



Dietary L-arginine supply during early gestation promotes myofiber hyperplasia

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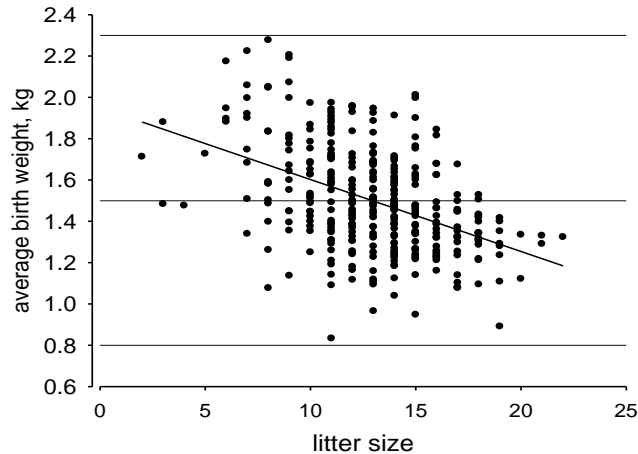
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Known relationships



- Litter size is negatively correlated with average litter birth weight

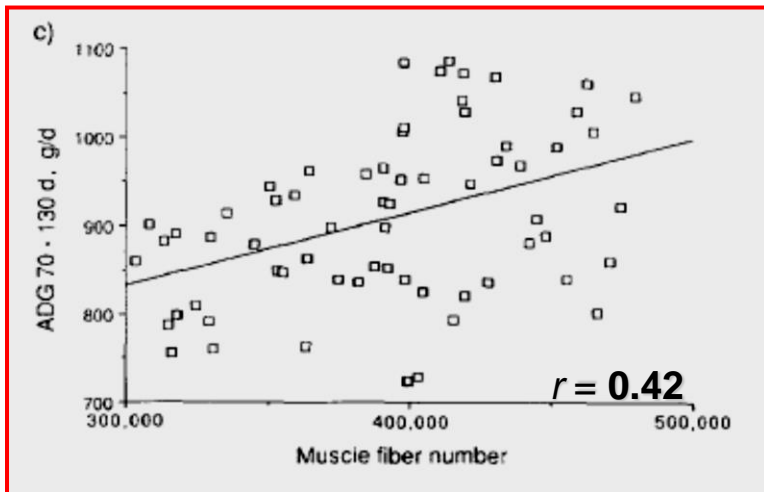


- Birth weight is negatively correlated with
 - survival rate in the first week after birth
 - postnatal growth
 - carcass leanness
 - some meat quality traits

myofiber number
myofiber size

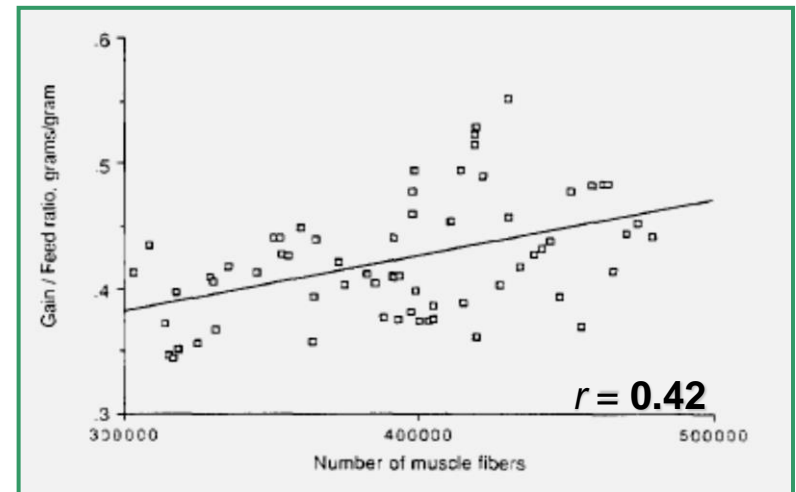
Total myofiber number (TFN)

