# NEWSLETTER 42

AUTUMN 2014

ELLEGAARD
GÖTTINGEN MINIPIGS

**Growth curve and growth data for the Göttingen Minipig**
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**Continued full AAALAC Accreditation**
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Clean pigs for clear results

## Dear Reader

Another summer has passed, and I hope you have had time to enjoy it with family and friends. In Denmark, the summer was long and warm, which we have thoroughly enjoyed.

We still have a few months left of the year 2014, and for us these months will be interesting. We will be attending several exhibitions and meetings where we will have the opportunity to disseminate knowledge about the Göttingen Minipig, but also to meet and interact with some of our many devoted customers and partners. In close collaboration with several of our customers and partners, we continue to develop new knowledge about the Göttingen Minipig, because we are keenly aware of the importance of available background data.

Recently, I visited Oriental Yeast Co. in Japan where the breeding of our Göttingen Minipigs is going very well. The staff in Japan are highly skilled and are very enthusiastic about and understand how to take care of the minipigs in the best possible way. It is always interesting for me to visit our partners in Japan and the USA because, even though we have many years of experience with minipigs, we can still learn from our partners.

Over the past year, we completed the renovation of our second minipig barrier. This renovation has improved the conditions for both minipigs and staff. It is a pleasure to observe how we can continue to refine the standards and adjust to the needs and normal behaviour of minipigs.
It is also very satisfying for me that our facility has just been fully AAALAC accredited once again, as it has been since 1998. AAALAC sets standards for animal welfare, and we are proud that we continue to meet the requirements and keep our accreditation.

Some of the content of this newsletter is based on information from our company, and I hope you will find it interesting to gain an insight into how our company is working and developing.

*Sincerely,*
*Jens Ellegaard, CEO*

## CONTENTS



# Renovation of our second minipig barrier

Over the past year, we completed the renovation of our second minipig barrier. The barrier was well-functioning and met all requirements. However, we find it very important to keep optimising the conditions for the minipigs so that their conditions and behaviour can be as close to normal as possible.
Improvements in the farrowing sections entail floor heating for the sows, larger pens and a new pen design with easier access to the piglets which reduces stress for the sows.
Several housing units now have more space in the corridors which is very beneficial when the minipigs are let out of the pens to have a walk/run in the corridor.
Through the years we have tested many different types of enrichment for the minipigs and we found out that the minipigs prefer a walk/run in the corridor. Not only is this enriching for the minipigs on the run but also for the other minipigs in the housing unit. Further information about this is available in our Newsletter no. 40 from Autumn 2013. It can be found on our website www.minipigs.dk.

More windows and brighter housing equipment have improved the working environment for the staff in the barrier. Other steps have further improved the working conditions, i.a. regarding cleaning of housing units and other daily working procedures.
You are welcome to contact us if you are interested in further information or if you would like to visit our facility (ellegaard@minipigs.dk)

# Continued full AAALAC Accreditation

Our facility has been fully AAALAC accredited once again.
We received our first AAALAC accreditation in 1998 and our facility has been fully reaccredited ever since.
AAALAC sets standards for animal welfare and the AAALAC quality management system is recognised internationally.
AAALAC accredits research institutions and producers of experimental animals which comply with its standards. The standards include regulations for the experimental animals, pen systems, safety for personnel and animals, training, facilities, equipment etc.
The AAALAC accreditation requires annual reporting and a detailed programme description must be submitted every three years, after which the facilities are reviewed by two AAALAC site visitors.
The quality system is an integral part of the production process. Our quality system is structured around AAALAC accreditation with the addition of relevant elements from the ISO 9000 standard and from GLP (Good Laboratory Practice). Our quality management system is documented in standard operating procedures (SOPs) covering all areas of our production process.
The animal welfare committee ensures that the production of minipigs takes place in accordance with national and European law and with internal guidelines including animal welfare.

# Growth curve and growth data for the Göttingen Minipig

The growth curve for the Göttingen Minipig has been updated with data derived from the past three years (June 2011-June 2014). The data represents all minipigs that matched the age +/- 7 days at the time of weighing. Data from breeding sows is excluded as the weight of these animals is fluctuating.

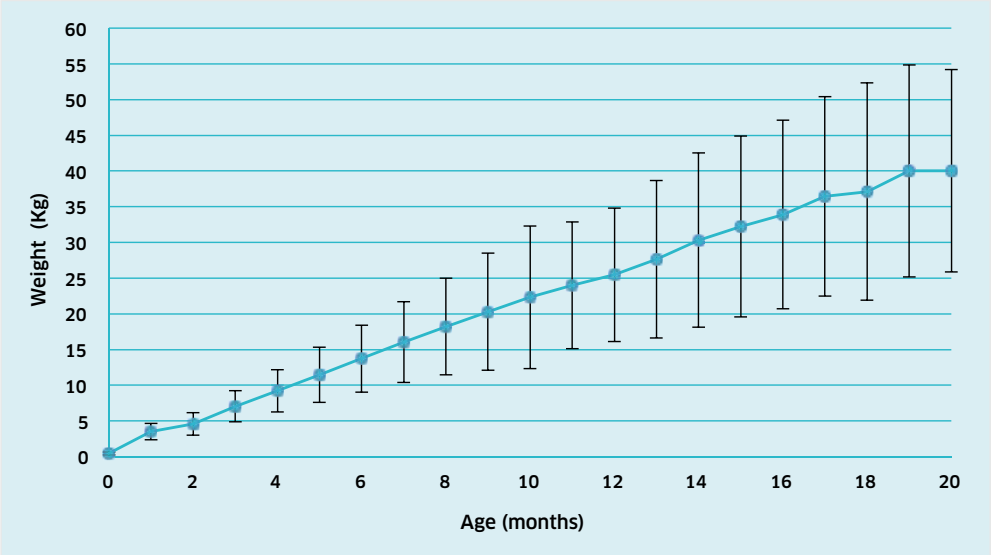
The 95% reference range presents the prediction interval between which the weight of 95% of the minipigs falls into. A minimum of normal minipigs are beyond the lower/upper limit of this interval.

The growth data and the growth curve are useful tools when deciding the suitable age/weight of minipigs for a study. They can also be used to predict the growth of the minipigs during a study and for measuring out the correct amount of diet for the minipigs.

The data has been processed in collaboration with the Georg-August University of Göttingen where the Göttingen Minipig was originally developed. The genetics for the entire breeding population of Göttingen Minipigs are still managed by the University in Göttingen.

For questions regarding the growth data or genetic management please contact Ellegaard Göttingen Minipigs: Tel.: +45 5818 5818, Email: ellegaard@minipigs.dk

## Growth Curve for the Göttingen Minipig




The vertical lines indicate the 95% referenge range which presents the prediction interval between which the weight of 95% of the minipigs falls into. A minimum of normal minipigs are beyond the lower/upper limit of this interval.

## Growth Data for the Göttingen Minipig

| Age | Sex | Number of animals | Mean weight (kg) | Standard deviation | 95% Reference Range |
|---|---|---|---|---|---|
| Birth | M | 12092 | 0,46 | 0,11 | 0,24 - 0,68 |
| | F | 11661 | 0,46 | 0,11 | 0,24 - 0,67 |
| 1 month | M | 9681 | 3,51 | 0,58 | 2,37 - 4,64 |
| | F | 9661 | 3,50 | 0,59 | 2,34 - 4,66 |
| 2 months | M | 5673 | 4,56 | 0,80 | 3,00 - 6,13 |
| | F | 6220 | 4,57 | 0,81 | 2,98 - 6,16 |
| 3 months | M | 5165 | 7,08 | 1,13 | 4,85 - 9,30 |
| | F | 5193 | 7,02 | 1,08 | 4,90 - 9,14 |
| 4 months | M | 3868 | 9,28 | 1,57 | 6,20 - 12,36 |
| | F | 3882 | 9,18 | 1,46 | 6,32 - 12,04 |
| 5 months | M | 3132 | 11,60 | 2,06 | 7,57 - 15,63 |
| | F | 3317 | 11,32 | 1,87 | 7,64 - 14,99 |
| 6 months | M | 2136 | 13,80 | 2,49 | 8,91 - 18,68 |
| | F | 2328 | 13,67 | 2,30 | 9,15 - 18,18 |
| 7 months | M | 1439 | 15,85 | 2,80 | 10,36 - 21,33 |
| | F | 1986 | 16,21 | 2,95 | 10,43 - 22,00 |
| 8 months | M | 1230 | 17,83 | 3,17 | 11,62 - 24,04 |
| | F | 1478 | 18,55 | 3,63 | 11,43 - 25,67 |
| 10 months | M | 615 | 21,52 | 3,92 | 13,84 - 29,20 |
| | F | 684 | 23,01 | 5,87 | 11,51 - 34,51 |
| 12 months | M | 382 | 24,84 | 4,46 | 16,11 - 33,58 |
| | F | 388 | 26,09 | 4,99 | 16,30 - 35,88 |
| 16 months | M | 96 | 34,26 | 6,70 | 21,13 - 47,39 |
| | F | 80 | 33,47 | 6,79 | 20,16 - 46,77 |
| 20 months | M | 46 | 41,25 | 6,49 | 28,53 - 53,96 |
| | F | 37 | 38,53 | 7,88 | 23,09 - 53,97 |

# Update from North America

The new housing facility will provide a significant increase in our available pen space.

