyrine	1.0	1.6 ± 0.7	0.84 ± 0.12	10 ± 0.3
	Buspirone	0.5	3.0 ± 0.1	0.99 ± 0.17	17 ± 2
	Carbamazepine	0.5	2.1 ± 0.3	1.11 ± 0.14	7.4 ± 1.1
	Diazepam	0.5	5.1 ± 0.4	1.96 ± 0.07	8.8 ± 0.7
	Way-100635	0.1	3.5 ± 0.7	2.44 ± 0.69	29 ± 3
Basic	Altanserin	0.1	4.7 ± 0.3	1.99 ± 0.06	17 ± 0.6
	Atenolol	0.3	4.4 ± 1.6	2.09 ± 0.68	11 ± 1
	Bupropion	0.1	3.6 ± 0.2	4.12 ± 0.29	28 ± 2
	Cimetidine	0.5	1.4 ± 0.1	0.92 ± 0.15	19 ± 3
	Citalopram	0.5	2.2 ± 0.02	4.36 ± 1.00	39 ± 8
	Diphenhydramine	0.2	1.9 ± 0.1	3.34 ± 0.83	42 ± 9
	Doxepin	0.1	2.4 ± 0.2	4.51 ± 1.38	40 ± 10
	Fluoxetine	0.3	3.3 ± 1.6	13.40 ± 1.99	47 ± 10
	Metoclopramide	0.3	8.5 ± 12.1	8.14 ± 7.05	36 ± 6
	N-desmethylclozapine	0.5	4.6 ± 0.1	8.91 ± 3.37	25 ± 9
	Propranolol	0.1	1.7 ± 0.3	1.45 ± 0.65	14 ± 4
	Risperidone	0.1	0.9 ± 0.03	1.02 ± 0.18	19 ± 1
	Verapamil	0.2	2.8 ± 0.2	2.08 ± 0.35	18 ± 2
Acidic	Indomethacin	0.375	5.5 ± 0.6	3.18 ± 0.31	19 ± 4
Zwitterionic	Gabapentin	0.5	4.0 ± 0.1	0.58 ± 0.04	2.0 ± 0.2

Table 1
In vivo PK parameters determined in female Göttingen minipigs for reference compounds (n=20).

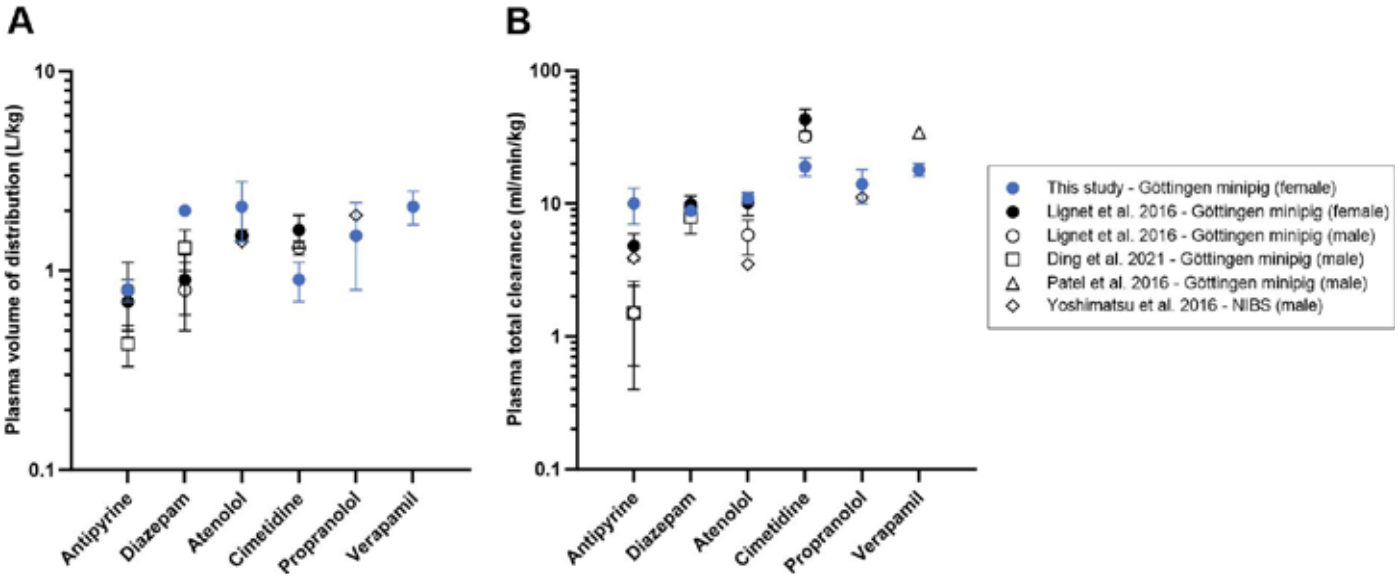

Figure 5
Comparison of data with published literature. A) Plasma volume of distribution (Vss). B) Plasma total clearance (CL).

Prediction of systemic CL based on IVIVE.
The IVIVE model was established using a more diverse reference set comprising of acids, zwitterions, and a broader coverage of human drug-metabolizing enzymes. As such, the reference set was supplemented with an additional 13 compounds (n=33) where published CL values were available for each.

IVIVE of CL using the well-stirred liver model was established. The predicted CL scaled from in vitro metabolic stability data (CLint) in hepatocyte was compared to the in vivo CL derived from the PK experiments. The IVIVE had a low prediction bias (AFE = 0.9) and high precision (AAFE = 1.4 and RMSE = 1.5). The strength of relationship was good (R2 = 0.70) with a high percentage of predicted CL values falling within 2-fold of the observed (96%).

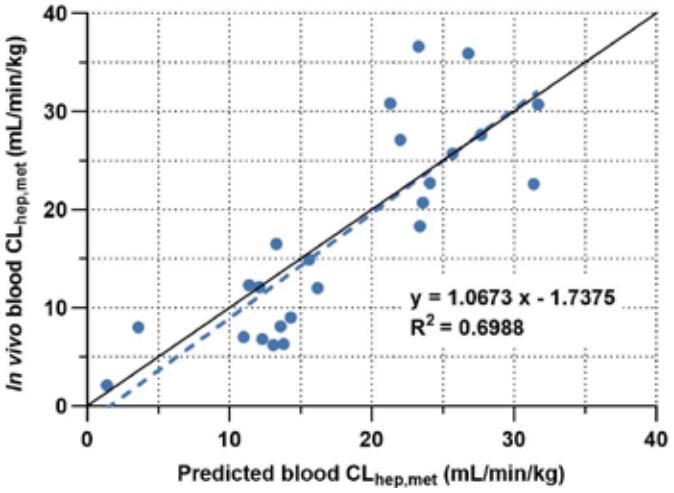

Figure 6
Correlation between log-transformed predicted in vivo blood clearance scaled from in vitro data and derived in vivo clearance for 24 reference compounds. From 33 compounds, 24 were selected for a regression corrected IVIVE, excluding those with significant extra-hepatic clearance (results demonstrated are from IVIVE method no. 4, the best-performing method (Langthaler et al., 2022)).

4. Discussion

The utilization of Göttingen minipigs in non-rodent PK studies and drug safety evaluations has grown in momentum in recent years (Suenderhauf and Parrott 2013; Suenderhauf et al. 2014; Lignet et al. 2016; Poulin et al. 2019; Ding et al. 2021). In light of potential interspecies variations in drug transporter expression at the BBB, there is an urgent need to verify the extent of brain penetration in minipigs to qualify the Göttingen minipig as an alternative species in brain drug research. To our knowledge this is the first work to be published determining the brain penetration in this species for a diverse set of reference compounds. Included were CNS and non-CNS acting drugs as well as known human P-glycoprotein substrates. As demonstrated in Figure 4A the reference set also spanned a relevant dynamic range (restricted to unrestricted and active brain uptake) providing a solid basis on which to assess brain penetration in Göttingen minipig.

Drugs identified as potential human P-glycoprotein substrates, such as cimetidine, risperidone, and its 9-hydroxyrisperidone metabolite, were shown to have restricted brain penetration (Kp,uu,brain values <0.3). A restricted Kp,uu,brain was also observed for drugs such as atenolol and gabapentin, which are often considered in literature as having low passive membrane permeability (measured in vitro in MDCKII-cells; Langthaler et al. 2024).

Species differences in brain penetration in vivo have been reported by several laboratories (Doran et al. 2012; Sato et al. 2021; Kido et al. 2022). The current study provides an expansive new dataset in Göttingen minipig to facilitate a comparison with other species (Figure 4A-B). A drug's brain penetration can be influenced by active processes such as uptake or efflux (controlled by transporters pumping drug in or out of the brain, respectively). These processes can differ across species due to transporter substrate affinity as well as transporter expression levels at the BBB. Therefore, brain penetration data was compiled from the literature for a subset of reference compounds allowing a broader comparison across species. Given published porcine P-glycoprotein expression levels were relatively high compared to other species we had initially anticipated that brain penetration could be much lower in minipig. However, there appeared no overall significant difference in the minipig 'brain penetration signature' (e.g. trend in Kp,uu,brain values across reference compounds) compared to NHP, dog and human.

The brain penetration for human P-glycoprotein non-substrates, such as antipyrine and propranolol, was consistent across NHP and dog as depicted in Figure 4B (also for rat, but data not shown here, see Langthaler et al. 2023). For known human P-glycoprotein substrates the brain penetration across species appeared more variable and compound dependent – for metoclopramide a relatively high Kp,uu,brain was observed in minipig versus other species whereas for risperidone a relatively low value was observed in minipig.