ADG vs. TFN



Dwyer et al. 1993
J. Anim. Sci. 1993, 71:3339-3343

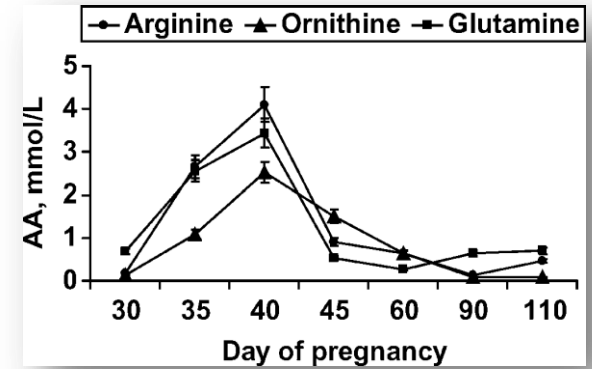
G/F vs. TFN



Dwyer et al. 1993
J. Anim. Sci. 1993, 71:3339-3343

ARGININE ↔ PRENATAL DEVELOPMENT

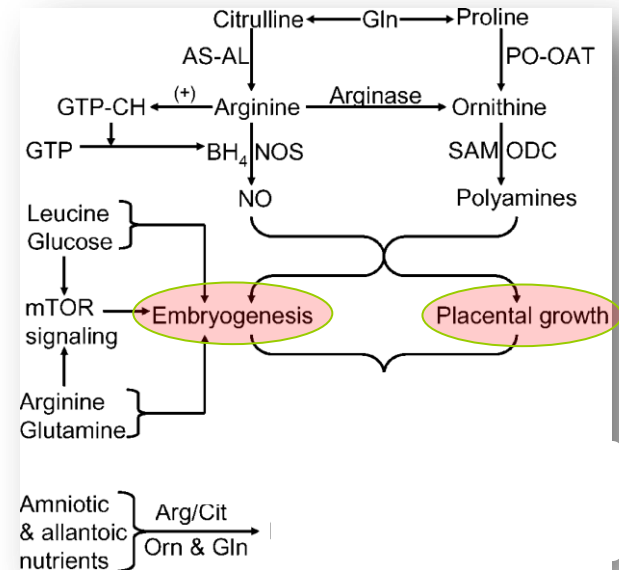
- In early pregnancy, arginine and ornithine level elevated in porcine amniotic and allantoic fluid



Wu et al. 2006

- Associated with a high syntheses rate of nitric oxide and polyamine in the porcine placenta

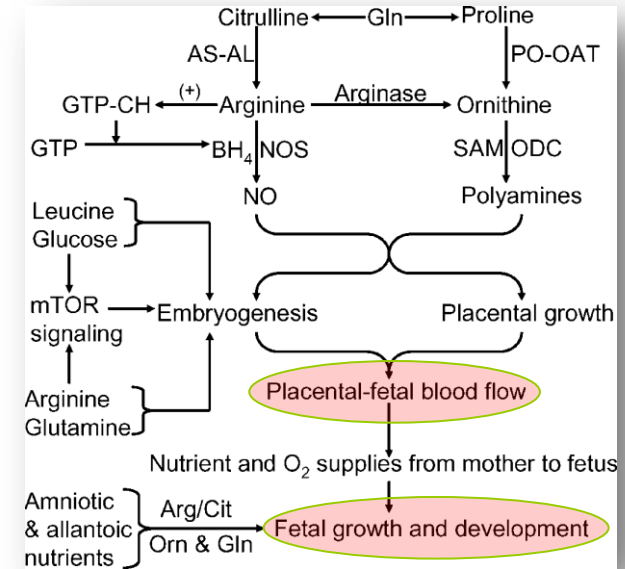
- Key role in angiogenesis = placental and embryonic development



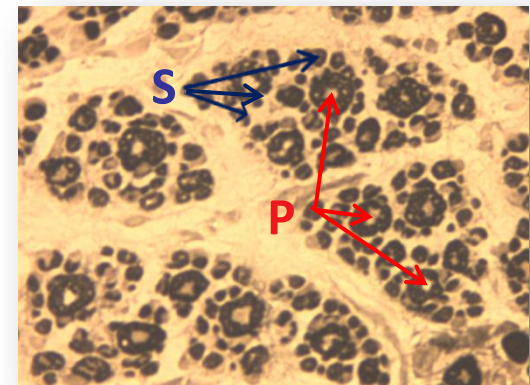
Wu et al. 2006

ARGININE ↔ MYOGENESIS

- Improved placental-fetal blood flow = improved fetal development
- Increased litter size without impact of birth weight
- Impact on muscle development?