Things have remained busy in Upstate New York, and demand for Gottingen Minipigs in North America has remained very strong this year. We are very excited to report that our new housing facility is almost complete, and we plan to populate the space in the coming month. This new barrier will allow significant production increases to accommodate rising demand, and in fact higher numbers of sows have already been bred in anticipation of the space. We've taken the opportunity to incorporate design changes to enhance animal care and enrichment, as well as efficient utilization of production space.

This past June we were also very happy to collaborate with Huntingdon Life Sciences to provide an overview of the background histopathology of the Gottingen Minipig, presented by Vasanthi Mowat, Director of Pathology at Huntingdon Life Sciences, at the annual Society of Toxicologic pathology conference in Washington, DC. Please contact nnavratil@marshallbio.com for more information. We also look forward to future opportunities to collaborate with Gottingen users across the industry to provide additional background data on the model.

Finally, we have realized there is a growing interest in the topic of "normal" pig behavior, and we have hosted several discussions about appropriate enrichment and socialization ideas within the past year. One significant area of interest is how to properly socialize minipigs that must be individually housed for study reasons. Swine, especially females, are very social animals that develop a complex hierarchy beginning at birth. Females in the wild remain in stable groups of related sows throughout their entire lives. Despite the fact that social bonds are disrupted in the laboratory setting, research suggests that swine rely heavily on the comfort of familiar conspecifics when coping with stress.[1] In Newsletter 39, Adrian Zeltner and Helle Lorentsen also demonstrated that individually housed minipigs are more fearful and less engaged by toys placed in their environment.[2] Therefore, social isolation in the laboratory can cause significant distress. We are interested in examining various options to allow animals to bond with conspecifics and remain enriched by their environment, even when study protocols require the animals be housed individually. We have found that allowing space between the bars for snout contact is a very good option. Some consideration of the spacing between the bars might be necessary, as larger spaces allow for greater contact but also provide neighbors with the ability to manipulate wound dressings or surgical sites more easily. It has been our experience that minipigs enjoy sleeping together in close contact, and individually housed minipigs will often rest next to the bars so they can be in physical contact with their neighbors. Therefore, providing resting areas that promote social contact with neighboring pens may provide a benefit. Some additional options to help promote social contact include allowing supervised play time each day, or socialization time while pens are being cleaned and other daily tasks are being performed by care takers in the room. Enrichment should promote rooting and exploring behaviors, and new toys should be rotated at least once daily to peak curiosity. Toys that smell like conspecifics, or tug-of-war-type toys affixed between pens to promote social interaction may promote greater engagement by the minipigs. We would be interested in hearing suggestions from other facilities as to how they promote appropriate social interaction and provide engaging enrichment in instances where minipigs must be individually housed. Please send your comments to nnavratil@marshallbio.com.

## References


1 Ruis, M.A.W., J. de Groot, J.H.A. te Brake, E.D. Ekkel, J.A. van de Burgwal, J.H.F. Erkens, B. Engel, W.G. Buist, H.J. Blokhuis & J.M. Koolhaas. (2001). Behavioral and physiological consequences of acute social defeat in growing gilts: effects of the social environment. Applied Animal Behavior Science 70: 201-225.

2 Zeltner, A. & H. Lorentsen. (2013). Minipig Activity – group housing vs single housing. Ellegaard Gottingen Minipigs Newsletter 39. http://minipigs.dk/fileadmin/filer/Newsletters/Nyhedsbrev_39.pdf.

# Minipig Welfare
# Acclimatisation and Socialisation

Ellegaard Göttingen Minipigs

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## Introduction

This text focuses on the time after the minipig is transported from the breeder up until the experimental procedures begin. Apart from general considerations regarding a beneficial acclimatisation period, this document provides specific instructions, including suggestions for a socialisation programme.
You probably know most of this already, but sometimes your routines deserve a closer look to reconsider some aspects and discuss whether there is room for improvement. Also, it is important to remember that it is not all or nothing, but rather small improvements and differences that do the trick!
The overall purpose is to improve general minipig welfare and conditions during the acclimatisation period. This will make the animals easier to handle during experiments and ultimately contribute to valid study results.

Once and for all, we wish to emphasise that acclimatisation is NOT a matter of leaving minipigs in peace without any disturbance. On the contrary, acclimatisation involves gradually accustoming the animals to experimental conditions in a way that takes account of animal wellbeing.

There is always room for improvement!

## Consider change as a stressor

Like most animals, minipigs perceive major changes in their environment as stressors. Therefore, any change in a minipig's environment should be introduced as gently as possible. At Ellegaard's breeding facilities, the first big change in a minipig's life is being weaned from the mother sow. Later in life, it may be logistically necessary to relocate the minipig within the barrier facility, including introducing it to a new group. The social hierarchy among minipigs is significant, and the process is undoubtedly stressful for the individual minipig, even if it is only being moved to a neighbouring pen.

Now imagine how drastic a change in a minipig's life it is to be removed from the barrier, be transported in a cage for hours and then be introduced to a new facility where everything is foreign: pen, bedding, food, pen mates, neighbours, staff, smells, sounds, etc.
These changes are likely to be perceived as possible stressors which can influence the general condition of the individual minipig. Stress is generally considered undesirable.

Yet unpredictability can also cause stress, whereas total predictability can result in boredom. So, to strike a balance, consider the major changes mentioned above as stressors and the minor changes, like the introduction of new toys and "a little walk in the corridor", as beneficial enrichment for the animal.

## Acclimatisation period

Some say that minipigs should be left alone without any disturbance to upset them.
We believe that the purpose of the acclimatisation period is to socialise the animals, gradually accustom them to the experimental conditions and, generally, to minimise stress for animals and staff alike.

As mentioned below, most physiological parameters need a few days in which to stabilise.

Possible infectious diseases or diarrhoea due to the new environment will typically show up during the first week and are incompatible with the experimental setup. It is also during the first week that preventive medication and vaccination can be prescribed. Some routinely give yoghurt or similar lactobacillus products in the first days after arrival to stabilise the intestinal microflora.

For these reasons, it makes sense to recommend at least one or two weeks of acclimatisation.

But the mental state of the minipigs is unlikely to stabilise within a week, especially if you expect them to cooperate in handling procedures and engage in close human contact.
In the following, various aspects of organising the acclimatisation period to maximise the benefits for both animal and staff are considered.

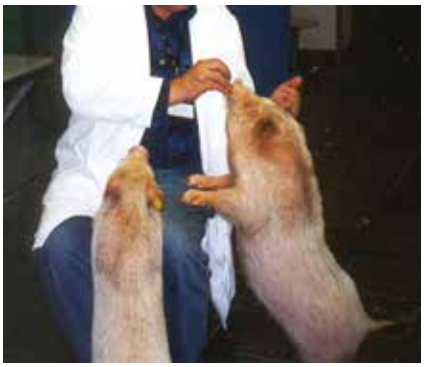
## General minipig behaviour

At Ellegaard we select only calm sows to be breeders, as we know that a sow's temperament has an impact on its offspring.

