

Voluntary locomotor activity promotes myogenic growth potential in domestic pigs

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Cork, May 2017



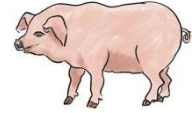
LEIBNIZ INSTITUTE
FOR FARM ANIMAL BIOLOGY



Background

- pigs are reared under limited space allowance
- standard requirement for fattening pigs: $< 1 \text{ m}^2$ (EU-DIRECTIVE 2008/120/EC)
- physical activity as a prerequisite for muscle health and as modulator for physiological characteristics
- BUT: difficulties to exactly monitor the locomotion behaviour
- a few studies regarding physical activity in farm animals – mostly associated with different housing conditions/rearing systems (Bee et al. 2004, Gondret et al. 2005)
- there are exercise-associated studies (laboratory animals or human)

Aim of our (pilot) study



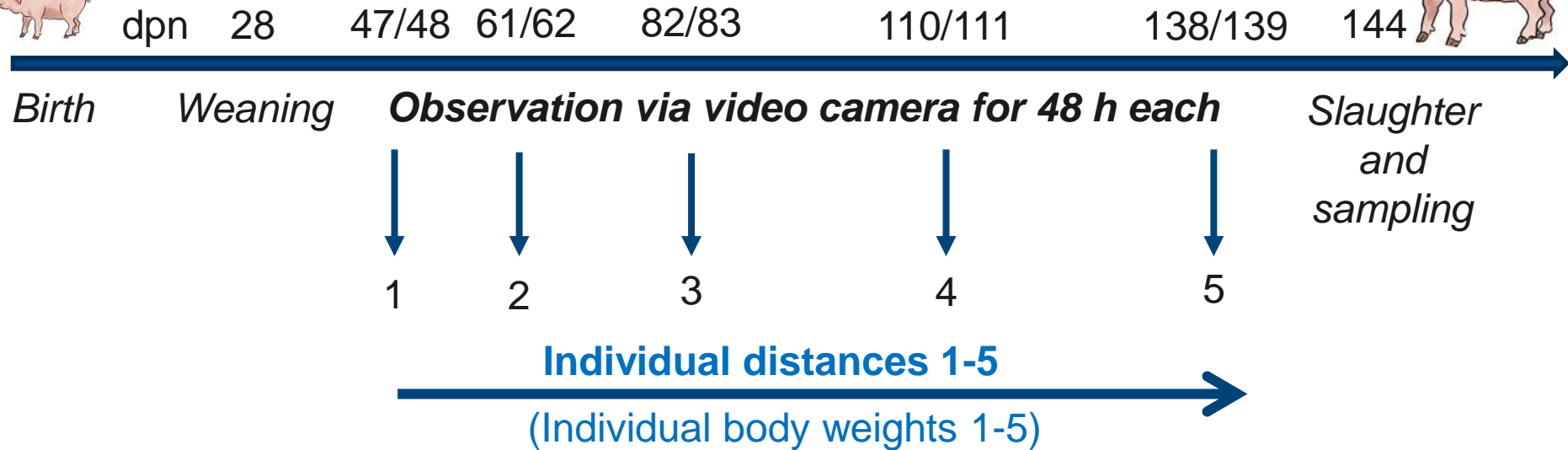
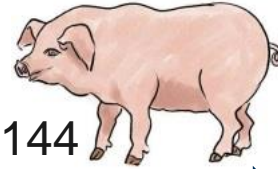
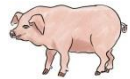
- Differences in voluntary activity in pigs reared under conventional conditions