Based on these data the minipig does not present itself as an outlier species for assessment of brain penetration. As such, it could be considered an alternative to dog or NHP in safety studies for development of CNS, as well as non-CNS, acting drugs. In-line with previous findings (Di et al. 2011) brain homogenate binding in the current study (Figure 3B) clearly showed that non-specific binding to brain components was consistent across species (Langthaler et al. 2023). This indicated that rat and minipig binding can be used inter-changeably, eliminating the need for testing in multiple species. Drug binding to in plasma can often show species differences (Colclough et al. 2014), influenced by different binding affinities for different plasma proteins. For the current reference set, drug binding in minipig and human plasma was closely aligned.

In pharmaceutical R&D IVIVE of CL across several species is often undertaken to build confidence that CL is predictable from scaled in vitro metabolic stability data and that is appropriate to apply the same scaling approach to make prospective prediction of human CL (Obach 1999; Ito and Houston 2004; Riley et al. 2005; Sohlenius-Sternbeck et al. 2010; Poulin 2013; Francis et al. 2021; Jones et al. 2022). Combining the CL data from this project with existing

literature data, we have successfully established an in vitro scaling method to predict in vivo CL in Gottingen minipig for a diverse set of reference compounds. As such minipig can be considered a useful addition to the PK species typically used to assess IVIVE performance underpinning robust prediction of human PK (Langthaler et al. 2022).

5. Conclusions

This PhD research project achieved several important milestones. For the first time, brain penetration has been characterized in minipig for a broad set of reference compounds. We have published an extensive new PK dataset for female Göttingen minipigs and demonstrated the beneficial impact of a cassette dosing paradigm (administering multiple compounds simultaneously at low doses to maximize the reference set tested whilst minimizing the number of animals used). Furthermore, we demonstrated that in vitro free fraction in rat brain homogenate or minipig could be used with free fraction in Göttingen minipig plasma and in vivo total brain to plasma ratios to estimate the Kp,uu,brain (extent of brain penetration).

Despite noticeable differences in transporter expression levels across species (Figure 2), these variations did not translate into notable species differences in brain penetration in vivo (Figure 4B). In addition to the successful approach for IVIVE of CL (Figure 6) it highlights the potential of the Göttingen minipig as a valuable non-rodent species for evaluating extent of brain penetration and performance of CL scaling methodologies; two important parameters underpinning human PK predictions for CNS drug candidates.

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Refined Post-Anesthetic Recovery Protocols for Göttingen Minipigs: Enhancing Animal Welfare and Procedural Outcomes

By Carina Anker[1] and Maja Ramløse[1]

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Anesthesia is frequently required in Göttingen Minipigs for surgical or other non-invasive interventions. The physiological and hemodynamic parameters of minipigs are impacted to varying degrees by the anesthetic protocols employed. Effective post-anesthetic care is essential for ensuring a smooth recovery and preventing post-procedural complications. This article discusses four feasible, non-pharmacological methods of post-anesthetic support: providing a calm and safe housing environment, monitoring vital parameters, ensuring supplemental heat, and using soft padding for physical protection. These measures contribute to a refined recovery process, continuing until the animal is alert, ambulatory, with restored swallowing reflexes, and stable cardiovascular and pulmonary function.

Introduction

The administration of anesthesia in Göttingen Minipigs is a common practice in biomedical research and veterinary procedures, aiming to minimize pain and distress during interventions. However, the recovery phase post-anesthesia is equally critical and requires careful management to avoid complications. The physiological and hemodynamic effects of anesthesia necessitate a structured post-anesthetic recovery plan to ensure optimal welfare and recovery outcomes for the animals.

This article outlines practical, non-pharmacological methods for enhancing the recovery process, emphasizing the importance of environmental modifications, vigilant monitoring, thermal support, and physical protection. These methods are intended to complement pharmacological strategies, such as preemptive analgesia, to achieve a comprehensive recovery approach.

Non-Pharmacological Post-Anesthetic Support Methods

Calm and Safe Housing

Immediately following an anesthetic procedure, the animal should be relocated to a dedicated recovery room. This room should be isolated from other animal housing areas, with only other recovering animals present to minimize disturbances. Key features of an ideal recovery room include cleanliness, dryness, and a quiet environment with dimmed lighting to reduce visual stimuli.

During recovery, minipigs should be housed singly to prevent injury from interactions with other animals. The transition back to their home pen should only occur once the animal has regained full alertness, including righting and swallowing reflexes, and when vital parameters are within normal ranges, ensuring patent airways and overall stability.

Image 1
Empty recovery pens located separately from other habitated pens.

Image 2
Göttingen Minipigs recovering from anesthesia are single housed and vital parameters monitored.

Image 3
Recovery pen fitted with plastic covered foam cushions, heated flooring with insulative blankets, heat lamp, extra oxygen supply- and monitoring equipment.

Monitoring of Vital Parameters

Continuous and frequent monitoring of vital signs is essential in the recovery process to allow for timely intervention should complications arise. Key parameters to monitor include end-tidal CO2 (EtCO2), oxygen saturation, heart rate, and body temperature. Close observation ensures that any deviations from the expected recovery trajectory are promptly addressed, increasing the likelihood of a successful outcome.

Additionally, delaying extubation until the swallowing reflex returns can help maintain patent airways, reducing the risk of respiratory complications. Following extubation, low-flow nasal cannula oxygen may be provided to enhance oxygenation, while intravenous catheters should be maintained for quick access in case of emergency interventions.

Supplemental Heat

Anesthesia commonly disrupts the thermoregulatory processes in minipigs, leading to a risk of hypothermia, which can prolong recovery and increase the likelihood of post-anesthetic complications such as wound infections. Göttingen Minipigs are particularly vulnerable due to their lack of an insulating fur coat, which exacerbates heat loss during and after anesthesia.

To mitigate these risks, supplemental heat should be provided through heated flooring and heat lamps. The height of the lamps should be adjusted to maximize heat distribution across the animal's body while avoiding thermal burns. Maintaining a slightly elevated room temperature and avoiding drafts are also crucial in preventing hypothermia.

Image 4
Keep the minipig warm by covering it with blankets or duvets.

Physical Protection

The anesthetic agents used during procedures impair the judgment, locomotion control, and reflexes of recovering minipigs, increasing their risk of self-inflicted injuries. Providing a soft, insulated surface such as a thick blanket or mattress in the recovery pen helps reduce discomfort from localized tissue pressure and minimizes heat loss. Padding the walls of the pen with materials such as customized foam cushions can further protect the animal, allowing it to stabilize its movements using its snout without injury.

Conclusion

A refined post-anesthetic recovery process is achievable through the implementation of non-pharmacological support methods. The four methods discussed—safe housing, vigilant monitoring, supplemental heat, and physical protection—are practical and feasible in various research and veterinary settings. While these methods are not exhaustive, they represent a significant step towards optimizing the welfare and recovery outcomes of Göttingen Minipigs. Additional measures, including preemptive analgesia and comprehensive welfare assessments, are critical components of a holistic recovery strategy.

By prioritizing these elements, animal caretakers, technicians, and scientists can contribute to improved post-anesthetic recovery, thereby enhancing both animal welfare and the reliability of experimental outcomes.

Image 5
Göttingen Minipig in post-anesthetic recovery, using a cushion hole for the snout to stabilize.

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Download poster

The Contents of this article has been presented as a poster during the yearly meeting of the Danish National Committee for the Protection of Animals used for Scientific Purposes, and came in on a shared first place.

Download the poster here:

Integrated Approaches in Pediatric Pharmacotherapy: Applications of the Neonatal Asphyxia Göttingen Minipig Model

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In the context of supporting drug development and pharmacotherapy for human neonates, this study was undertaken to enhance our understanding of the effects of perinatal asphyxia (PA) and therapeutic hypothermia (TH). The overarching goal of this research, using the neonatal Göttingen Minipig model, was to disentangle the effects of systemic hypoxia and TH on the pharmacokinetics (PK) of four drugs used in the neonatal intensive care unit (NICU), and on drug enzyme functionality. Such insights can subsequently inform the development of a physiologically-based pharmacokinetic (PBPK) model, which is instrumental for dose precision in human neonates. The data below are a summary of the PhD research of Marina Stroe [1].

Introduction

The neonatal population presents challenging dose predictions due to the pronounced growth and development during early life stages, affecting drug exposure. These complexities are further amplified in neonates affected by perinatal asphyxia (PA), a condition that impacts multiple organ systems and often necessitates intensive care interventions such as therapeutic hypothermia (TH). TH, the standard treatment for hypoxic-ischemic encephalopathy, improves survival and neurodevelopmental outcomes by modulating metabolic processes and reducing oxygen consumption. However, TH also alters drug disposition through its effects on cardiac output, organ blood flow, and enzymatic activity, necessitating tailored pharmacotherapy to optimize safety and efficacy.