In addition, we conduct daily socialisation of weaned pigs and new breeders to accustom them to human contact.

Minipigs are clever, curious and social animals

In general, minipigs are clever, curious and social animals, not only as regards their pen mates but human beings as well. Why then do some minipigs exhibit the speciesspecific behaviour of screaming when they are picked up and handled?



Some theories claim that screaming is a congenital, lifesaving mechanism for piglets to prevent them from being crushed to death by the mother sow.

Regardless of whether this is true, the volume of the screaming when being handled is rarely caused by pain or discomfort but seems more like a communication of "Don't fuss around with me!"

With the right technique (and treats), however, it is possible to gain the minipig's confidence along the lines of "If you scratch my back, I'll scratch yours!"

Furthermore, as minipigs are easily startled, any approach to and handling of them should be done quietly and calmly.

## From transportation to new facilities

Like any other species, it is well known that transporting minipigs induces changes in their cardiovascular, immune, endocrine and central nervous systems. These parameters normally return to baseline within a couple of days.
Ellegaard has considered every aspect of the process to make it as pleasant and gentle as possible as regards the physical, physiological and psychological conditions.

Unloading should be as gentle as possible

After arriving at the destination, the unloading should be as gentle as possible by avoiding unnecessary bumps, shocks and stressful conditions.
Unfortunately the lid of the transport cage is difficult to remove, which poses quite a challenge to getting the pig out of the box.

The ideal situation is to place the open cage inside or outside the pen and wait for the pig to leave on its own volition. Some customers have implemented this routine, as it gives the minipig the opportunity to leave the cage when it feels safe.

Other customers report that there is no time for this procedure, as the cages need to be returned to the vehicle as soon as possible. If so, you might as well get it over with as quickly as possible by lifting the minipig out of the cage and saving your energy and efforts for other activities that may be more beneficial to overall minipig socialisation.

Each minipig should be given a clinical examination after arrival to make sure that it has not been injured during transport and to identify each animal.

Here, too, some customers perform this examination right after unloading, before the minipig enters its new pen. It is also possible to wait a few hours or postpone this until the next day when things have settled down a bit, but it is generally hard to say which is best. In any case, it is often beneficial to consider this – and any procedure for that matter – from a welfare perspective and, if necessary, change it.

A little trick: reuse the wood shavings from the transport cage by spreading them on the floor in the pen. It may cover up the new smells a little. Furthermore, offering food in the shavings may distract the minipigs' attention from the new environment.

## Housing

Being social animals, minipigs prefer being housed in groups. This trait should be seriously considered when planning a study.

If individual housing is necessary for experimental reasons, each minipig should have tactile, olfactory or at least visual contact with other minipigs

Be aware of the fact that individually housed minipigs may need more human contact than those in groups.

Some tend to think that male minipigs cannot be housed in groups. In fact, they can live in groups until they are about one year old. For female minipigs, it is important to monitor the wellbeing of each individual in the group and take action if any animal is not thriving.

Minipigs should be provided with some kind of bedding or rooting material. At Ellegaard, they are used to chopped straw, but any other material which satisfies their natural urge to root will be appreciated.

If possible, offer feed on the floor. Scattering it in the bedding material makes it even more interesting to search for.

If you have planned to house the minipigs in groups, please indicate this when you place your order with Ellegaard. We will then do our best to select minipigs from the same pen and, if possible, transport them in the same transport cage.

Sometimes it may be necessary to form new groups of minipigs or introduce a single animal to an already stable group. This will interfere with the social hierarchy within the group up to now and sometimes result in fighting, thus requiring extra attention by the staff.

The best way is to mix them in a “neutral” area, i.e. in a pen of considerable size which is new to all the minipigs involved.
Offer lots of food scattered in the bedding.
Observe them closely during the first one or two hours. If the hierarchy has not been established during the first 24 hours, it is not likely to happen.

You can help the minipigs to adjust to the new environments by placing the drinking devices in the defecation area and infrared light at the opposite end of the pen to mark the resting and sleeping area.

Pen design will not be included in this text. Instead, we suggest the corridor as a perfect place for mini-pigs to stay!
As a general rule, one minipig should have the run of the corridor at any time during daytime hours at least. This minipig will run up and down saying hello to all its mates. This routine is the best social enrichment you can offer minipigs! It breaks up the monotony during the day for the “walkabout” pig, as well as for all the other pigs in the room. A little scratch from the staff when they pass by is an extra bonus.

**Remember:**
the best environmental enrichment for a minipig is to be together or have physical contact with other minipigs! Enrichment such as toys and other things to play with is a poor substitute for contact with conspecifics

Every contact
with a minipig
is a training situation

## Socialising and training minipigs

As mentioned above, it is important to use the acclimatisation period efficiently for socialisation and general training.

Some tend to think that training is a question of whether to implement clicker training, which is not true.

As they are smart creatures, minipigs will memorise good and bad experiences alike. Unlike dogs, minipigs are not quite as forgiving. Therefore it is important to do your best to make a good impression from the outset and engender trust.

It may be a good idea to formalise and document the socialisation and training process. There are numerous advantages of doing this: it reminds you that it is important, it visualises the process, it can be organised and scheduled, you can follow the progress for each individual animal, etc.

## Where should socialisation and training take place?

One view is that the pen is to a minipig like a basket is to a dog: a sanctuary where it feels safe. Therefore, every procedure should be performed outside the pen.
The other view is that any change in environment is undesirable. Therefore, as many procedures as possible should be performed inside or near the pen.

The truth is somewhere in between, depending on the kind of procedure to be performed. For example, oral gavage is considered very stressful and should not be performed in the presence of other minipigs.
General socialisation should take place in the pen, whereas training in the sling or training of dermal application could easily be done in the corridor or somewhere near the pen.

*An example of a socialisation programme is provided in the Rethink report, www.rethink-eu.dk:*

**Step 1 (Day 0):** The new arrivals are left for 2–5 hours to settle in; the technicians have minimal interaction with the new arrivals as they tend to be nervous.

**Step 2 (Days 0–5):** The technicians sit in the pen with a food reward (diet/apples) and wait until the pigs come to them for the reward. Food reward must be used with caution. Once the pigs take their reward, the technicians can start to touch the pigs to accustom them to physical contact with humans.

**Step 3 (Days 2–8):** The pigs are trained to be handled by picking them up; this is done in the pen a couple of times a day – this accustoms the pig to human interaction and to being touched and handled. Each time the pig is handled it should be given a verbal and/or patting reward, and can, after a full procedure, be given a food reward.
The animals are trained to walk up and down the corridors in the pig bays and to step onto a balance in the procedure room. A verbal and/or patting reward should be given for performing the required behaviour; alternatively a food reward can be given.

**Step 4:** Training for blood sampling can be started as soon as the pig is comfortable with being picked up and carried. The pig is walked to the procedure room and placed on its back on the table using the 'V' shaped restrainer to mimic restraining for bleeds. The pigs receive a lot of positive physical contact and verbal praise. A food reward is given each time after having been to the procedure room (if the research protocol allows).

*The table below is an example of record-keeping for each minipig and may inspire you to prepare your own chart.*

### Socialising Minipigs

Room: Week/Year: Responsible:

| Pig no. | Behaviour | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Touching | | | Eats out of hand | On arm | | | In sling | | | In box or on scale | | | Other | | |
| | * | ** | *** | ✓ | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| 1 | | | | | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | | | | | |
| 5 | | | | | | | | | | | | | | | | |
| 6 | | | | | | | | | | | | | | | | |
| 7 | | | | | | | | | | | | | | | | |
| 8 | | | | | | | | | | | | | | | | |
| 9 | | | | | | | | | | | | | | | | |
| 10 | | | | | | | | | | | | | | | | |

| | |
|---|---|
| * | shy, moves away when touched, does not seek contact |
| ** | seeks contact, accepts some touching |
| *** | actively looks for contact and likes to be touched and scratched |
| | |
| 1 | fights all the time and tries to get out of the situation |
| 2 | some struggling, but relaxes after a while |
| 3 | calm and relaxed during the whole procedure |

It can be discussed whether training of experimental procedures, e.g. habituation to the sling, should be performed inside or outside the pen.