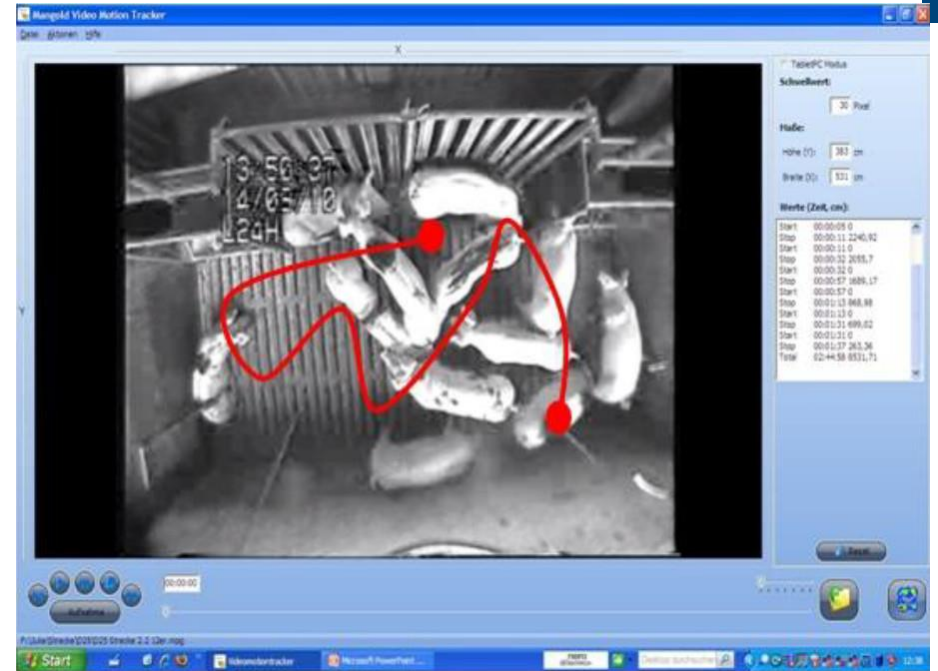
- Reflection on muscular cellularity and underneath the sub-cellular level
 - ➔ Muscle structure and biochemistry (*M. semitendinosus*)
 - ➔ Gene expression at mRNA level (genes associated with myogenesis and muscle metabolism)

Experimental Design

12 focus animals out of 48 pigs
(in 2 replicates)



Methods - VideoMotionTracker®



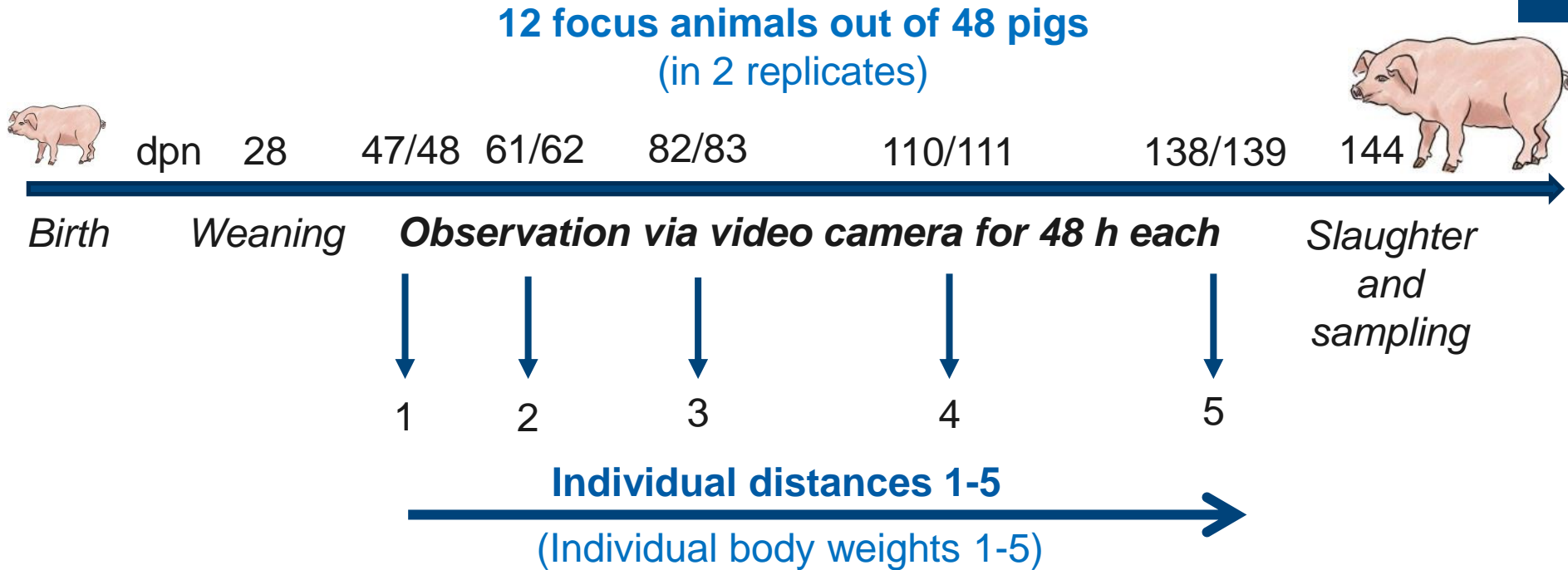
Individually marked pigs were observed via video camera

Methods - VideoMotionTracker®



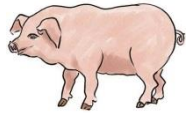
Digitized videos were analyzed with the VideoMotionTracker® (Brendle and Hoy 2011)

Experimental Design



➔ For each focus animal: digitized videos were analysed for daily distances at every time point, summed up over five time points