Pediatric drug therapy faces significant challenges due to limited clinical data and ethical barriers to conducting trials in neonates, often resulting in the off-label use of medications

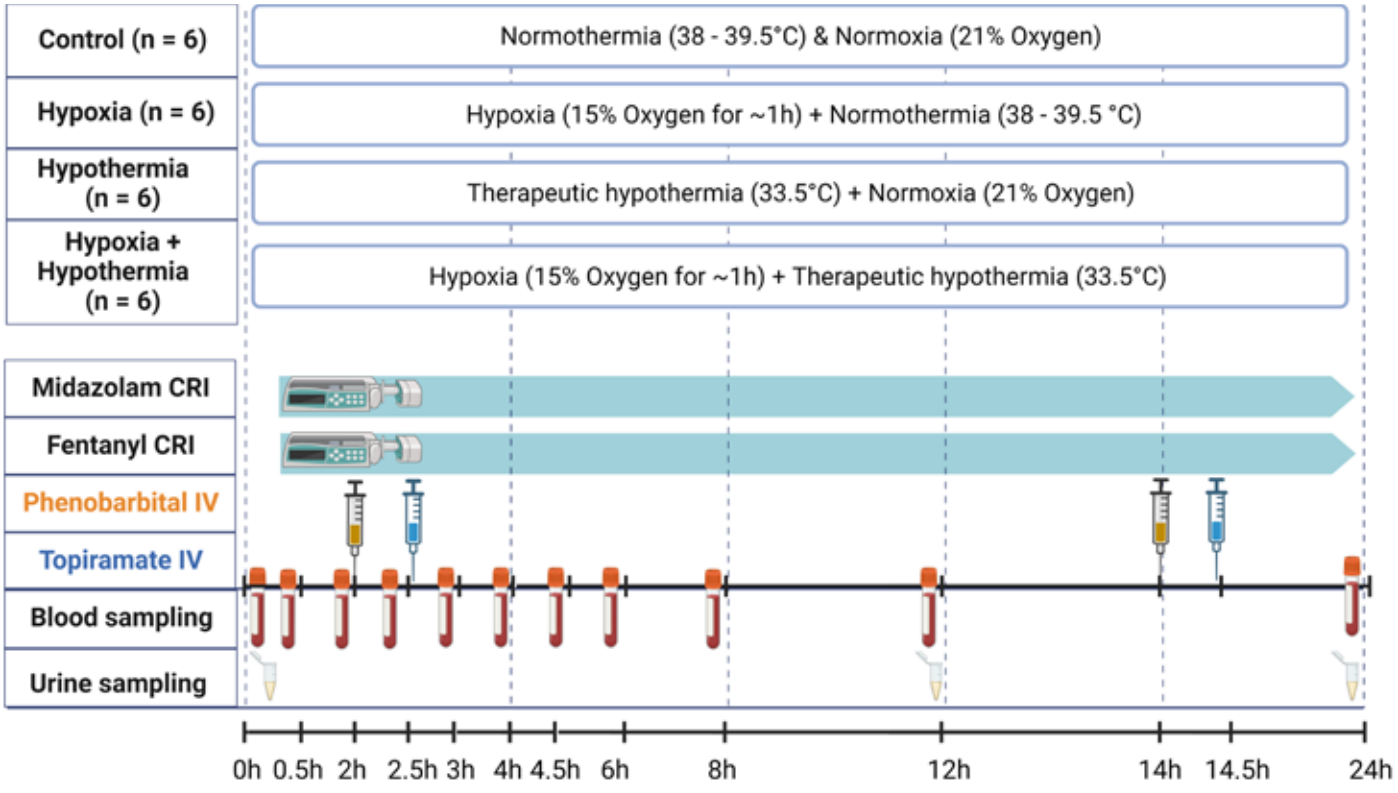

Figure 1
Neonatal Göttingen Minipig groups with their therapeutic interventions and sampling schedule. Four conditions were investigated, i.e., control (C), therapeutic hypothermia (TH), hypoxia (H), hypoxia and TH (H+TH), with six piglets per condition (n=6). A blood sample was withdrawn pre-drug administration and subsequently, 10 times after the drug administration, resulting in a total of 11 samples over the study period for each Göttingen Minipig. Urine samples were collected at 12 hours and 24 hours via cystocentesis. Figure created with BioRender.com. * Degree Celsius (°C); hour (h); constant rate infusion (CRI); intravenous (IV).

without robust evidence. In this context, animal models, such as the Göttingen Minipig, could provide a critical solution for understanding the pharmacokinetics (PK) in this population. Göttingen Minipigs exhibit physiological and anatomical similarities to humans, including comparable gastrointestinal, hepatic, and cardiovascular development, and their larger size facilitates sampling without significantly impacting physiology [2]. Additionally, their genetic consistency and high homology to human drug-metabolizing enzymes, such as Cytochrome P450 (CYP), make them an ideal model for neonatal studies [3,4].

The I-PREDICT project: Innovative physiology-based pharmacokinetic (PBPK) model to predict drug exposure in neonates undergoing cooling therapy (Senior research grant from the Research Scientific Foundation-Flanders (FWO), Belgium - G0D0520N) aimed to refine dosing strategies for neonates with PA undergoing TH by developing a PBPK model using the Göttingen Minipig model [5]. The first hypothesis stated in this project was that systemic hypoxia and TH reduce metabolic drug clearance, necessitating dosing adjustments. To test this, an experimental study was conducted at the research facility of Ellegaard Göttingen Minipigs A/S, Dalmose, Denmark [6], examining the PK of midazolam, fentanyl, phenobarbital, and topiramate under four conditions: control, hypoxia (H), TH, and combined hypoxia and TH (H+TH). Neonatal Göttingen Minipigs, within 24 hours of birth, were subjected to these conditions, with drug administration and blood sampling performed under anesthesia (Figure 1). The gene and protein expression, as well as the activity of specific CYP enzymes were evaluated using in vitro methods. Hepatic CYP expression levels and activity were assessed in both adult and neonatal Göttingen Minipigs, in addition to the 24 experimental Göttingen Minipigs.

In Vivo: Drug Disposition in Neonatal Göttingen Minipigs with Asphyxia and Hypothermia

Midazolam, fentanyl, phenobarbital and topiramate were selected as model drugs based on their different physicochemical and/or PK characteristics and clinical relevance: midazolam (CYP3A4; intermediate extraction ratio (ER)), fentanyl (CYP3A4; high ER); phenobarbital (CYP2C19; low ER), and topiramate (largely renally excreted unchanged). The PK was investigated under four conditions i.e., hypoxia (group H), therapeutic hypothermia (group TH), hypoxia and therapeutic hypothermia (group H+TH) and controls (group C) (Figure 2).

A. Impact of therapeutic hypothermia on drug disposition

In this study [7], a statistically significant decrease in fentanyl clearance by 66% was observed, leading to an approximately 3-fold longer half-life in the TH group compared to the C group. Additionally, the 24-hour plasma concentrations were statistically significantly higher in the TH group compared to the three other groups investigated. According to thermopharmacological principles, CYP activity decreases at lower body temperatures [8]. As summarized by Tortorici et al (using non-clinical data), TH can decrease the systemic clearance of drugs metabolized by CYPs, by 7-22% per degree Celsius below 37 °C [9]. Therefore, the TH impact on fentanyl metabolism could be attributed to the reduced CYP3A activity. However, it could also be associated with the diminished liver blood flow [10,11]. Trends towards lower clearance and longer half-life of midazolam and phenobarbital due to TH, and the inability to reach a steady state, provide evidence of the limited drug removal capacity in neonatal Göttingen Minipigs. Regarding phenobarbital, only the H+TH group demonstrated a potential trend, with a 30% decrease in clearance with a 35% longer half-life compared to the C group. A possible reason for the discrepancy between the TH and H+TH groups may lie in the small sample size, which limits the ability to draw robust conclusions. Future studies should include a larger sample size and a longer duration of the experiment to better assess these trends and clarify the effects of TH on drug disposition.

Concerning drugs undergoing little or no hepatic metabolism (i.e., topiramate), existing evidence suggests that TH following hypoxic-ischemic injury does not influence the PK [12,13]. This hypothesis was confirmed in our study since we could not detect a significant effect of TH on topiramate PK in neonatal Göttingen Minipigs. In our study a decrease in topiramate clearance of 28% for the H group was detected compared to the C group. These PK changes induced by hypoxia are expected since the hypoxic-ischemic injury itself can induce renal impairment (e.g., gentamicin in neonatal pigs [13] and human neonates [12]).

B. Impact of therapeutic hypothermia on cardiovascular processes

In addition to the potential direct impact on drug disposition, TH also has an impact on cardiovascular parameters [14,15]. Firstly, high ER drugs, such as fentanyl, are flow limited drugs meaning that upon IV administration, the systemic clearance is highly dependent on the liver blood flow. Data on organ blood flow, cardiac index, or cardiac output are typically needed to draw such conclusions. Although our study did not directly measure these parameters, we recorded the heart rate (HR) and detected statistically significant lower values in the hypothermic groups: statistically significant lower HR ($P<0.0001$) and body temperature ($P=0.0051$) were detected for both TH and H+TH groups compared to C and H groups. As HR impacts cardiac output, the observed lower HR in our study could be linked to the lower capacity of drug removal from the body, as a possible mechanistic hypothesis. This trend was observed for fentanyl (high ER), while drugs with intermediate (midazolam) and low (phenobarbital) ER were less impacted. Therefore, this might explain the more pronounced and statistically significant TH impact on fentanyl PK compared to midazolam, even though both drugs share the same metabolic pathway (i.e., hepatic CYP3A22, CYP3A29, CYP3A46 Göttingen Minipig metabolism [16]). Future experiments measuring organ blood flow, cardiac index, or cardiac output would provide more comprehensive insights, especially in the context of PBPK modelling.

In Vitro: Cytochrome P450 Drug Metabolism in Göttingen Minipigs

It is crucial to acknowledge that CYPs can be evaluated at various levels, including gene expression, protein abundance, and enzyme activity. All these levels are important particularly in distinguishing between acute and prolonged temperature-induced modifications: while alterations in expression levels require a period for manifestation, changes in enzymatic activity can manifest promptly. Therefore, this study [17] aimed to investigate the impact of age, hypothermia, and hypoxia on hepatic CYP expression, abundance, and activity in Göttingen Minipigs.