The main point here is that, rather than doing things routinely, both minipig and staff can reduce stress by considering the best conditions for each training session or experimental procedure

Ellegaard's Newsletter no. 33 (www.minipigs.dk) presented a calculation of how the clicker training of minipigs can save time and money. There is reason to believe that the same calculation could be done regarding the economic aspect of the overall acclimatisation, socialisation and general prestudy training.

After reading this text, we hope you have become more aware of the importance of the acclimatisation period. And hopefully you will be inspired to implement new socialisation procedures (and schedule time for them!) to benefit from them in the long term.

### Good socialisation and training save time and money!

As a breeder of the Göttingen Minipig, it is of utmost importance to us that everyone who comes in contact with the minipig is well informed about correct housing, husbandry and handling. Even so, many customers tell us that it is difficult to find the time for socialisation and training – and time is money.

Please feel welcome to contact Ellegaard Göttingen Minipigs if you would like further details or wish to schedule a visit to your premises (handling@minipigs.dk). We can help you to kickstart the process or move forward with any procedures you have already implemented.

# CYP substrate specificity in the liver microsom

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## Abbreviations:

EROD: ethoxyresorufin, COU: coumarin, PROD: pentoxyresorufin, OME: omeprazole, TOL: tolbutamide, PAC: paclitaxel, BUF: bufuralol, DEX: dextromethorphan, CLX: chlorzoxazone, BUP: bupropion, TESTO: testosterone, NIF: nifedipine.

## Abstract

New drugs and chemicals have to undergo pharmacological and toxicological testing, and for the in vivo part of this testing, different species have to be chosen. The pig is becoming the non-rodent species of preference. As metabolism may play a key role in pharmacological and toxicological properties, it is important to know the metabolic capacity of the test species compared to human capacity to enable extrapolation. In humans, an array of marker substrates for the different CYP isoenzymes has been identified. However, it is not known how specific these marker substrates are to the different porcine CYP isoenzymes. The aim of this review, therefore, is to analyse the specificity of 12 different human marker substrates in microsomes from both minipigs and conventional pigs. The metabolism rates for the different substrates are correlated with the apoprotein level of the different enzymes. However, the picture is not that clear for minipigs or conventional pigs alone, probably due to the limited number of animals and variations within the groups. However, the results of the analysis for all pigs revealed that ethoxyresorufin, coumarin, bupropion (0.5 mM), bufuralol, omeprazole, and testosterone or nifedipine can be used as marker substrates for CYP1A, CYP2A, CYP2B1/2, CYP2B6, CYP2C9, and CYP3A respectively.

## Introduction

Drugs and new chemicals have to be toxicologically tested to characterise their toxicological properties. Furthermore, the pharmacological properties, such as pharmacokinetics and pharmacodynamics, have to be determined for new drugs. These studies are carried out in vitro and/or in vivo using various test species. The search for an ideal model for estimating these parameters in humans is ongoing, as rodent models were generally shown not to be ideal, due to their metabolic properties, i.e. activity of the cytochrome P450 complex does not correspond well with human activities.[1,2] As metabolism may be crucial with respect to toxic effects and pharmacokinetics, it is important that the species used is well-characterised with respect to metabolic activity to enable better extrapolation of results from laboratory animals to humans. The phase I liver metabolism is mainly catalysed by the cytochrome P450 complex. The cytochrome P450 complex consists of an array of isoenzymes divided into three main families: CYP1, CYP2 and CYP3. The activity of these isoenzymes has been studied extensively in human liver microsomes, and specific marker substrates for measuring their activity have been identified (Table 1).[3-5] As the pig, and particularly the minipig, is becoming a species of choice due to its physiology, it is important to obtain more knowledge of porcine-liver CYP activities. These activities have mainly been studied in minipigs, but the activity of some of these isoenzymes has also been measured in conventional pigs, as reviewed by Skaanild.[6] However, substrate specificity in pigs has not been determined for many of the human-marker substrates. The objective of this review is to determine how specific these reactions are to the different isoenzymes and then compare this to human specificity. Substrate specificity is determined by correlating previously published data on metabolism activity and apoprotein level for different substrates and isoenzymes.

## Materials and methods

### Animals

In all studies, 8 sexually mature Göttingen minipigs (4 females and 4 males aged 4 months) and 12 conventional pigs (4 females, 4 males and 4 castrates aged 3.5 months) previously described were used.[7]

### Chemicals

All antibodies and 6-alpha hydroxypaclitaxel were obtained from Gentest (Massachusetts, USA), hydroxy bupropion from BD Biosciences (Bedford, USA), and the 5-hydroxy metabolite of omeprazole was a gift from AstraZeneca. All other chemicals were of analytical grade and were obtained from either GE Healthcare or Sigma (St Louis, USA).

Isolation of liver microsomes

Microsomes were isolated according to Olsen et al.[8] Briefly, the liver was homogenised in a 50 mM Tris-HCl buffer containing 0.25 M sucrose and 1 mM EDTA. The homogenate was centrifuged and the supernatant was transferred to new tubes and centrifuged once more at 105,000 x g at 40C for 60 minutes. The pellet containing the microsomes was homogenised in a storage buffer and frozen in liquid nitrogen.

Microsomal protein concentration was determined using a modified Lowry method.[9]

### Total P450 activity

The microsomal solution was mixed with a 3-ml glycerin buffer (20% glycerin, 80% phosphate buffer, pH 7.4) and kept in ice. One sample was mixed with 50 ul sodium dithionite and aerated with CO (3–5 bubbles per second) for 4 minutes. Another sample was treated in the same way but without CO aeration. The samples were scanned spectrophotometrically from 400–500 nm and the difference spectrum was used to calculate the P450 concentration according to Omuro & Sato, 1964.[10]

# es of minipigs and conventional pigs

**Enzyme assay**

The microsomal mixture for all assays consisted of a buffer containing 32 mM K-phosphate pH 7.46, 2.5 mM MgCl2, 15 mM glucose-6-phosphate, 10 U glucose-6-P-dehydrogenase/ml, 1.1 mM NADP and 0.91 mg microsomal protein in a total volume of 1.075 ml. The mixture was pre-incubated for 5 minutes at 370C before the test substrate was added. Most of the reactions were stopped by adding 1 vol methanol and centrifuged at 8,500 x g for 5 minutes.

The metabolism measurement of the different substrates has been described in previous publications.[7, 11–13]

**Immunoblotting**

Microsomes isolated from the pig liver were used for blotting. In short, microsomal protein was separated by PAGE SDS gel electrophoresis and blotted to Hybond-ECL nitro-cellulose membranes. Further, the hybridisation conditions have been described in previous publications.[7, 11–13] Antibody binding was detected by chemiluminescence, using a biotinylated secondary antibody followed by a streptavidin-horseradish peroxide conjugate. After development, the blots were exposed to HyperfilmECL and the signal was quantified using QuantiScan software from Biosoft (Cambridge, UK).

## Results

The total P450 activities measured in minipig microsomes were significantly higher (p = 0.002) than in microsomes from conventional pigs, 809 pmol/mg ± 164 compared to 455 pmol/mg ± 77. Furthermore, the metabolism of the different human-marker substrates was analysed and the results are listed in Table 1, expressed as mean ±SD. It can be seen the standard deviations are quite high for most of the substrates. The analysis revealed that the metabolism rates in minipigs were significantly higher than in conventional pigs with respect to the following substrates: paclitaxel (PAC), bufuralol (BUF), dextromethorphan (DEX), bupropion (BUP), testosterone (TEST) and nifedipine (NIF) (Table 1). No differences were seen for the rest of the test substrates. The metabolism in pigs was comparable to activity in humans regarding the metabolism of ethoxyresorufin (EROD), coumarin (COU), pentoxyresorufin (PROD), tolbutamide (TOL), and bupropion (BUP) (5 mM). The capacity to metabolise PAC, chlorzoxazone (CLX), and TEST was lower in pig than in humans, whereas the opposite was seen as regards omeprazole (OME) and DEX.

The apoprotein level of the different isoenzymes was estimated using anti-human antibodies, and as the affinity of these antibodies against the porcine enzymes is not known, the results are given in units/mg protein. The apoprotein levels were correlated with the metabolism rates measured for the different substrates. The results of the correlation analysis for the minipigs, conventional pigs, and all pigs are shown in Tables 2, 3 and 4 respectively.