➔ Pigs were assigned to three categories according to their total distance walked:
long distance (LD), **medium distance (MD)**, **short distance (SD)**; n = 4 each



Histological and histochemical analyses


capillarisation - staining for alkaline phosphatase/eosin


fibre type classification – combined staining for diaphorase and acid pre-incubated ATPase

intramuscular fat proportion – oilred staining

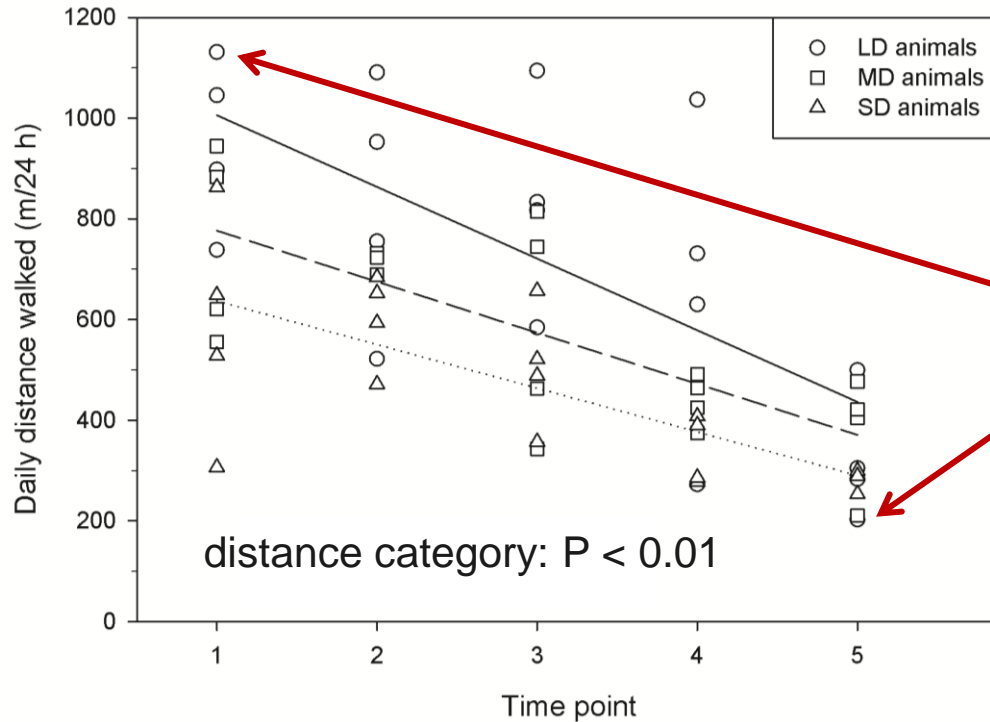
Biochemical analyses

DNA, RNA, protein and specific enzyme activities

 **mRNA expression of genes** encoding for myogenic transcription factors, growth factors and muscle structure or metabolism associated proteins

 **Statistical analysis:** ANOVA (MIXED, distance category as fixed factor, slaughter weight as co-variable, *post hoc* Tukey test) and Spearman rank correlation (CORR)

Results – Walking distance



Distances walked:

time point: $P < 0.001$

largest = 1131 m (TP1)

shortest = 202,4 m (TP5) → distance reduction with proceeding age



Total distance walked (Σ 5 time points):
 $P_{LD-SD} < 0.01$, $P_{LD-MD} < 0.05$, $P_{MD-SD} = 0.12$



LD > 3000 m (mean = 3604.6 m),

MD = 2500-3000 m (mean = 2867.7 m),

SD < 2500 m (mean = 2316.3 m)

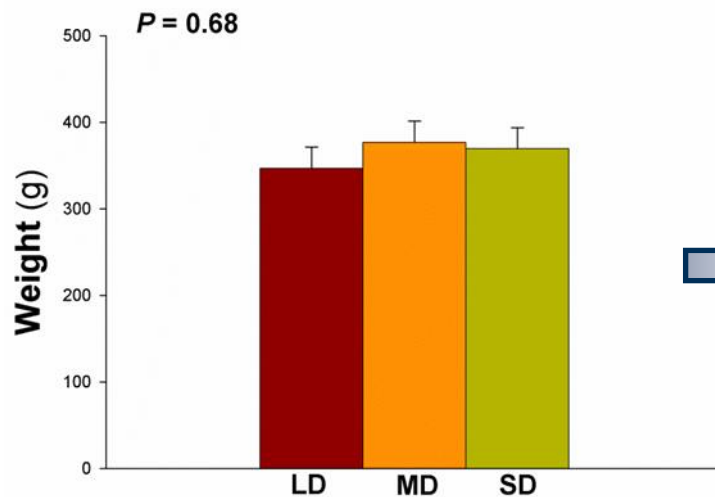
Results – Body & muscle weights

Body weight:

➤ increased with proceeding age (time point: $P < 0.001$)

➤ unaffected by walking distance (distance category: $P = 0.741$)

Muscle weight of *M. semitendinosus*:

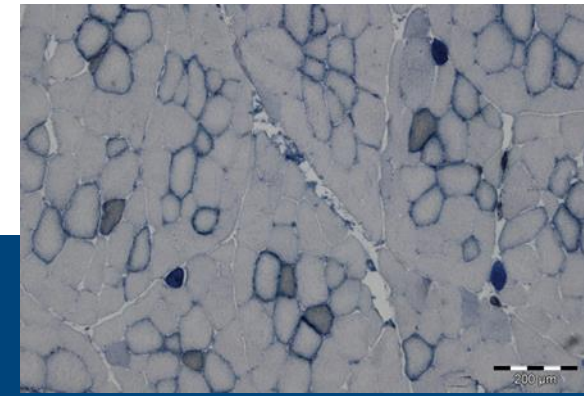


Muscle weight and circumference (not shown) were unaffected by walking distance.

Results – Histoanalysis (*M. semitendinosus*)

	<u>LD</u>	<u>MD</u>	<u>SD</u>	<u>SE</u>	<u>P</u>
Total fibre number (×1000)	953	880	895	34	0.33
Fibre cross-sectional area (µm²)					
STO	4,049	4,384	4,887	449	0.45
FTO	3,222	4,036	3,696	338	0.29
FTG	3,468	3,553	3,627	213	0.87
Pathologic	2,605	1,678	1,435	706	0.46
Average	3,456	3,847	3,864	270	0.51
Fibre type proportion (%)					
STO	20.1	22.2	17.2	2.6	0.44
FTO	28.1	28.7	29.5	1.4	0.78
FTG	49.7	47.9	52.4	1.8	0.27
Pathologic	2.2	1.2	0.9	0.7	0.39

➔ TFN, FCSA, fibre type distribution and capillarisation (not shown) **remained unchanged** by distance categories.

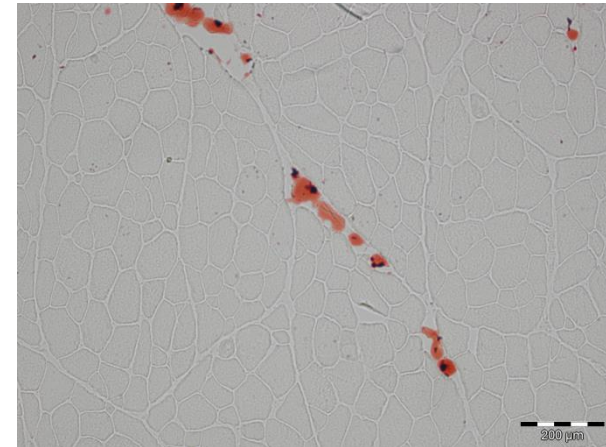
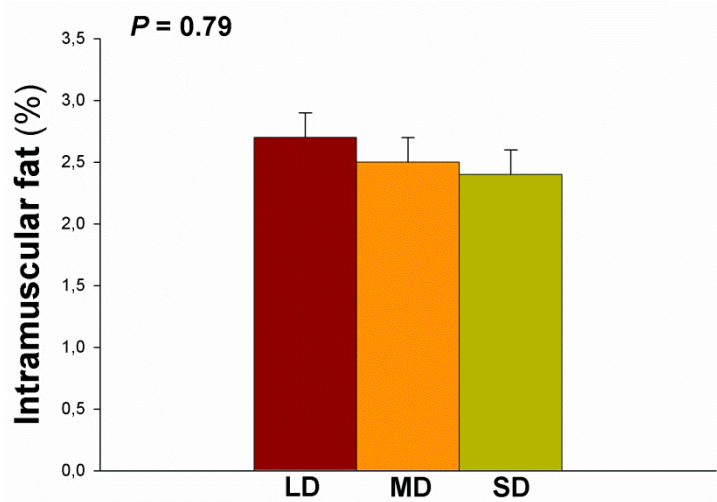


Results – Biochemical analysis (*M. semitendinosus*)

	<u>LD</u>	<u>MD</u>	<u>SD</u>	<u>SE</u>	<u>P</u>
Total DNA (mg)	273.71	293.31	304.02	19.06	0.54
Total RNA (mg)	165.08	176.89	167.82	9.81	0.69
Total protein (g)	52.55	55.67	56.58	3.39	0.69
Specific isocitrate dehydrogenase activity (IU/g protein)	16.13	15.75	14.70	1.10	0.65
Specific lactate dehydrogenase activity (IU/mg protein)	3.32	3.42	3.54	0.13	0.52
Specific creatine kinase activity (IU/mg protein)	26.11	26.20	26.63	0.98	0.92

➔ The content of DNA, RNA and protein or specific enzymes activities were **not affected** by walking categories.