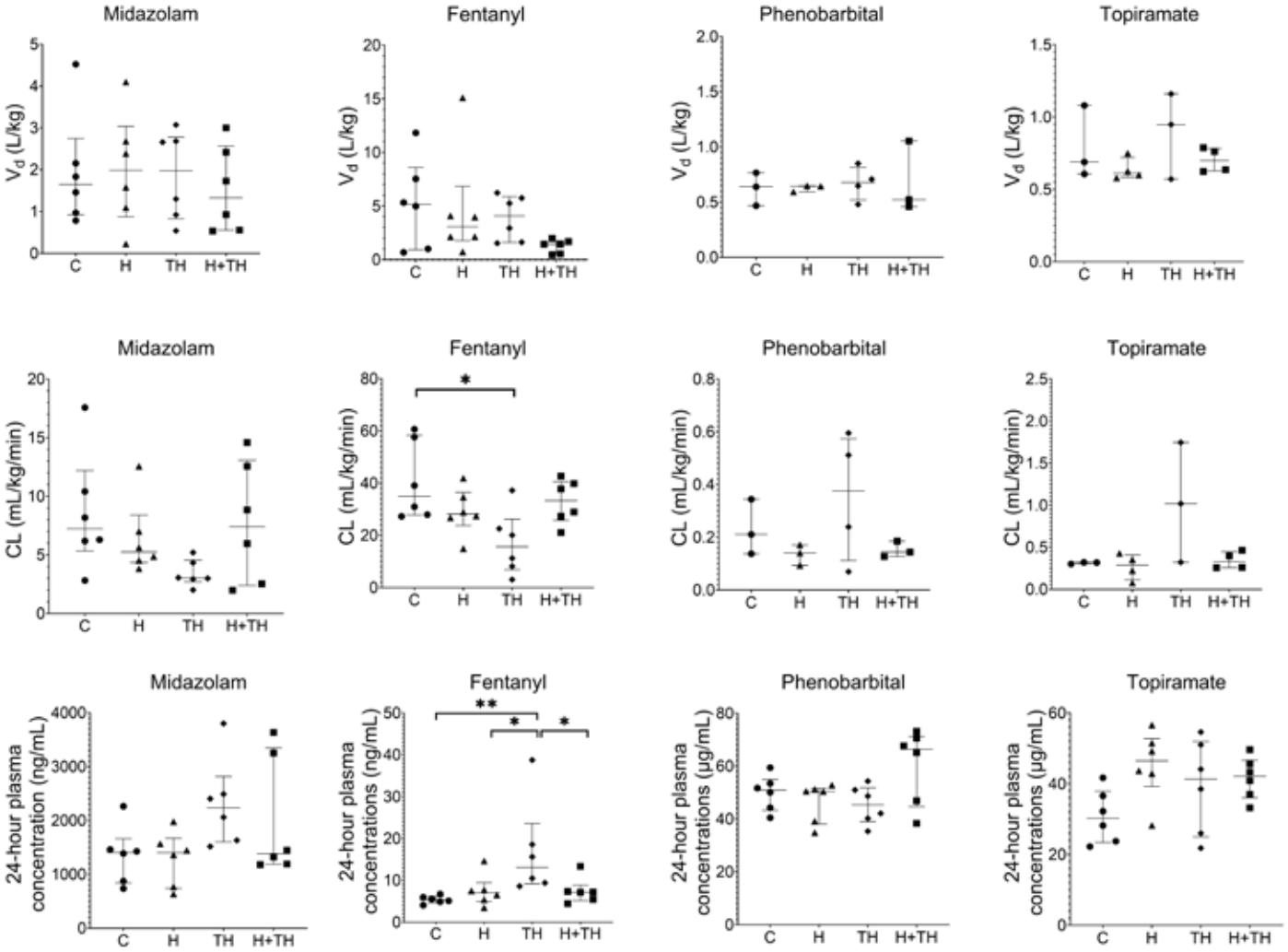

Figure 2
Pharmacokinetic parameter estimates of the studied drugs in neonatal Göttingen Minipigs. The names of each group were abbreviated as follows - control (C), therapeutic hypothermia (TH), hypoxia (H), hypoxia and TH (H+TH). Data are presented as scatter plots, median with the whiskers presenting the interquartile range, which is the range between the first (25th percentile) and third quartiles (75th percentile), for: volume of distribution (Vd); clearance (CL); 24-hour plasma concentrations. The number of animals used in this analysis was six per group for each PK parameter and drug, except for phenobarbital and topiramate, where three and four individuals, respectively, were used for volume of distribution and clearance. Datapoints that lie beyond the whiskers are outliers. Statistically significant differences were only found for fentanyl, with a significantly lower clearance (P=0.0099) in the TH group compared to the control. Additionally, the 24-hour plasma concentrations were statistically significantly higher in the TH group compared to C (P=0.0026), H (P=0.0271) and H+TH (P=0.0337) groups. p-value: *, P<0.05; **, P<0.005; ***, P<0.0005; ****, P<0.0001.

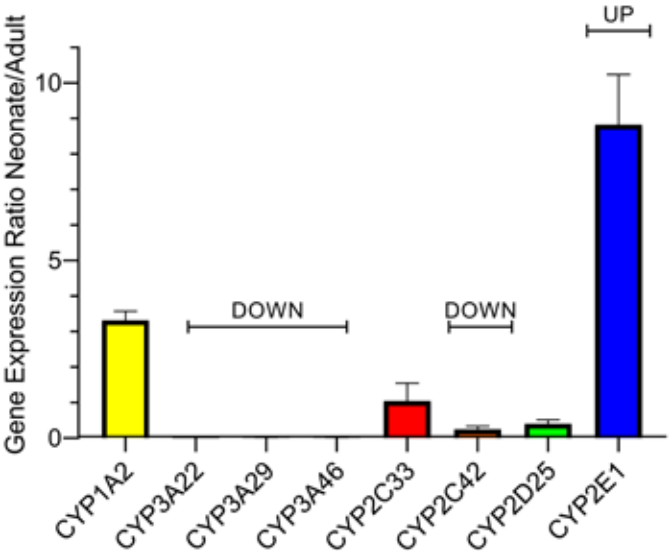

Figure 3
The gene expression ratios and standard errors following the relative expression software tool for eight target genes in Göttingen Minipigs: the age-related differences: adult (n=6) versus neonatal (<24 hours of age denoted as postnatal day, PND1; n=6) male Göttingen Minipigs. * Cytochrome P450 (CYP).

Significantly lower CYP3A22, CYP3A29, CYP3A46, CYP2C42, and higher CYP2E1 expressions were observed in neonates compared to adult Göttingen Minipigs and no differences were noted for CYP1A2, CYP2C33, and CYP2D25 (Figure 3). Additionally, the CYP activity in neonatal Göttingen Minipig liver microsomes was 75% lower than in adults (Figure 4).

During hypothermia, most cellular processes are slowed down, including the activity of drug metabolizing enzymes [18]. Our results showed that general CYP activity in adult Göttingen Minipig liver microsomes decreased significantly (by 36%) when exposed to in vitro hypothermia (33 °C) (Figure 4). Our in vivo drug disposition data in neonatal Göttingen Minipigs sustain this [7].

It is well known that acute systemic hypoxia in humans down-regulates specific CYP isoforms and up-regulates CYP3A4 and P-glycoprotein, changing the drug clearance and the kinetics [19]. Our study results, resumed in Table 1, align with these findings regarding hypoxia-induced changes in CYP3A. We observed a statistically significant up-regulation of CYP3A29, the only CYP3A Göttingen Minipig isoform that showed significant changes in gene expression in response to hypoxia.

Our study revealed increased gene expression of CYP3A29 and CYP2C33 in the H group, with hypoxia combined with TH further elevating CYP2C33 and CYP2C42 expression compared to in vivo controls. However, these changes were not consistently reflected at the protein level. Similar variability has been observed in other studies, such as in rats, where the impact of hypoxia on CYP2C protein abundance and activity depended on the severity and duration of hypoxia [20]. These findings highlight that the effects of hypoxia on CYP2C enzymes vary with the isoform, the experimental conditions, and the type and intensity of hypoxia.

Lastly, CYP2E1, recognized for its high oxidase activity, plays a significant role in hypoxic injury, with hypoxia leading to a