The CYP1A-like porcine enzyme level correlates well with the metabolism rates of ethoxyresorufin (EROD), coumarin (COU) and pentoxyresorufin (PROD) in minipigs. In conventional pigs, however, no significant correlation was found. As regards the CYP2A-like enzyme, it correlates well with the COU metabolism in both strains, but also with ethoxyresorufin (EROD) and PROD in minipigs and chlorzoxazone (CLX) in conventional pigs. The CYP2B(1/2)-like enzyme level only correlates significantly with the BUP (0.5 mM) in minipigs, whereas the CYP2B6-like protein correlates with COU, CLX and BUF metabolism in minipigs. Correlations were found between the CYP2C8 protein level EROD, CLX and BUF, but only in minipigs. The CYP2C9-like protein concentration correlated with omeprazole (OME) metabolism in both porcine strains, but also with tolbutamide (TOL) and BUP in conventional pigs. No significant correlation between the CYP2C19-like protein level correlated and the metabolism rates for the test substrates was found in conventional pigs. However, the apoprotein level correlates with EROD, COU, and CLX metabolism rates in minipigs. Regarding the CYP2E1-like enzyme, none of the substrate reactions correlated with the CYP2E apoprotein level. Finally, the CYP3A4-like protein correlated with CLX metabolism in both strains and with the metabolism of NIF and TESTO in conventional pigs.

When considering the results for all 20 pigs, significant correlations are seen between CYP1A and the metabolism rates of EROD and PROD; CYP2A and the metabolism of COU and CLX; CYP2B1/2 and BUP metabolism; CYP2B6 and the metabolism of BUP 5 mM, BUF, DEX, NIF, and TESTO; and CYP2C9 and the metabolism of PAC, OME and TOL. Also the CYP3A level correlates with the metabolism of NIF and TESTO.

## Discussion

The metabolic capacity of microsomes isolated from minipigs and conventional pigs was reviewed, looking at total P450 activity and the metabolic rates of several human marker substrates for the individual isoenzymes. Differences in total P450 content were seen, with minipigs exhibiting the highest activity. These differences were also seen in humans, as the content in Caucasian subjects was higher than in Japanese (0.43 nmol/mg protein compared to 0.26 nmol/mg protein.[3] These values also show that porcine values are comparable to human values, as the values found for the conventional pig were the same as for Caucasians. The activity of some individual isoenzymes was also significantly higher in minipigs compared to conventional pigs, which could be expected as total P-450 content is higher. Significant differences in human races were not seen, but Hispanics tend to have higher activities of CYP2A6, CYP2B6 and CYP2C8.[14]

The activity of individual isoenzymes was analysed using 12 different test substrates. As regards minipigs, several marker-substrate reactions correlated to one isoenzyme or vice versa, whereas fewer correlations were found for conventional pigs. This could be due to either low specificity reactions or the number of animals in each group. Variations in metabolism rates could also be a reason if the spread in metabolism rates was small. The latter seems to be the explanation, as a much clearer picture evolved when the correlation analysis was made using all 20 pigs. This gave a higher number of animals and especially for some reactions, where a significant difference in activity rates was seen, there was a much wider interval between high and low metabolism rates. For the EROD assay, the marker assay for human CYP1A2, the correlation analysis showed that this metabolism correlated with several isoenzymes in minipigs, but with none in conventional pigs. When looking at all 20 pigs, it is clear that this substrate is specific for CYP1A, as the highest correlation coefficient was found between CYP1A and EROD as seen for humans.[3] The capacity also corresponds to that measured in human microsomes.[14] Coumarin, a marker substrate for human CYP2A6, could also be metabolised by porcine microsomes, and good correlations were found between the metabolism rate and the protein level for CYP2A in both strains. However, the protein level in minipigs also correlates to EROD and PROD metabolism, and for conventional pigs, a correlation to the metabolism of CLX was seen. This indicates that CYP2A in pigs is involved in more reactions than in humans, which is in agreement with recent results showing that CYP2A is the most abundant CYP subfamily in pigs.[17] When looking at all 20 pigs, correlations with both coumarin and CLX were found. This indicates that COU is the best substrate for measuring CYP2A activity in pigs, as no other CYP is involved in COU metabolism because no correlations were seen for any other CYP apoprotein level. However, CLX is not a specific substrate for CYP2E, as the hydroxylation activity of coumarin correlates better with the CYP2A protein level than with CYP2E. This is in accordance with previous studies.[12] In humans, the PROD assay is used to estimate CYP2B6 activity, but no correlation was found between PROD metabolism and the CYP2B protein level determined using an anti-rat CYP2B 1/2 and an anti–human CYP2B6 antibody. This indicates that the PROD is probably not a good marker substrate in pigs. At the low substrate concentration, the minipig CYP2B1/2 can catalyse the metabolism of BUP, another human CYP2B6 substrate. The correlation for all pigs revealed that BUP (0.5 mM) correlated with CYP2B1/2 and BUP (5 mM) correlated with CYP2B6. However, a better correlation was found when using BUF and DEX, two human CYP2D substrates, indicating concordance with previous studies that these substrates can be used as marker substrates for CYP2B in pigs, as no correlations were found with any other apoprotein value. Porcine CYP2B (6) also correlates with the metabolism of two CYP3A test substrates, NIF and TESTO, which was also seen for human microsomes.[3] The correlation coefficient for these two substrates was the same in human and porcine microsomes when correlated to CYP2B (6) and CYP3A apoprotein levels. However, these substrates are more specific in conventional pigs and in all pigs when it comes to measuring the activity of CYP3A. One human CYP2E substrate CLX was also tested. CLX is not a very specific substrate as the metabolism rate of this substrate correlates to several different CYP apoprotein levels. The best correlation was with CYP2A in minipigs and all pigs, indicating that this substrate should not be used for estimating the activity of CYP2E, which corresponds to previously published results,[12] Furthermore, a recent study using cDNA for the different CYP isoenzymes also shows that CYP2A19, CYP2E1 and CYP2C333v4 can metabolise CLX.[16] In human microsomes, the CYP2C isoenzyme activities of C8, C9 and C19 are estimated using PAC, TOL and OME respectively. The porcine metabolism rates of these substrates correlate with the protein level estimated using the anti-human CYP2C9 antibody, indicating that these substrates may all be used for determining the CYP2C9 activity in porcine microsomes. OME is the best substrate, as the metabolism of OME gave the highest correlation coefficient for both minipigs and all pigs. Apparently, none of the substrates can be used for measuring the activities of CYP2C8 and CYP2C19, which makes it necessary to evaluate and test more CYP2C human substrates, as both immunoblotting and mRNA results indicate that these enzymes exist in pigs.[13] The best substrates for measuring CYP3A activity in conventional pigs are nifedipine and testosterone giving the best correlation. For minipigs, however, CLX seems to be the best, as this substrate gives the best correlation with the CYP3A apoprotein level. When all 20 pigs are analysed together, the best substrates are nifedipine and testosterone. In conclusion, the picture is not that clear for minipigs or conventional pigs when considering the individual strains, probably due to the number of animals and the variations in the activities. However, when grouping all pigs together, it can be concluded that EROD, COU, BUP (0.5 mM), BUF, OME, and TESTO or NIF can be used as marker substrates for CYP1A, CYP2A, CYP2B1/2, CYP2B6, CYP2C9, CYP2E and CYP3A respectively.