Results – Intramuscular fat

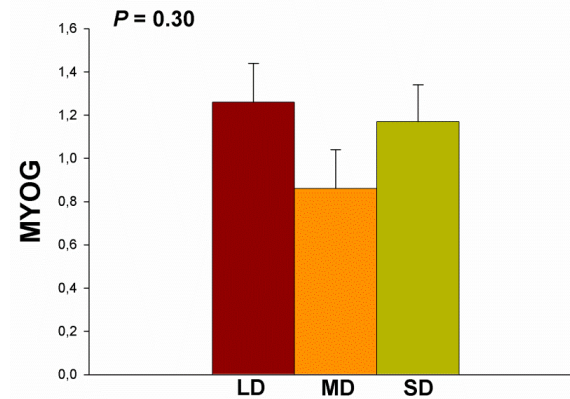
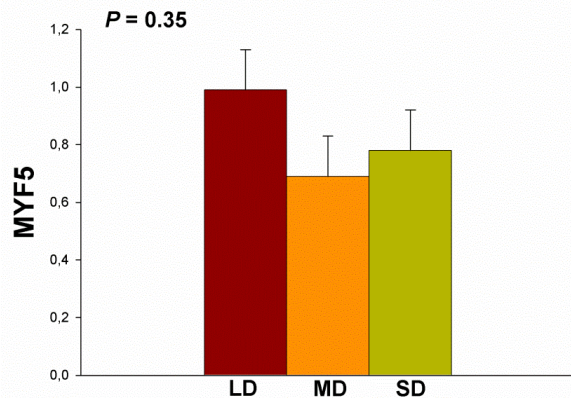
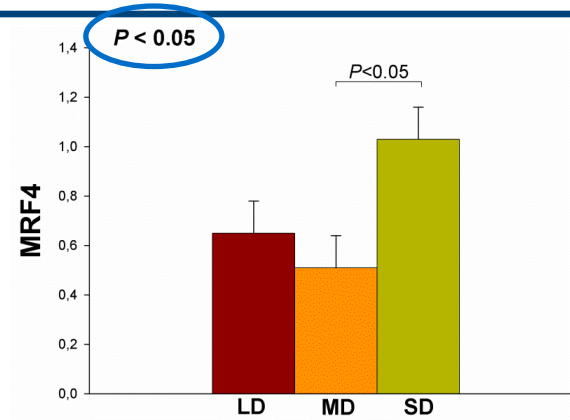
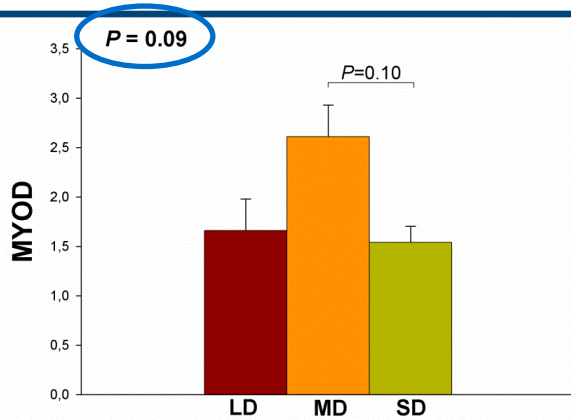