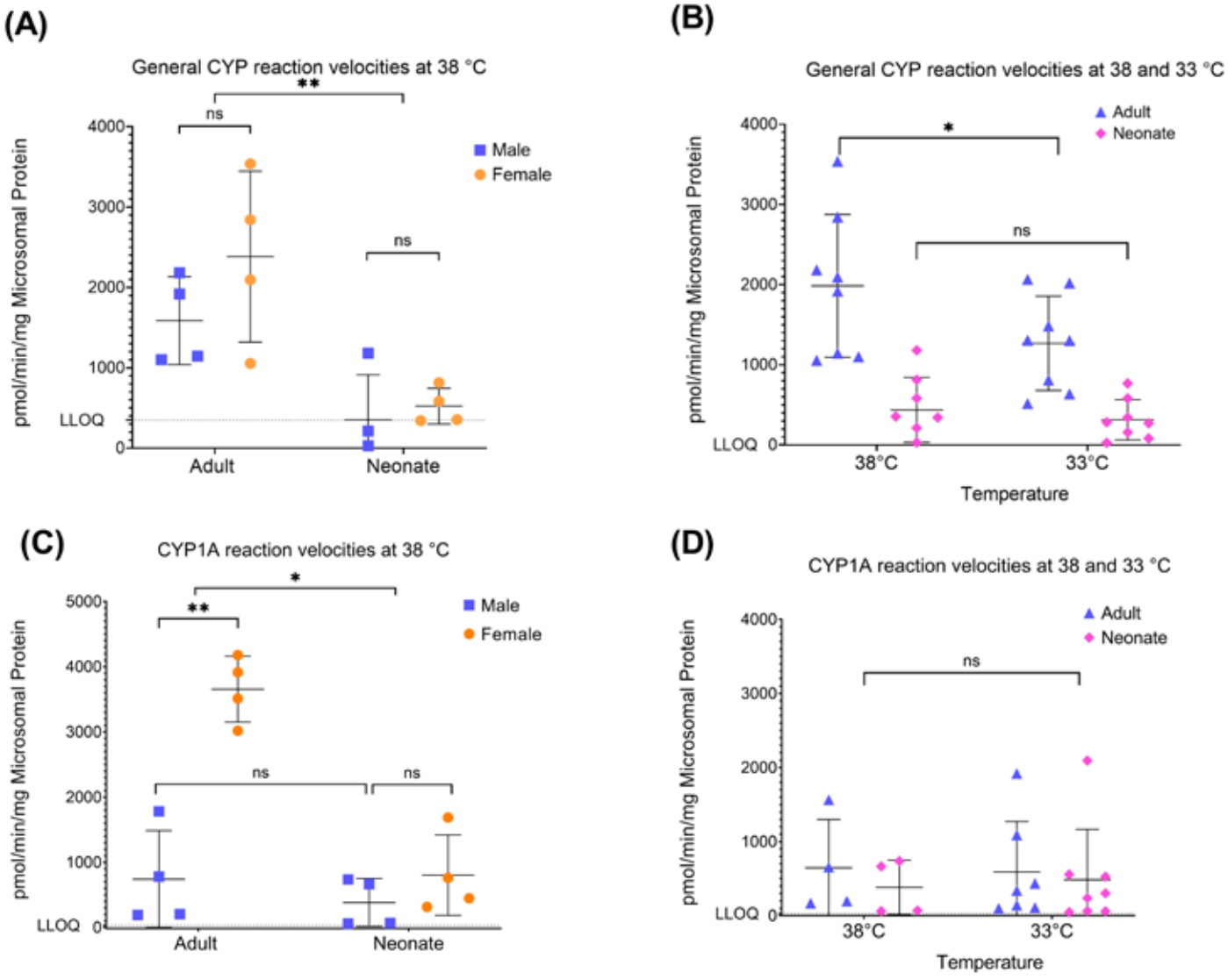

Figure 4
Hepatic Cytochrome P450 (CYP) activity in Göttingen Minipig liver microsomes: The effect of age, sex, and temperature (immediate) on the general CYP and CYP1A activity. General CYP activity was significantly influenced by age at both 33 °C (P =0.0001) and 38 °C (P=0.0006), with a 75% difference in reaction velocities between adults and neonates. Temperature had a significant effect only in adults, with a 36% decrease in reaction velocity at 33 °C compared to 38 °C (P=0.0153), while no temperature effect was observed in neonates. For CYP1A activity, sex (P=0.0004) and age (P=0.0240) were significant factors, while temperature had no effect.* Lower limit of quantification (LLOQ); p-value: *, P<0.05; **, P<0.005; ***, P<0.0005; ****, P<0.0001; ns, non-significant.

marked increase in its expression, catalytic activity, reactive oxygen species production, and associated cell death [21]. Our results indicated a statistically significant increase in CYP2E1 protein abundance in the H group compared to controls, aligning with these previous findings. These results show that CYP2E1 in the liver may be involved in hypoxic injury.

Conclusion

In this study, a neonatal Göttingen Minipig model for dose precision in PA was developed in which the impact of systemic hypoxia and TH on drug disposition and enzyme functionality could be studied separately. TH increased fentanyl plasma levels by reducing hepatic clearance and prolonging half-life, likely due to impaired liver blood flow and decreased CYP3A metabolism. Midazolam showed similar trends, with increased plasma levels and reduced clearance under TH. Additionally, TH lowered heart rate, particularly impacting high ER drugs like fentanyl. In vitro studies revealed differences in CYP gene expression and activity between neonatal and adult Göttingen Minipigs, highlighting one more time the role of maturation in drug metabolism. The exposure to in vitro hypothermia reduced CYP activity in adult liver microsomes by 36%, while hypoxia in neonates altered CYP3A29 expression and CYP2E1 abundance, though no gene expression changes were observed with in vivo TH. Despite the success of several challenging techniques, the model has limitations, such as the 24-hour survival period, which excludes a rewarming phase and limits the PK profile. Enhanced diagnostic tools would improve the evaluation hypoxic-ischemic encephalopathy in this setting. This research provides valuable insights, difficult to obtain clinically, into the different effects of hypoxia and TH on drug metabolism. These findings can inform PBPK models to improve precision dosing in human neonates.

Level	Target CYP	Treatment Group	Control Group	Expression Ratio	*P*	Result
Gene expression	CYP3A29	H	C	5.147	0.026	Up-regulated
Gene expression	CYP2C33	H	C	3.229	0.002	Up-regulated
Gene expression	CYP2C33	H+TH	C	2.491	0.003	Up-regulated
Gene expression	CYP2C42	H+TH	C	4.019	0.040	Up-regulated
Protein abundance	CYP2D6	H	C	-	0.014	Lower
Protein abundance	CYP2D6	H	TH	-	0.008	Lower
Protein abundance	CYP2D6	H	H+TH	-	0.011	Lower
Protein abundance	CYP2E1	H	C	-	0.036	Higher

Table 1
Overview of the statistically significant results for gene and protein expression impacted by hypoxia. * Cytochrome P450 (CYP).

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Investigation of large volumes and high flow rates in subcutaneous dosing in Göttingen Minipigs

ABOUT STUDY INSIGHTS: Göttingen Minipigs are increasingly selected for all aspects of biomedical research and are fully recognized as a reliable and established animal model by all regulatory authorities worldwide. This section aims at providing an insight into the wide use of Göttingen Minipigs within biological research.

Insight provided by:
Andres Eskjær Jensen, DVM | Ellegaard Göttingen Minipigs A/S, Denmark

What is the study about?
There is a need for developing new technologies to enable sub cutaneous (SC) delivery of larger volumes and higher concentrations in relation to SC treatments being preferred over IV. This study is about investigating large and fast SC delivery in Göttingen Minipigs.

What is the purpose of the study?
Testing the feasibility of SC delivery of various volumes and at different flow rates in the Göttingen Minipigs.

Why is it important?
A lot of effort is being put into designing large volume autoinjectors and novel formulation technologies for SC delivery. These devices and technologies need to be tested to ensure safety and functionality. Such testing should be performed in animal models before tests in humans are to be initiated.

What makes this study particularly interesting?
There are certain physical limitations to autoinjectors, that limits the force which can be used for delivering the drug into the SC space. Highly concentrated formulations bring high viscosities, which require high forces. Imagine trying to force syrup through a needle and you need to push 5 ml through in 20 seconds – it will require substantial force… and the more force you add to the mix, the more risks will follow. This study investigated how much force it required to inject saline at different very fast flowrates.

Which challenges have you met during the study?
The usual suspect, namely biology. The SC space is a multiplex of different structures with varying parameters. Unfortunately, it's not a homogenous place like the blood pool you access via IV. So, there's an inherent variation in the output data that grows once you push the boundaries of speed and volume. Especially the position of the needle point is important, as just the tiniest obstruction of the lumen of the needle has huge implications for the output data.

How do you recommend going about species selection?
When it comes to SC delivery, the Göttingen Minipig is the golden standard, as its skin is very comparable to human, especially when it comes to the SC space. Two things to consider however is the presence of musculus panniculus carnosus that can interfere in the SC and the hair follicles that can cause an issue for autoinjectors if the needle hits one dead on.

Any learnings you would like to share?
Expect variation in data and take care not to investigate too many things at the same time.

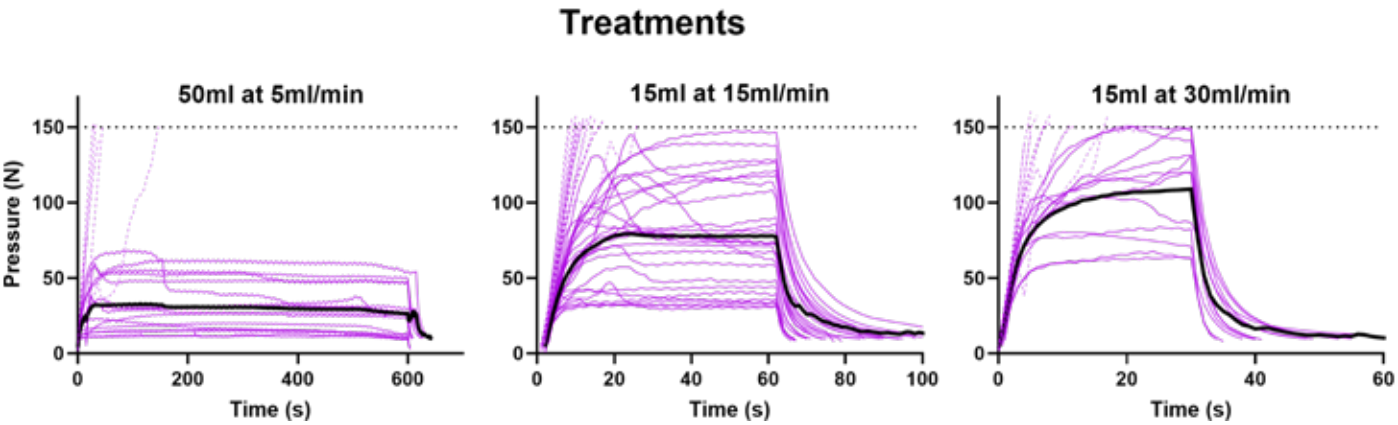


Image 1
Three different treatments (flow rate and volume) and the respective pressure required for injection over time.