**Table 1**

This table shows the in vitro capacity of humans, minipigs, and conventional pigs to metabolise 12 different human CYP marker substrates.

| Substrate | Human CYP | Reaction | Minipig activity | Con. pig activity | Human activity |
|---|---|---|---|---|---|
| Ethoxyresorufin | CYP1A2 | O-deethylation | 51.3 ± 36.6 | 39.04 ± 7.06 | 49.9 ± 38* |
| Coumarin | CYP2A6 | 7-hydroxylation | 330 ± 130 | 200 ± 108 | 939 ± 910*/ 298 ± 146** |
| Pentoxyresorufin | CYP2B6 | dealkylation | 1.02 ± 0.27 | 1.02 ± 0.47 | 0.29 ± 0.44** |
| Omeprazole | CYP2C9 | 4-hydroxylation | 210.2 ± 97.6 | 176.5 ± 62.4 | 46 ± 33** |
| Tolbutamide | CYP2C19 | 4-hydroxylation | 247.3 ± 208.6 | 144.6 ± 69.77 | 138 ± 48** |
| Paclitaxel | CYP2C8 | 6- hydroxylation | *2.48 ± 0.97* | *1.46 ± 0.66* | 348 ± 340* |
| Bufuralol | CYP2D6 | 1-hydroxylation | *705 ± 320* | *320 ± 90* | |
| Dextromethorphan | CYP2D6 | O-demethylation | *4420 ± 1800* | *2370 ± 600* | 299 ± 202* |
| Chlorzoxazone | CYP2E1 | 6-hydroxylation | 3.54 ± 2.54 | 4.1 ± 0.6 | 1950 ± 980* |
| Bupropion (0.5 mM) | CYP2E1 | hydroxylation | *295.77 ± 143.9* | *81.22 ± 32.77* | |
| Bupropion (5 mM) | CYP2B6 | hydroxylation | *214.12 ± 86.67* | *87.67± 25.7* | 131 ± 120** |
| Testosterone | CYP3A4 | 6β-hydroxylation | *920 ± 190* | *490 ± 170* | 3630 ± 3470* |
| Nifedipine | CYP3A4 | N-oxidation | *3270± 1060* | *1140 ± 1360* | |

Activity units pmol/min/mg prot

*Italic:* Significant differences between minipig and conventional pigs (p< 0.5)

* Parkinson et al. (2004), ** Turpeinen et al. (2007).

**Table 2. Isoenzyme levels estimated using Western blotting**

Correlations (r) between enzyme activity and apoprotein levels of different CYPs for 8 minipigs (* p ≤ 0.05).

| Substrates | CYP1A | CYP2A | CYP2B1/2 | CYP2B | CYP2C8 | CYP2C9 | CYP2C19 | CYP2E | CYP3A |
|---|---|---|---|---|---|---|---|---|---|
| EROD | 0.88 * | 0.92 * | (-)0.88 * | 0,52 | 0.9 * | 0,44 | 0.91 * | 0,52 | 0,58 |
| Coumarin | 0.97 * | 0.82 * | (-)0.7 * | 0.7 * | (-)0.9 * | (-)0.4 | 0.94 * | 0,63 | 0,67 |
| PROD | 0.71 * | 0.71 * | (-)0.7 * | 0.6 * | 0,5 | (-)0.2 | 0,51 | 0,44 | 0,4 |
| BUP 0.5mM | (-)0.9 * | (-)0.8 | 0.7 * | (-)0.4 | (-)0.9 | 0,65 | (-)0.89 * | (-)0.6 | (-)0.4 |
| BUP 5 mM | 0,4 | 0,5 | (-)0.2 | 0,4 | 0,2 | 0,4 | 0,17 | (-)0.1 | 0,1 |
| CLX | 0.9 * | 0,66 | (-)0.6 | 0.76 * | 0.7 * | (-)0.4 | 0.83 * | 0,6 | 0.75 * |
| Paclitaxel | (-)0.44 | (-)0.3 | 0,2 | (-)0.2 | (-)0.3 | 0,6 | (-)0.37 | (-)0.3 | 0 |
| Omeprazole | 0,2 | (-)0.1 | 0,46 | 0,11 | (-)0.3 | 0.87 * | 0,24 | (-)0.2 | 0,1 |
| Tolbutamide | (-)0.5 | (-)0.4 | 0,27 | (-)0.2 | (-)0.55 | 0,52 | 0,56 | (-)0.1 | (-)0.2 |
| Bufuralol | 0,54 | 0,1 | 0 | 0.88 * | 0,01 | 0,27 | 0,29 | 0,62 | 0,47 |
| Dextro. | 0,28 | (-)0.2 | 0,23 | 0,62 | 0,03 | 0,1 | 0,09 | 0,48 | 0,38 |
| Nifedipine | 0,53 | 0,65 | (-)0.5 | 0,5 | 0,43 | 0,23 | 0,49 | 0,2 | 0,6 |
| Testo. | 0,42 | 0,57 | (-)0.4 | 0,4 | 0,47 | 0,24 | 0,52 | 0,1 | 0,6 |

**Table 3. Isoenzyme levels estimated using Western blotting**

Correlations (r) between enzyme activity and apoprotein levels of different CYPs for 12 conventional pigs (*p ≤ 0.05).

| Substrates | CYP1A | CYP2A | CYP2B1/2 | CYP2B | CYP2C8 | CYP2C9 | CYP2C19 | CYP2E | CYP3A |
|---|---|---|---|---|---|---|---|---|---|
| EROD | 0,22 | (-)0.24 | 0,33 | 0,39 | 0,19 | 0,24 | 0,1 | (-)0.3 | 0,04 |
| Coumarin | (-)0.1 | 0.7 * | 0,27 | 0,55 | 0,01 | (-)0.5 | 0,1 | (-)0.3 | 0,7 |
| PROD | 0,25 | (-)0.44 | (-)0.1 | (-)0.2 | (-)0.1 | 0 | (-)0.2 | 0,3 | (-)0.2 |
| BUP 0.5mM | (-)0.1 | 0,18 | 0,03 | 0,15 | (-)0.2 | 0.72 * | (-)0.2 | 0,23 | (-)0.2 |
| BUP 5 mM | (-)0.1 | 0,02 | 0,5 | 0,3 | (-)0.04 | 0,45 | (-)0.3 | 0,5 | 0,01 |
| CLX | (-)0.1 | 0.61 * | 0,2 | 0,1 | 0 | (-)0.5 | 0,1 | (-)0.3 | 0.57 * |
| Paclitaxel | (-)0.5 | 0,1 | 0,16 | 0 | (-)0.6 | 0,4 | (-)0.4 | (-)0.2 | (-)0.1 |
| Omeprazole | (-)0.4 | (-)0.4 | 0,34 | 0,25 | (-)0.6 | 0.55 * | (-)0.5 | 0,5 | (-)0.1 |
| Tolbutamide | (-)0.5 | (-)0.5 | 0,22 | 0 | (-)0.4 | 0.66 * | (-)0.3 | 0,25 | (-)0.4 |
| Bufuralol | (-)0.23 | (-)0.3 | 0,43 | 0,26 | 0,16 | 0,24 | 0,12 | 0,24 | 0,1 |
| Dextro. | 0,35 | 0 | 0,33 | 0,07 | 0,17 | (-)0.3 | 0,31 | (-)0.1 | 0,3 |
| Nifedipine | 0,05 | 0,4 | 0,28 | 0,21 | (-)0.45 | (-)0.43 | (-)0.35 | 0,22 | 0.7 * |
| Testo. | 0,1 | 0,4 | 0,21 | 0,24 | (-)0.3 | (-)0.45 | (-)0.27 | 0,24 | 0.7 * |

**Table 4. Isoenzyme levels estimated using Western blotting.**

Correlations (r) between enzyme activity and apoprotein levels of different CYPs for all 20 pigs (*p ≤ 0.05).