Oilred staining



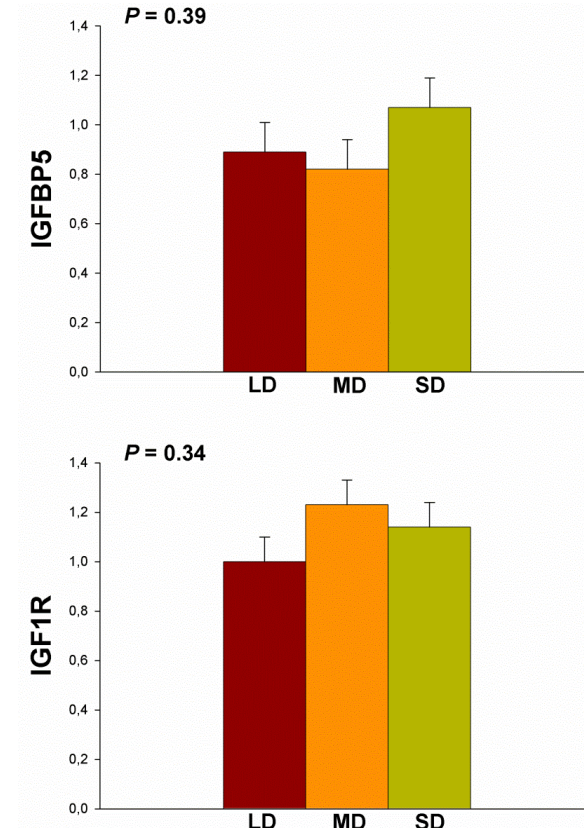
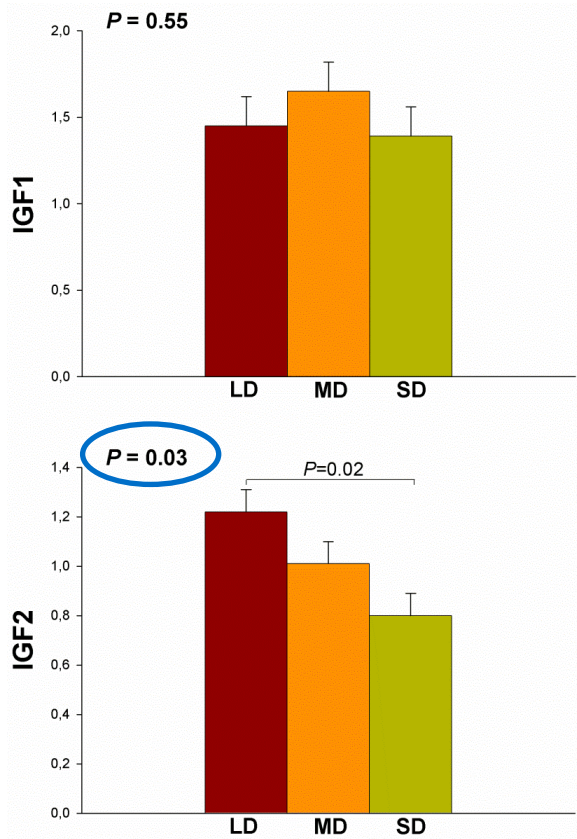
Intramuscular fat content of *M. semitendinosus* at slaughter age was **unaffected** by distance category.

Results – mRNA expression (MRF)



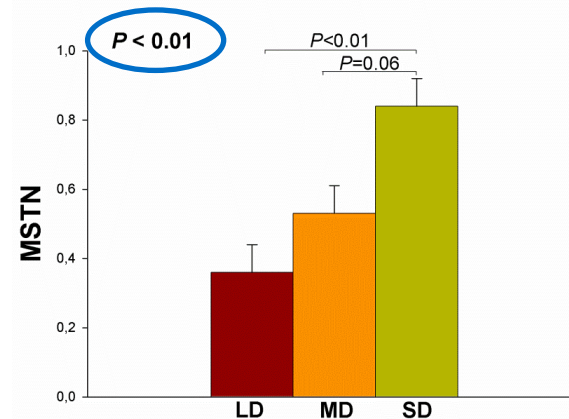
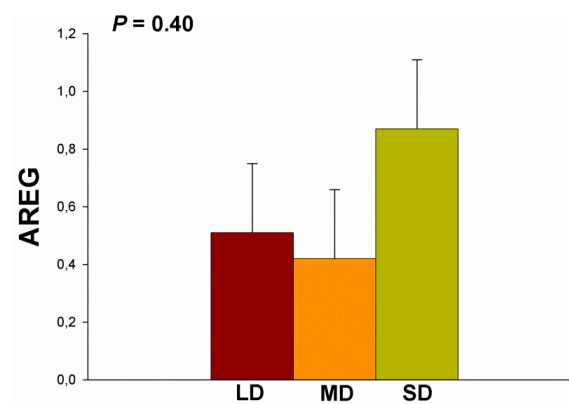
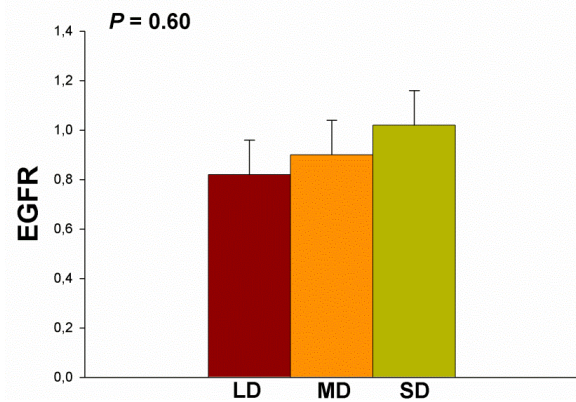
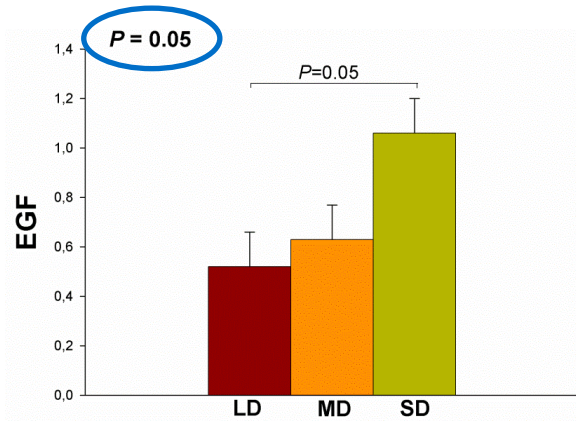
SD pigs exhibited a lower MYOD (by trend) and higher MRF4 mRNA expression than MD pigs. MYF5, MYOG and PAX7 (not shown) remained unchanged by walking distance.