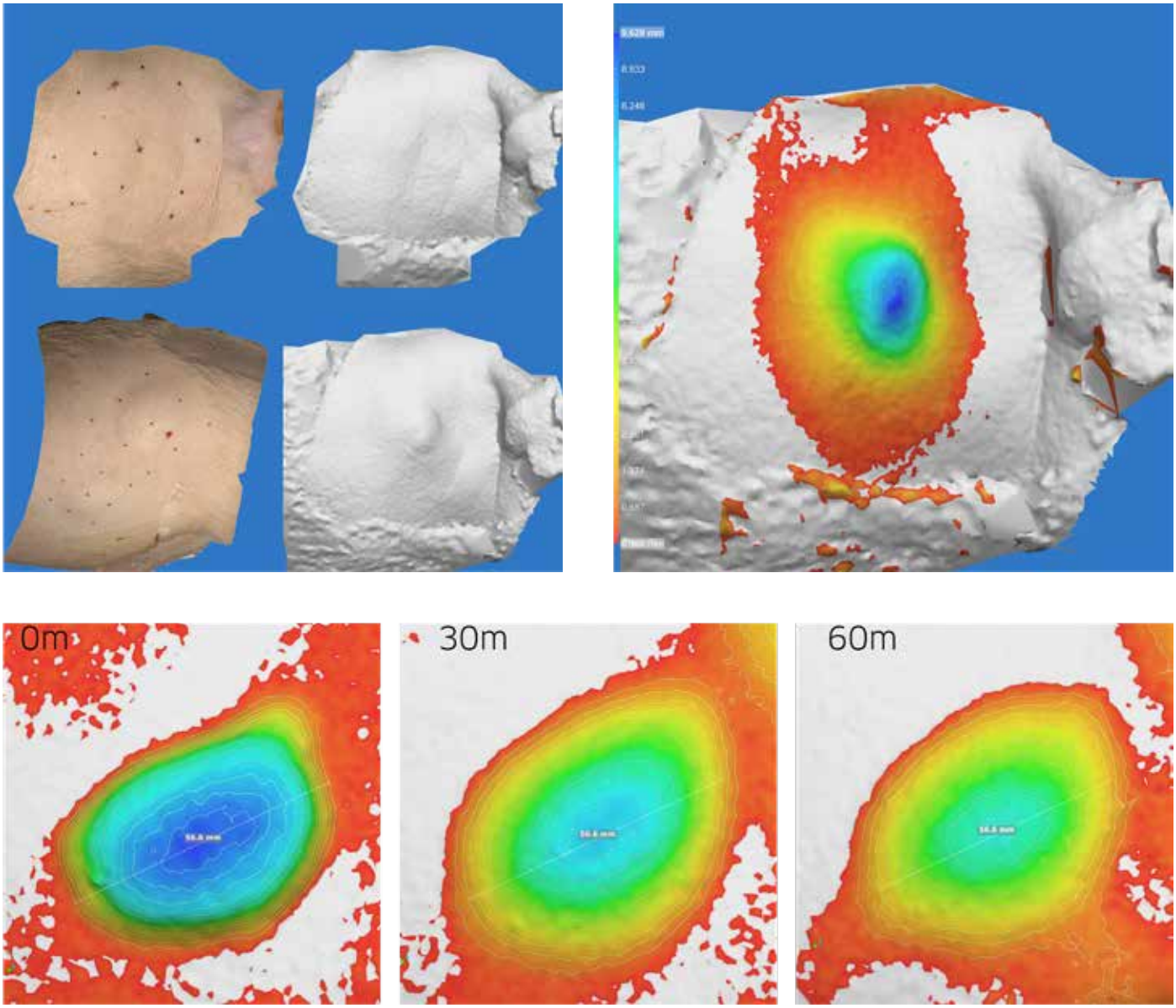


Image 2
Top left: 3D images from before and after injection.
Top right: Topographic heatmap of bleb height.
Bottom: Topographic development of bleb over time after injection.

Spotlights

Tech Week 2025

In the 5th week of 2025, Ellegaard Göttingen Minipigs once again joined in celebrating the International Laboratory Animal Technician Week.

The purpose of Tech Week is to pay tribute and recognise the skills and hard work of Laboratory Animal Technicians caring for animals in biomedical research. "It is important to remember and acknowledge the vital contribution to scientific research brought by Laboratory Animal Technicians every day. Not just this week, but all year round, as Animal Technicians create the best possible environments for laboratory animals, and thereby clear the path for optimal scientific results" explains Maja Ramløse, Principal Laboratory Animal Veterinarian at Ellegaard Göttingen Minipigs.

Throughout the week, the Animal Caretakers invited their colleagues for a peak in their daily routines, and colleagues shared examples of their appreciation in return, with examples of extraordinary efforts from internal projects and delivery preparations.

This year, the Animal Caretakers in the Research Barrier explained the huge part they play during a nephrectomy in the surgical suite by taking colleagues through their preparations, actions during surgery, and post-surgical care and monitoring of the minipigs.

A returning and always popular event during TechWeek is the yearly open house in the breeding barriers. This year, visitors (employees at Ellegaard Göttingen Minipigs only) could choose between a variety of daily procedures to attend, for example, socialisation, feeding and bedding, breeding evaluation, farrowing, quarantines, enrichments, etc. As always, the minipigs greeted their visitors with curiosity and excitement.

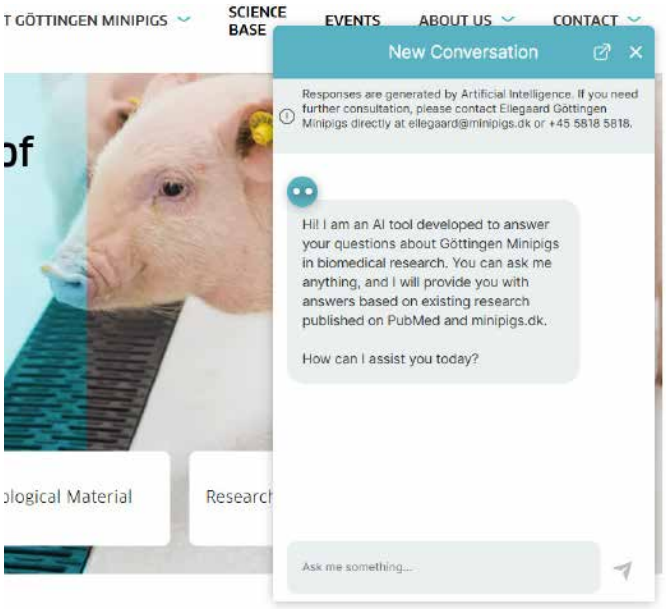
New scientific AI tool answers questions about Göttingen Minipigs

Ellegaard Göttingen Minipigs A/S has developed a new AI Chatbot that can answer any question relating to Göttingen Minipigs in biomedical research - and the answers are always based on published research material.

The AI Chatbot has been programmed to look for answers at PubMed and minipigs.dk as the only two validated sources. When answering, it adds links to the sources used to create the response, enabling the user to read more in the full publications.

This tool is relevant for all everyone working with Göttingen Minipigs, no matter level of experience. Use the tool to get started on new research or clarify uncertainties about the application and use of Göttingen Minipigs in biomedical research.

Go to minipigs.dk to try the new scientific AI Chatbot.



Göttingen Minipigs Symposium in Boston

The success from the two Göttingen Minipigs Symposiums in San Diego and San Francisco in January 2025 will be repeated, this time in **Boston, Massachusetts on 8 May 2025**.

Join a full day dedicated to knowledge-sharing about Göttingen Minipigs in biomedical research. This one-day scientific conference presents minipig users with a unique opportunity to meet, discuss, and share experiences on minipigs, but also attend scientific lectures targeted at both experienced minipig users and those still exploring the opportunities and benefits of Göttingen Minipigs as a large animal model.

Save the date, and follow Ellegaard Bioresearch on LinkedIn for more information on the scientific programme and registration.

Health Monitoring Report: December 2024

Every 6 months the Health Monitoring Report (HMR), based on FELASA recommendations, is published for all three barriers at Ellegaard Göttingen Minipigs.

Principal Laboratory Animal Veterinarian at Ellegaard Göttingen Minipigs, Maja Ramløse, who is responsible for reviewing the overall health monitoring plan, collecting, accumulating, and reporting the results, says: "We monitor the health of our colonies twice a year for a wide range of pathogens. In May/June we screen for selected agents, and in November/December we perform an extended analysis. For the latest report we are very pleased to confirm, that the December 2024 report shows no changes in the overall health status at our facility."

Download the full report and exclusion list from: minipigs.dk/about-gottingen-minipigs/health-status.

Invitation to the 17th

Minipig Research Forum

Amsterdam, The Netherlands
9-11 April 2025

Join the 17th Minipig Research Forum - a unique opportunity for minipig users to meet, discuss and share knowledge and experiences within all areas of minipig use related to biomedical research. Take part in this conference packed with scientific lectures, poster presentations and the opportunity to network with minipig users from all over the world.

SCIENTIFIC SESSIONS AND WORKING GROUP UPDATES

- **Keynote presentation:**
  "Pig omics technologies to uncover molecular mechanisms in health and disease", by Associate Professor Peter Karlskov-Mortensen, University of Copenhagen, Denmark
- **Göttingen Minipigs in Metabolism Research**
- **Practical Challenges and Solutions with Dosing and Evaluating Minipigs** - interactive session
- **Species Selection - Requirements, Case Stories and Rising Models**
- **Update from:**
  Biomarker Group (MRF 2023)
  Research of Pain Group (MRF 2024)
  IgG Humanized GM-Paper Group (MRF 2024)
  Göttingen Micropigs (by Ellegaard Göttingen Minipigs)

Each session contains presentations by speakers sharing the latest data and knowledge within their specialist areas. See the full scientific program on page 2 or at minipigresearchforum.org.

BREAKOUT SESSIONS

Attendees can choose between two different breakout sessions. See the topics on page 2. Breakout Session 1 (BS1) contains a continuing education topic.

Note: The online ticket for Session 2 includes online participation in BS1. Both sessions will be broadcast live/no recordings will be shared.