| Substrates | CYP1A | CYP2A | CYP2B1/2 | CYP2B | CYP2C8 | CYP2C9 | CYP2C19 | CYP2E | CYP3A |
|---|---|---|---|---|---|---|---|---|---|
| EROD | 0.85 * | 0.67 * | (-)0.2 | 0,51 | 0,5 | (-)0.1 | 0.75 * | 0,35 | 0,43 |
| Coumarin | 0,45 | 0.75 * | (-)0.2 | 0,01 | 0,14 | (-)0.54 | 0,43 | 0,23 | 0,23 |
| PROD | 0.56 * | 0,34 | (-)0.3 | 0,24 | 0,1 | (-)0.1 | 0,27 | 0,36 | 0,1 |
| BUP 0.5mM | (-)0.2 | (-)0.5 | 0.54 * | 0.45 * | 0 | 0.7 * | (-)0.3 | (-)0.3 | 0,33 |
| BUP 5 mM | 0,44 | 0,2 | 0,4 | 0.74 * | 0,2 | 0.6 * | 0,23 | 0 | 0,5 |
| CLX | 0.61 * | 0.65 * | (-)0.2 | 0.3 * | 0,2 | (-)0.5 | 0.54 * | 0,23 | 0,4 |
| Pacllitaxel | (-)0.2 | (-)0.2 | 0,4 | 0,4 | (-)0.2 | 0.6 * | (-)0.2 | (-)0.25 | 0,3 |
| Omeprazole | (-)0.1 | (-)0.24 | 0,4 | 0,3 | (-)0.3 | 0.73 * | (-)0.3 | 0,1 | 0,1 |
| Tolbutamide | 0,32 | (-)0.44 | 0,3 | 0,2 | (-)0.2 | 0.6 * | (-)0.4 | 0 | 0 |
| Bufuralol | 0,53 | (-)0.1 | 0,4 | 0.9 * | 0,4 | 0,34 | 0,35 | 0,24 | 0.6 * |
| Dextro. | 0,36 | (-)0.2 | 0,43 | 0.76 * | 0,26 | 0,29 | 0,26 | 0,14 | 0.6 * |
| Nifedipine | 0,52 | 0,2 | 0,32 | 0.81 * | 0,28 | 0,41 | 0,36 | 0,01 | 0.75 * |
| Testo. | 0,43 | 0,16 | 0,37 | 0.74 * | 0,23 | 0,32 | 0,3 | 0 | 0.8 * |

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# Coronary and peripheral arteries of eight-month-old Göttingen Minipigs are suitable for transcatheter cardiovascular procedures: The results of quantitative vascular angiography

## Introduction

The dynamic development of and high demand put on quality and performance measures of medical devices in interventional cardiovascular medicine require careful investigation in a pre-clinical setting.

Available in-stent restenosis animal models enable evaluation of neointimal formation and tissue effects in response to angioplasty procedures. Minipigs are similar to humans regarding cardiovascular anatomy, heart rate and blood pressure, and according to Recommendations for Preclinical Evaluation of Drug-Eluting Stents in Preclinical Studies, the porcine model is recommended as the animal model of choice. Recently, the US Food and Drug Administration recommended a longer follow-up (up to 6 months) for most preclinical stent studies, which creates a problem of significant weight increase in domestic swine during this period. This could be resolved by using the minipig model.

## Aim, methods and results

Therefore, in our study, we aimed to analyse the diameter of the main arteries of the Göttingen Minipig. The entire study was conducted at the Centre for Cardiovascular Research and Development (CCRD) of the American Heart of Poland Inc. and was approved by the Local Animal Research Ethics Committee. Five Göttingen Minipigs weighing 30–33 kg, with an average age of 8.5 months, were included in the study. Intravascular access was obtained via femoral artery using a standard Seldinger technique. To assess the vessel size, quantitative vascular analysis and coronary angiography (QVA/QCA) were performed utilising the CMS-QVA/QCA software (Medis), and all angiograms were recorded in DICOM format. Two contralateral projections were chosen for analysis. The following arteries were examined: left anterior descending coronary artery (LAD), left circumflex (LCx) coronary artery, right coronary artery (RCA), common carotid artery, renal arteries, common iliac arteries and femoral arteries. Angiographic results are summarised in Table 1. The reference diameter (RD) of coronary arteries is approximately 2.7 mm, while common carotid and common iliac arteries are about 50% wider, which is comparable to adult patients.

## Conclusions

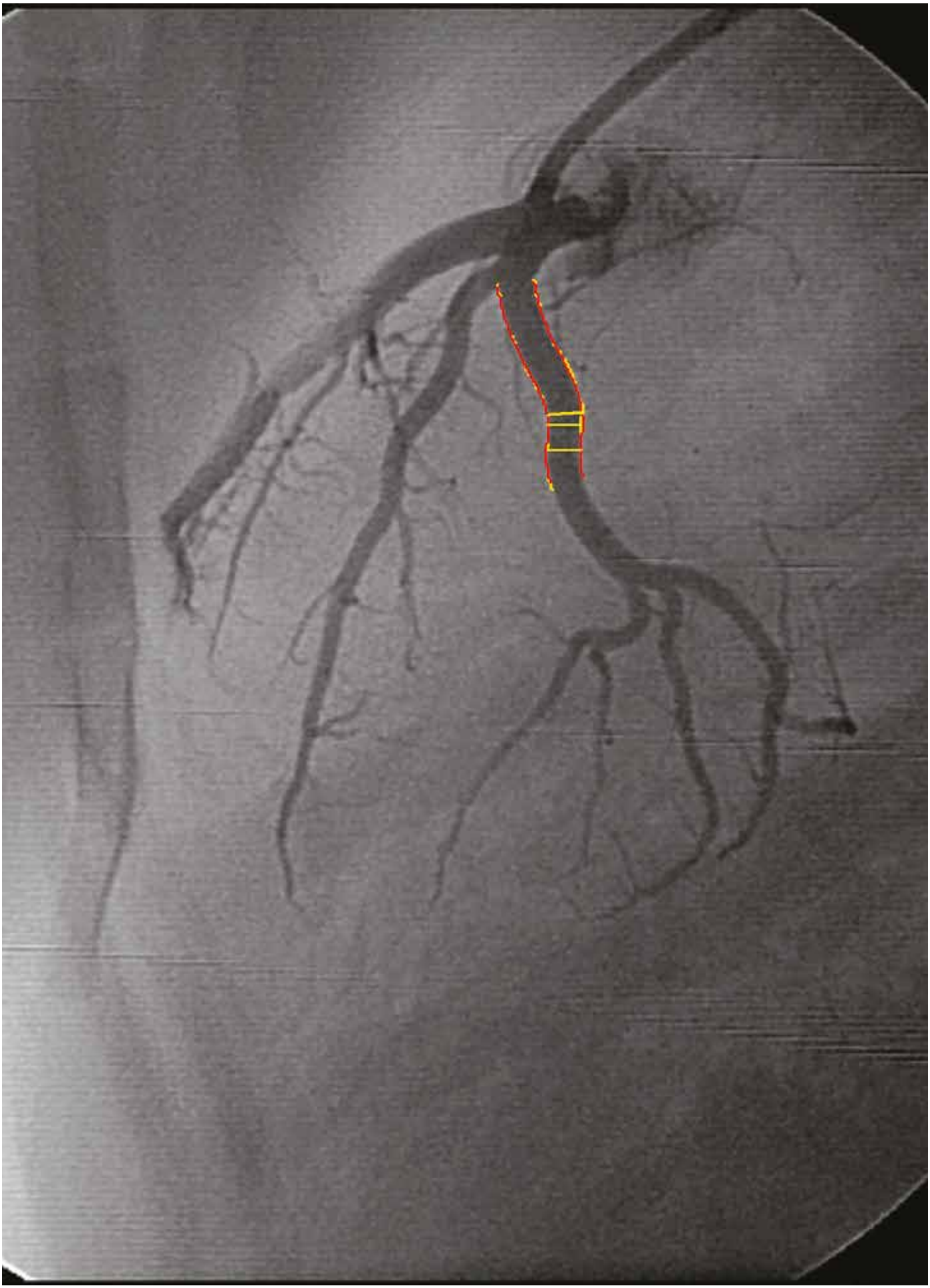
The angiographic results showed us that the size of the arteries in Göttingen Minipigs is perfect for experimental evaluation of transcatheter cardiovascular devices: i.e. stents, drug coated balloons, balloons, wires, catheters and intravascular imaging devices. Furthermore, the minipigs maintain a low weight during follow up which makes the Göttingen Minipig a very suitable model for studies of longer duration.

The CCRD was launched in May 2004 and offers consultancy services for companies planning to introduce new cardiovascular devices, treatment methods and drugs into the European market, including the design and completion of preclinical and clinical studies as well as the setup of a product portfolio required for CE-mark application. CCRD ensures the highest ethical standards and closely monitors the compliance of all procedures with European regulations and requirements of the Local Ethical Committee. Our Preclinical Department provides protocol preparation, complete project realisation (including histopathological, angiographic, IVUS, OCT, statistical analysis) and final report writing.