Results – mRNA expression (growth factors)



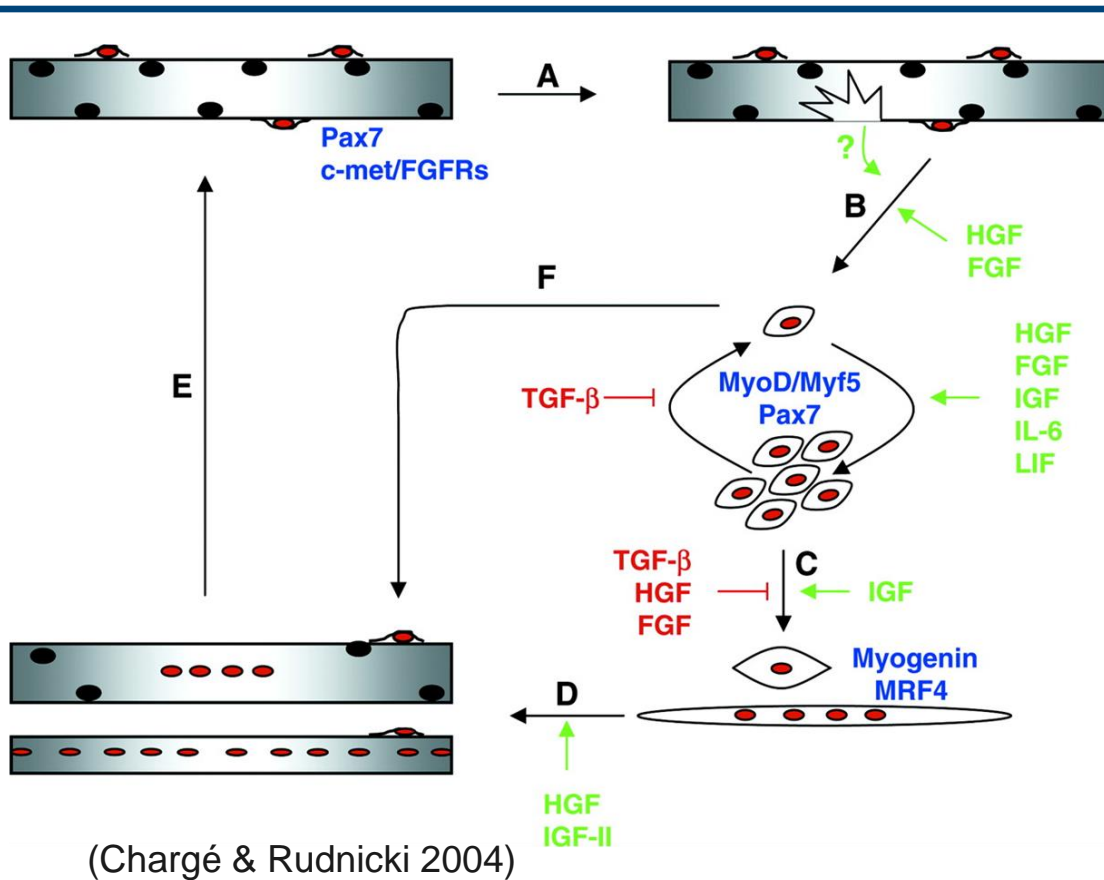
SD pigs exhibited a lower IGF2 mRNA expression than LD pigs. IGF1, IGF1R and IGFBP5 remained unchanged by walking distance.

Results – mRNA expression (growth factors II)



EGF and MSTN mRNA was increased in SD compared with LD pigs, whereas AREG and EGFR remained unchanged.