PRACTICALITIES

Starts at	**9 April 2025** (Wednesday) 15:00 hrs CEST
Ends at	**11 April 2025** (Friday) 12:00 hrs CEST
Registration fee	Registration fee: €595 Reduced sponsor ticket fee: €450 * Online ticket: Session 2+BS1: €95 * * Log in to your MRF account for more information.
Register	To register, log in to your MRF account and follow the link to the registration page: minipigresearchforum.org
Venue	NN Amsterdam Schiphol Hotel Kruisweg 495 2132 NA Hoofddorp, The Netherlands (Free shuttlebus from/to the airport)
Accommodation	Accommodation is available at the venue hotel at a special conference rate of €149 per night incl. breakfast when booking via this link.

***IMPORTANT NOTICE:* Hotel rooms are limited. Please make your reservation before 9 March 2025 due to the flower season in Amsterdam.**

POSTER VIEWING SESSION

The very popular poster viewing session is of course part of the program. Posters are accepted either with technical (e.g., tips and tricks) or scientific content (must contain data). Commercial posters without data and/or technical information will be declined. Read the poster guidelines with format requirements at minipigresearchforum.org. **The deadline for submitting a poster abstract is 21 March 2025.** We also encourage young scientists to present a poster as a good opportunity to display their work and results. As a tradition, the best poster(s) will be elected during the MRF meeting by the MRF Steering Committee.

The MRF is a non-profit organization with more than 500 members worldwide working with minipigs in industry, academia, and regulatory bodies. Participation in the annual MRF conference requires membership (free of charge). The MRF conference requires physical attendance from the attendees (with Session 2+ BS1 as an exception for MRF 2025). Read more and apply for membership at www.minipigresearchforum.org

KEYNOTE PRESENTATION (Wednesday)

Pig omics technologies to uncover molecular mechanisms in health and disease	Peter Karlskov-Mortensen, University of Copenhagen, Dept. Of Veterinary and Animal Sciences, Denmark

POSTER SESSION (Wednesday)

Poster introduction by authors and poster viewing session	Deadline for submission: 21 March 2025 Poster Guidelines on minipigresearchforum.org

SESSION 1 | GÖTTINGEN MINIPIGS IN METABOLISM RESEARCH (Thursday)

Measuring energy expenditure in Göttingen Minipigs using indirect calorimetry: Validation and methodological considerations	Simon Krogh Bredum, Embark Laboratories, Denmark
Background and experiences from using Göttingen Minipigs as the large animal model throughout discovery and safety/tox studies for a biotech project in mid-stage clinical development	Rasmus Jørgensen, Cytoki Pharma, Denmark
Obesity development and signs of metabolic abnormalities in young Göttingen Minipigs	Mihai Curtasu, Aarhus University, Denmark

UPDATES ON MRF WORKING GROUPS – PART I (Thursday)

Biomarker Discovery in the European Initiative on Minipig and Micropig Models - NHPig[1]	Allan Valenzuala, University of Antwerp, Belgium
Research of Pain Group (MRF 2024)	Sigal Meilin, MD Bioresources, Israel

SESSION 2 | PRACTICAL CHALLENGES AND SOLUTIONS WITH DOSING AND EVALUATING MINIPIGS - AN INTERACTIVE SESSION (Thursday)

Oral dose application | Practical perspective about training for oral dosing with tube and oral dosing by capsule	Kim Paul Broens, CRL Den-Bosch, The Netherlands
Ocular evaluations | Performing in-life indirect ophthalmoscopy and a slit-lamp examination and presenting normal background findings	Nikoline Folmer Jensen, Scantox, Denmark
SC dosing | Subcutaneous administrative device and imaging technology to characterize and assess the distribution of administered materials post dose	Eleanor McRae, AstraZeneca, United Kingdom
Dermal dosing | Practical challenges and contamination risks associated with dermal dosing in minipigs	Michela Di Carlo, ERBC, Italy

BREAKOUT SESSIONS (Thursday) Abstracts and format on minipigresearchforum.org

BS1: Agony Aunt: Tackling practical challenges in minipig research	Supported by subject matter experts from various industries
BS2: Intracerebral administration in Göttingen Minipigs: anatomical considerations	Introductory presentation by Gael Quesseveur, CRL Lyon, France

UPDATES ON MRF WORKING GROUPS – PART II (Friday)

IgG Humanized Göttingen Minipigs - Paper Group (MRF 2024)	Pramila Singh, CRL Lyon, France
Status on Göttingen Micropigs and status on NHPig[1] project	Lars Siim Madsen, Ellegaard Göttingen Minipigs, Denmark; and Henrik Duelund Pedersen, Novo Nordisk, Denmark

SESSION 3 | SPECIES SELECTION - REQUIREMENTS, CASE STORIES AND RISING MODELS (Friday)

Required justifications for species selection	Sean O'Halloran, Labcorp, United Kingdom
Results from an immunogenicity assessment of two IgG1 drug candidates in humanized IgG1/4 and in standard Göttingen Minipigs	Christian Sommer, Ferring Pharmaceuticals, Denmark
Non-rodent Species Selection for Nonclinical Safety Assessment of Pharmaceuticals: Minipig or not Minipig	Maryam Rafie-Kolpin, AstraZeneca, United Kingdom
Additional data for species selection - Spatial transcriptomics of the minipig liver	Björn Jacobsen, F. Hoffmann-La Roche, Switzerland

[1] NHPig: The European Initiative on Minipig and Micropig Models

GÖTTINGEN MINIPIGS ACADEMY

Customised courses

Courses are booked on demand so the contents can be costumised according to the participants' level of knowledge and experience. Courses can be scheduled as personal or team sessions, and you can combine more courses in an individualised, consecutive structure.

Ask about course opportunities

Contact academy@minipigs.dk for a conversation about how we can meet your training needs, or see minipigs.dk for a full course overview.

Who can join?

The academy facilitates seminars, workshops, and webinars around Göttingen Minipigs, designed for those working within the life sciences industry. Courses target a diverse audience and are carefully selected to accommodate the needs of:

- **Animal Technicians**
- **Animal Caretakers**
- **Laboratory Technicians**
- **Veterinarians**
- **Researchers**
- **Scientists**
- **Study Directors**
- **Sponsor Monitors**
- **Surgical Staff**

Yet, anyone with an interest in Göttingen Minipigs in biomedical research can benefit from the courses.

Course: Handling & Dosing

Course: Vascular Access

Former course participants

Time well spent - a good mix of theory and hands-on training for new as well as experienced employees, boosting the qualifications of our minipig facility team.
Gubra | Principal Scientist, Pharmacology Research

Perfect course day with a good overview of the most common dosing methods followed by practical training. Very rewarding!
AstraZeneca | Animal Technician

Being able to test new methods for fixing and dosing minipigs for one day gave our team a good start to develop new knowledge and skills that we use daily in our work.
AstraZeneca | Veterinary Nurse

Thanks to both of you again for a good course and your hospitality. Made our journey worthwhile.
Pharmaron | Senior Animal Technician

I have been working with Göttingen Minipigs for more than 10 years, and one thing I know, is that there is always new things to learn. The workshop was a good combination of theory and hands on work – and one of the best things was for sure that the workshop was adapted to us course participants and conveyed by very skilled personnel. I will recommend everyone working with the minipig to participate in one of the workshops – there is always more to learn, and the Göttingen Minipigs Academy is the place to learn it.
Minerva Imaging | DVM and Senior Scientist

Veterinary Mngmt, Welfare & Culture of Care

Duration 6 hours
Participants Veterinarians, Animal Caretakers, Laboratory Technicians
Location Copenhagen or Dalmose, Denmark
Price Contact academy@minipigs.dk

In this advanced learner's course, we will go into detail with animal welfare aspects tailored to fit the specific needs of Göttingen Minipigs, incl. valuable acclimatisation, socialization, and thorough behavioral management programs addressing various enrichment elements, etc. We will discuss the importance of working with a Culture of Care and suggestions on how to promote it. We will look at clinical issues you might encounter in Göttingen Minipigs and suggested treatment protocols. You will learn how to maintain healthy minipigs, including setting up effective biosecurity measures and performing adequate health monitoring.

You will learn:

- Göttingen Minipigs biology and behaviour
- Animal welfare incl. acclimatisation, socialization, behavioral management programs, enrichment elements, etc.
- Culture of Care and how to promote it
- Clinical issues and treatment protocols
- How to maintain healthy Göttingen Minipigs, effective biosecurity measures, and adequate health monitoring

Handling & Dosing

Date 6 hours
Participants Veterinarians, Animal Caretakers, Laboratory Technicians
Location Dalmose, Denmark
Price Contact academy@minipigs.dk

In this practical course, you get to work on your technique and train in various procedures. Learn to understand the behavior of Göttingen Minipigs, the importance of socialization and training, and an introduction to how to create an enriched habitat that supports the biological needs of Göttingen Minipigs. Engage in hands-on learning as you approach, lift, and carry the minipigs under supervision. Try various restraint and dosing techniques, and discover the practicality of slings when performing blood sampling. You will also learn about humane euthanasia in the course. Depending on your experience level, we can go through more specific training like endotracheal intubation and catheterization methods for vascular access.

You will learn:

- Behaviour of Göttingen Minipigs, socialisation, and training (theory)
- How to handle the minipigs physically (practice)
- Blood sampling, restraint, and dosing techniques (practice)
- Humane euthanasia (practice)
- Endotracheal intubation and catheterization methods for vascular access (experienced course participants)

Anaesthesia & Analgesia

Duration 3 hours
Participants Veterinarians, Animal Caretakers, Laboratory Technicians, Surgical Staff
Location Copenhagen or Dalmose, Denmark
Price Contact academy@minipigs.dk

This course is well suited for beginners, but more experienced participants may also appreciate the explained aspects of the anaesthesia for Göttingen Minipigs. This theoretical course is usually conducted as a 3-hour course with physical attendance.