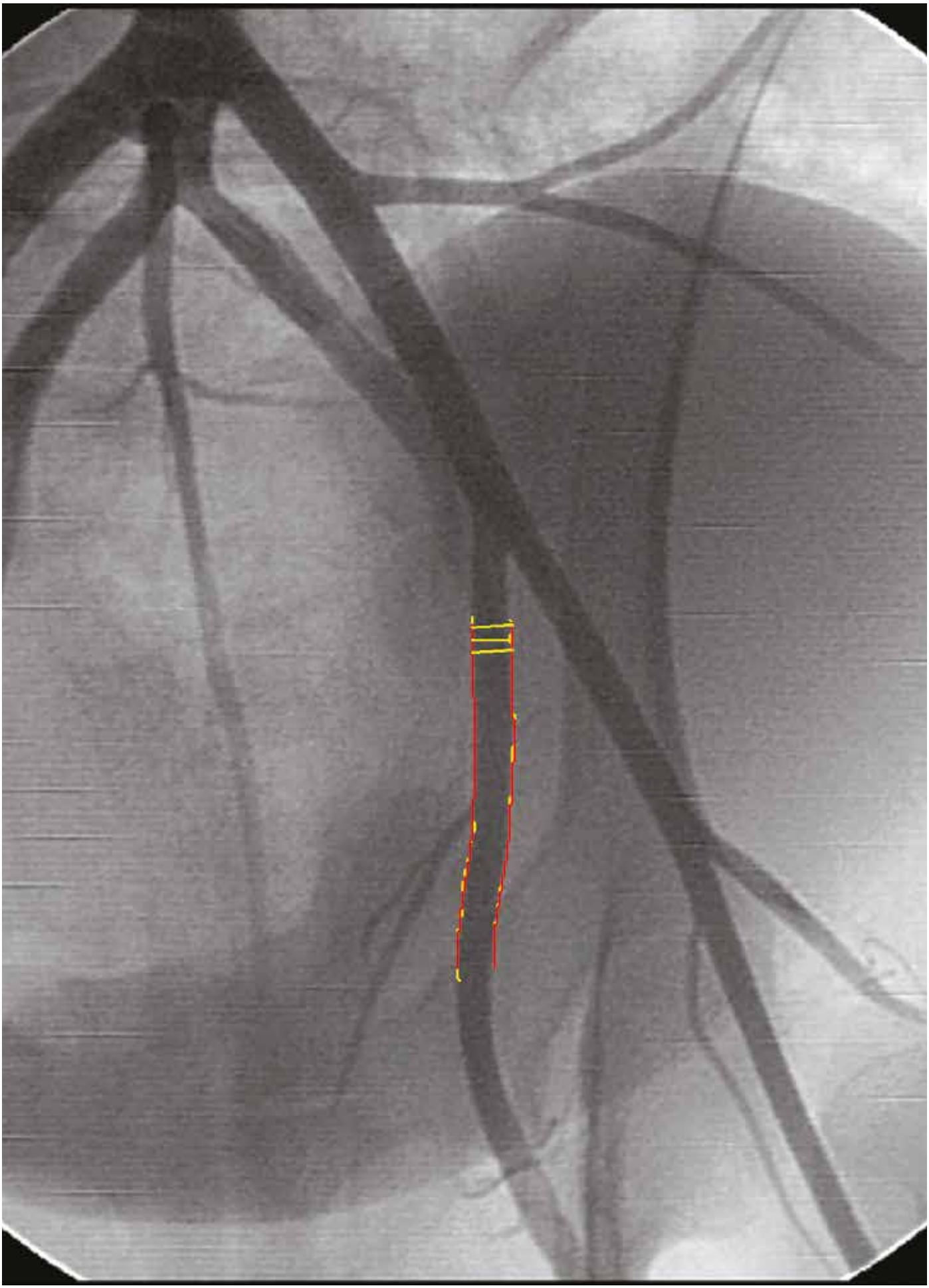
**Table 1. QVA/QCA analysis in Göttingen Minipigs aged 8.5 months**

| Artery | Reference Diameter mm (RD)* | Standard Deviation (SD) |
|---|---|---|
| LAD | 2.65 | 0.24 |
| LCx | 2.33 | 0.52 |
| RCA | 2.45 | 0.38 |
| Common Carotid Artery | 4.66 | 0.37 |
| Renal Artery | 4.24 | 0.78 |
| Common Iliac | 3.85 | 0.68 |
| Femoral Artery | 2.75 | 0.41 |

*The reference diameter is calculated as the mean diameter of individual vessels in 5 minipigs



Angiography. Left circumflex coronary artery in 8.5 month old Göttingen minipig”



Angiography. Left circumflex coronary artery in 8.5 month old Göttingen minipig”

# Göttingen Minipig Technical Symposium 2014

On the 5th of June 2014 Adrian Zeltner hosted the first Göttingen Minipig Technical Symposium in Manchester, UK. Here is a review of the symposium from John Chambers, Covance Laboratories:

The first UK Mini Pig Technical Symposium was a great success!

On the 6th June 2014 at the hotel Jurys Inn Manchester the first minipig technical Symposium in the UK took place, hosted by Adrian Zeltner. All attending the meeting were invited the night before to meet for dinner and drinks in the hotel restaurant and bar. Nearly all involved were able to make the meal and this proved to be a great way of networking and introducing ourselves before the meeting the following day. I have to say a great idea from Adrian was mixing up the seating plan and certainly helped people to get to know one another and engage in some interesting topics over dinner and drinks. Meeting all involved before the day (of which many people were presenting) definitely helped break the ice and during the meeting itself it helped provide lots of open and informal discussions.

The meeting itself started with introductions from Adrian and Anette from Ellegaard, Adrian stressed from the outset that this was very much "our" day and that lots of open discussions and as much participation from all involved would really get the best out of the day. I for one was extremely happy with the relaxed atmosphere and sharing of information from all involved.

On a personal level it was excellent to see so many interesting presentations and hear from experts in their fields. Being from a CRO I was particularly interested and pleased with the presentations regarding enrichment, acclimatisation, general care and housing and technical procedures. It was great to be able to ask so many questions and have such open and honest answers on a whole variety of subjects. I have been to many minipig conferences over the years but as an animal technician myself I found this one to be without doubt the most beneficial and relevant to be involved in.

I would like to take the opportunity to thank Adrian for being an excellent host and providing an excellent group of participants to engage in some great discussions with.

I look forward to the next one!

*John Chambers, Covance Laboratories*

# Rational Selection of the Non-Rodent Species: Toxicology, Pathology and Relevance to Man

The 29th annual meeting of the British Society of Toxicological Pathology (BSTP) will be held jointly with the Association of Comparative Clinical Pathology (ACCP) and the Minipig Research Forum (MRF).
The meeting will take place on Thursday 13th and Friday 14th November 2014 at the Alderley Park Conference Centre, Cheshire, UK and the topic will be "Rational Selection of the Non-Rodent Species: Toxicology, Pathology and Relevance to Man."
The Royal College of Pathologists has awarded 9 CPD credits for full attendance at the meeting.

The programme includes many informative presentations and also several about minipigs, among others:
"Minipigs in the Safety Assessment of Drugs" - Peter Glerup, CiToxLAB Scantox
"Minipigs or Primates in the Safety Assessment of Biopharmaceuticals?" – Warren Harvey, Charles River Laboratories
"The Skin of the Minipig: Arguments for Selection as a Non-Rodent Species in Dermal Studies" – Beatrice Gauthier, Galderma

The meeting will address the various factors to be considered when choosing the non-rodent species and provide a comparative overview of the relative benefits of each non-rodent species. The scientific programme will include a poster session, interactive case presentations, a trade exhibition and sponsor presentations.
You can meet Ellegaard Göttingen Minipigs at this meeting and at stand no. 6 in the exhibition area. Further information and a registration form can be found at the BSTP website (www.bstp.org.uk) or the MRF website (www.minipigresearch-forum.org).

# Meeting calendar

| NAME | DATE | LOCATION |
|---|---|---|
| European Teratology Society | 1-4 September | Hamburg, Germany |
| EuroTox | 7-10 September | Edinburgh, United Kingdom |
| GV-SOLAS/IGTp | 10-12 September | Frankfurt, Germany |
| HSBLAS/ESLAV/ECLAM | 22-23 September | Athens, Greece |
| BSTP/ACCP/MRF Meeting | 13-14 November | Alderley Park, Cheshire, UK |

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