Possible role of IGF2 and MSTN



- Postnatal IGF2 mRNA expression reflects muscular phenotype in pigs (van den Maagdenberg et al. 2008, Rehfeldt et al. 2012, Paredes et al. 2013)
- MSTN may negatively regulate IGF2 expression to control postnatal skeletal muscle growth (MSTN^{-/-}, WT, Clark et al. 2015)
- Low IGF2/MSTN ratio in rats after spaceflight associated with muscle loss (Lalani et al. 2000)

➔ IGF2/MSTN ratio as indicator of homeostatic balance that maintains muscle mass.

Summary

- Individual walking distances within 24 h by 12 focus animals were measured at five different time points using a video tracking method. The **daily distance walked** by each pig **was reduced with proceeding age**.
- Pigs could be assigned to **three categories** regarding their voluntary locomotor activity: **LD > 3000 m, MD = 2500-3000 m, SD < 2500 m**.
- At slaughter (144d of age) **no differences due to walking categories** were found in **muscle microstructure** (fibre number and size, capillaries, fibre type distribution) **and biochemistry** (DNA, RNA, protein, enzyme activities) of *M. semitendinosus*.
- **Distance-dependent differences in mRNA expression** of genes encoding growth (**IGF2, EGF, MSTN**) and transcription factors (**MRF4, MYOD**) were found. While the mRNA expression of muscle structure and metabolism associated genes remained unchanged (not shown).
- **IGF2/MSTN ratio was positively correlated with the distance walked**.

THANKS TO....



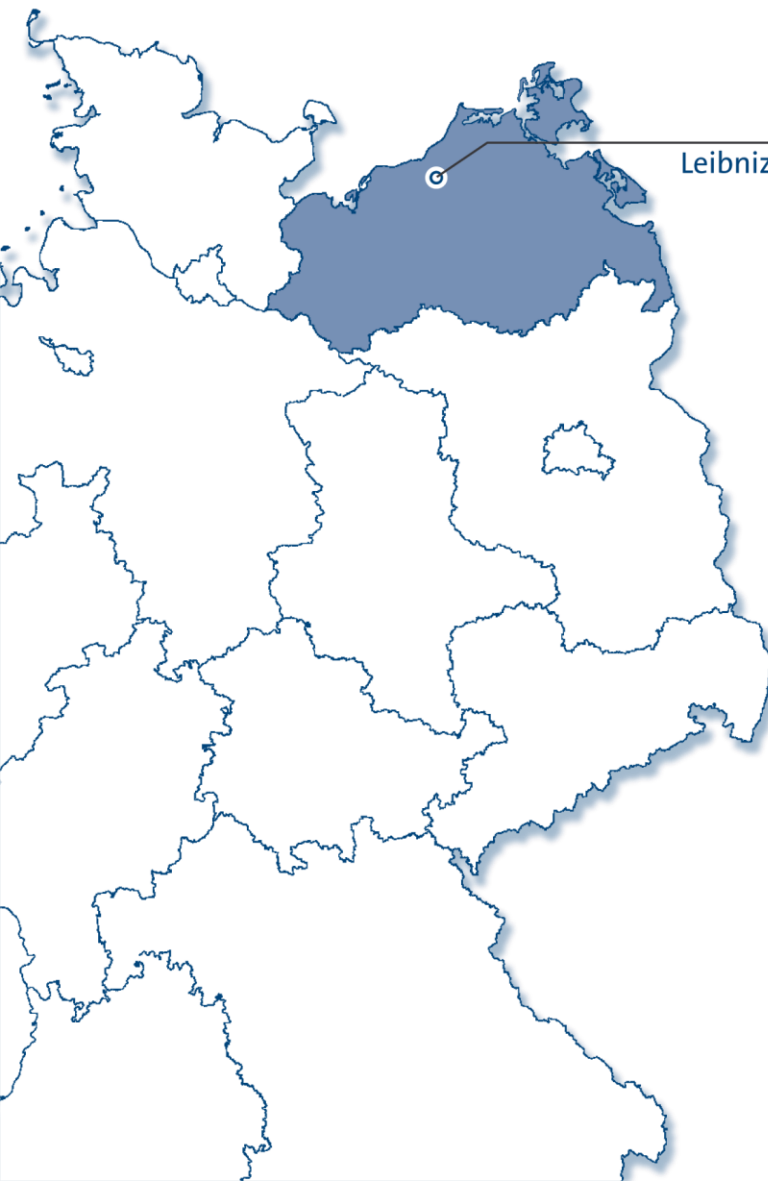
our pigs that moved around despite of the limited space allowance.

our colleagues of the institutional pig facility and slaughterhouse for animal care and help.

Anne Berndt, Kerstin Niemann and Marie Jugert-Lund for excellent technical assistance.

THANK YOU FOR YOUR ATTENTION!





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