You will learn:

- Göttingen Minipigs anatomy and characteristics for anaesthesia
- Basic physiology for anaesthetics
- Pharmacology of the most used drugs
- Protocol design and what we wish to achieve with it
- How to monitor vital parameters, manage ventilation and fluid balance
- Equipment and methods for administering anaesthetic compounds, as well as explain their advantages and limitations
- Emergency handling and acute treatment procedures

Vascular Access

Duration 6 hours
Participants Laboratory Technicians, Veterinarians, Animal Caretakers, Scientists
Location Dalmose, Denmark
Price Contact academy@minipigs.dk

(Mini)pig vascular access can be challenging due to the lack of superficial veins. Göttingen Minipigs are no exception, and careful consideration has to be given already during the design process of the study. In this practical course, we will present the possibilities and tools that can be applied to make the study successful. The overall outcome is that you learn to surgically implant jugular catheters, first demonstrated by one of our surgeons, and afterward on your own.

You will learn:

- Blood sampling from sling and catheters
- Establishing IV access
- Placement of peripheral catheters
- Endotracheal intubation
- Surgical implantation of jugular catheters

NEWS FROM

Ellegaard Göttingen Minipigs A/S

Ellegaard Göttingen Minipigs A/S is a leading international company supplying Göttingen Minipigs for biomedical research around the world. From our AAALAC accredited facility in Denmark we breed Göttingen Minipigs and enable the development of safer and more effective medicines, all based on our core values: Animal welfare, quality, respect, and collaboration.

Appointments and anniversaries

21 May 2024 Olivier Dillenseger started as new Regional Manager.

Olivier supports our clients on Göttingen Minipigs, genetically modified models and our research services in France, Switzerland, and the Southern part of Germany.

1 June 2024 Anders Köppen Nygaard started as new Operations Manager.

Anders is responsible for our production, order, and delivery set-up and ensures that our facility in Dalmose is running smoothly and effectively.

1 August 2024 Charlotte Hoeg Thygesen started as new Scientific Director.

Charlotte safeguards our high health status and level of animal welfare as well as develops, prepares, and aligns our Research Service capabilities to market demand.

12 August 2024 Kamillah Linnea Seefeld started as office trainee.

As part of her education to become an administrative assistant, Kamillah will take part in the daily routines in finance, HR, sales, orders, and marketing.

1 September 2024 Lars Siim Madsen started as new Chief Scientific Officer.

Lars focuses on business development, external collaborations, and potential partnerships, helping to shape the strategic direction of our scientific advancement.

1 October 2024 Sarah Olivia Danielsen Hansen started as Animal Caretaker trainee.

Sarah has joined forces with our team of Animal Caretakers in our research barrier to ensure that our Göttingen Minipigs thrive and have everything they need.

1 June 2024 Sofie Nørrelund Kirchhoff celebrated her 5th anniversary with us.

Sofie is one of our experienced Animal Caretakers in the breeding barriers, who ensures the well-being of our Göttingen Minipigs every day.

1 August 2024 Maria Bonnesen celebrated her 5th anniversary with us.

Maria is our Head of Marketing and handles all marketing and communication affairs, ensuring that knowledge about our Göttingen Minipigs is available to the market.

Where to meet us in 2025

CONFERENCE	DATE	LOCATION
Göttingen Minipigs Symposium	21 Jan	San Diego, CA, USA
Göttingen Minipigs Symposium	23 Jan	San Francisco, CA, USA
SOT and ToxExpo	16-20 Mar	Orlando, FL, USA
Janssen Juvenile Tox Symposium	3-4 Apr	Beerse, Belgium
Minipig Research Forum	9-11 Apr	Amsterdam, The Netherlands
Göttingen Minipigs Symposium	8 May	Boston, MA, USA
FELASA	2-5 Jun	Athens, Greece
EUROTOX	14-17 Sep	Athens, Greece
SPS	13-15 Oct	Jaarbeurs, Utrecht, The Netherlands
EARA Conference	6-7 Nov	Berlin, Germany
ACT	16-19 Nov	Phoenix, AZ, USA
AFSTAL	19-21 Nov	Nantes, France
LASACON	TBA	India
STP-I	TBA	India

Expansion of facility: Genetically modified Göttingen Minipigs a huge success

Genetically modified Göttingen Minipigs are becoming more and more sought after, particularly the IgG 1/4 Humanized Göttingen Minipigs. Also, the breeding of the still new strain of Göttingen Micropigs requires separate pens, hence, the research barrier at Ellegaard Göttingen Minipigs A/S (where the genetically modified models are bred) is running out of space.

Therefore, Ellegaard Göttingen Minipigs has been investigating opportunities to expand the research barrier in order to accommodate the growing demand of genetically modified Göttingen Minipigs and continued development and breeding of Göttingen Micropigs. Operations Manager, Anders Köppen Nygaard elaborates: "We have been looking for a flexible solution to expand the current barrier capacity and still be able to adjust and adapt to learnings while breeding the IgG 1/4 Humanized Göttingen Minipigs and developing the Göttingen Micropig. Therefore, we decided to install a prefabricated solution consisting of three custom-made modular buildings of around 300 square meters in total. The modules comply with our high standards on animal welfare, infection control, and working environment that have been established and developed throughout the company's history."

The construction is performed by entrepreneurs that is known by Ellegaard Göttingen Minipigs from earlier expansion projects and general maintenance at the company facilities, while also adding new entrepreneurs to the necessary extend in order to ensure the highest possible know-how end experience within relevant areas.

The last modular buildings will arrive in the first quarter of 2025 and hereafter the remaining work and last preparations will take place, before the barrier is fully operational in mid-2025.

Ensuring safety first during transport

In February 2025, all drivers at Ellegaard Göttingen Minipigs A/S attended a driving course at the local road safety center, to brush up their driving skills under challenged conditions. “It’s important that our drivers’ skills behind the wheels are maintained and certified regularly, so both they and the minipigs they transport across Europe are ensured a safe and comfortable journey”, says Anders Köppen Nygaard, Operations Manager at Ellegaard Göttingen Minipigs.

Likewise, the drivers appreciate the prioritisation of the practical courses. After the course Jørn Frydenberg, Driver at Ellegaard Göttingen Minipigs, said: “You become an experienced driver when driving about 100.000 km around Europe every year. However, attending a course like this adds something else as we become updated on the latest security features in the cars, get to test them to the “limit” under secure conditions, and learn how the cars will react in an emergency situation. Also, it is great to spend a day with your colleagues and share experiences, as we spend a lot of time alone on the roads.”

Christmas sponsorships

Every year at Christmas, Ellegaard Göttingen Minipigs makes donations to a selection of charities. The size of the donations is decided through an internal vote amongst all employees, with the aim of creating ownership and unity.

The charities are selected to support the organisational focuses on corporate values, purpose, or UN Global Goals. This Christmas the recipients of the Christmas donations were:

- Danish Cancer Society and Danish Hospital Clowns, supporting the purpose of enabling the development of safer and more effective medicines.
- Animal Protection Denmark, supporting the core value "Animal Welfare".
- Forests of the World and SOS Children's Villages, supporting the UN Global Goals for Sustainable Development.

New publications on Göttingen Minipigs

Ellegaard Göttingen Minipigs gives high priority to collaborative projects that aim to better characterize and validate Göttingen Minipigs as a translational animal model and which facilitate and refine the use of Göttingen Minipigs in research projects and safety testing. Do you have an idea for such a collaborative project? Please contact ellegaard@minipigs.dk.

Eisler W, Held M, Rahmanian-Schwarz A, et al.
The Goettingen minipig as an experimental model in wound-healing studies
Published: April 2024
DOI: 10.1016/j.jpra.2024.03.011

Neimat JS, Bina RW, Koenig SC, et al.
A Novel Closed-Loop Electrical Brain Stimulation Device Featuring Wireless Low-Energy Ultrasound Power and Communication
Published: May 2024
DOI: 10.1016/j.neurom.2024.02.008

Hartmann KT, Nielsen RL, Mikkelsen FC, et al.
Bacterial micro-aggregates as inoculum in animal models of implant-associated infections
Published: May 2024
DOI: 10.1016/j.bioflm.2024.100200

Bergamo ETP, Witek L, Ramalho IS, et al.
Sustained Release of Salicylic Acid for Halting Peri-Implantitis Progression in Healthy and Hyperglycemic Systemic Conditions: A Gottingen Minipig Model
Published: May 2024
DOI: 10.1021/acsbiomaterials.4c00161

Langston JL, Moffett MC, Pennington MR, Myers TM
Pharmacokinetics and pharmacodynamics of standard nerve agent medical countermeasures in Göttingen Minipigs
Published: June 2024
DOI: 10.1016/j.toxlet.2024.04.014

Hartung CB, Visscher C, Grone R, Kamphues J
The ileal, total tract and postileal digestibility of compound feeds either rich in wheat or rye in an ileo-caecal fistulated minipig model
Published: June 2024
DOI: 10.1080/1745039X.2024.2406434

Jia H, Chang Y, Song J
The pig as an optimal animal model for cardiovascular research
Published: June 2024
DOI: 10.1038/s41684-024-01377-4

Liu F, Habibollahi M, Wu Y, et al.
A multi-channel stimulator with an active electrode array implant for vagal-cardiac neuromodulation studies
Published: July 2024
DOI: 10.1186/s42234-024-00148-3

Jhelum H, Kaufer B, Denner J
Application of Methods Detecting Xenotransplantation-Relevant Viruses for Screening German Slaughterhouse Pigs
Published: July 2024
DOI: 10.3390/v16071119

Petrucci M, de Brot S, Casoni D
Case report: Side effects of etomidate in propylene glycol in five Göttingen Minipigs
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