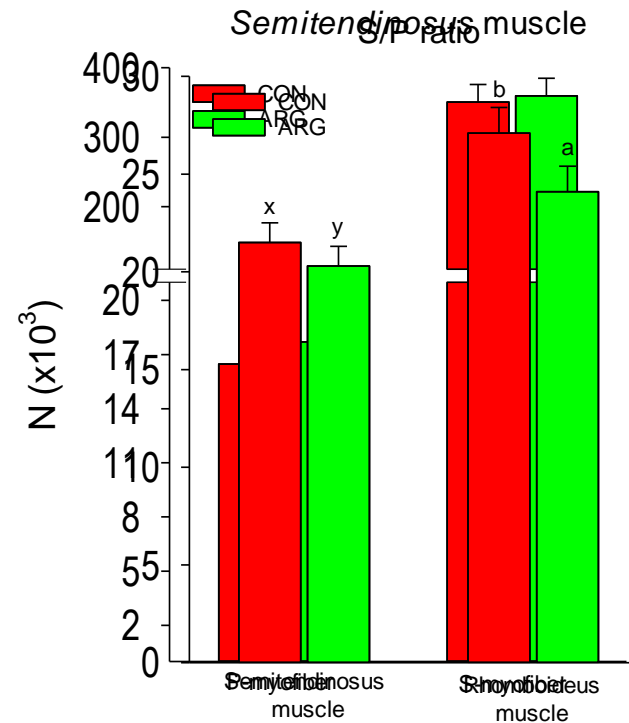
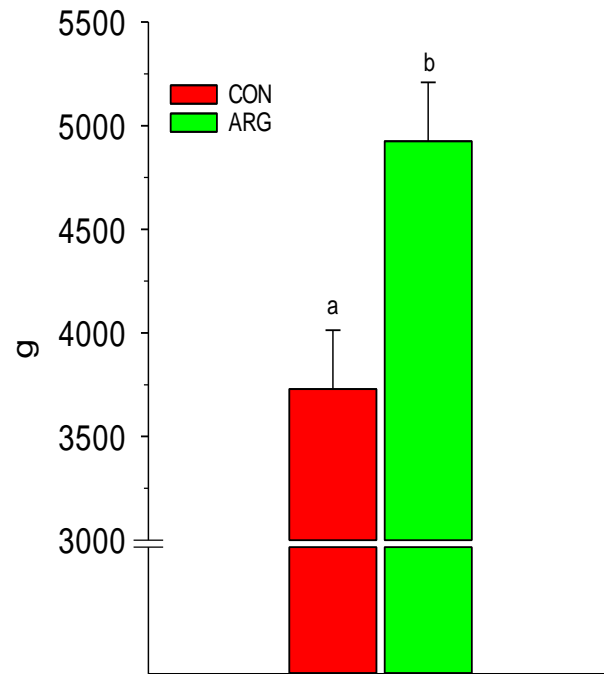


Wu et al. 2006



Dietary manipulation of myofiber development

Average fetal weight (d 75 of gestation)



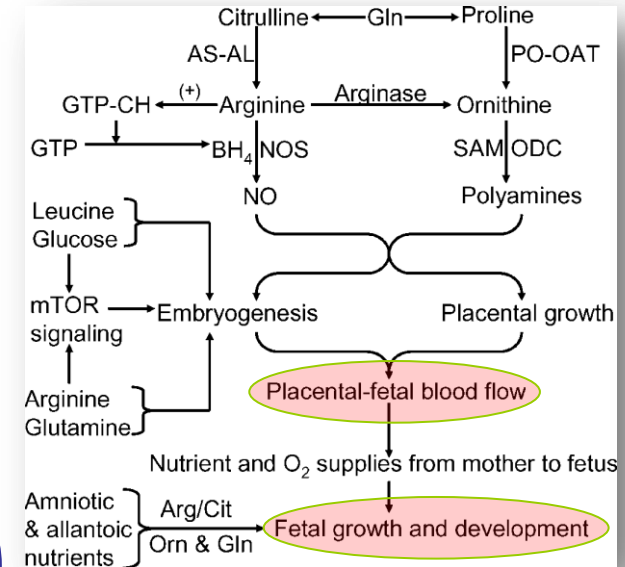
Animal (2010), 4:10, pp 1680–1687

ARGININE ↔ MYOGENESIS

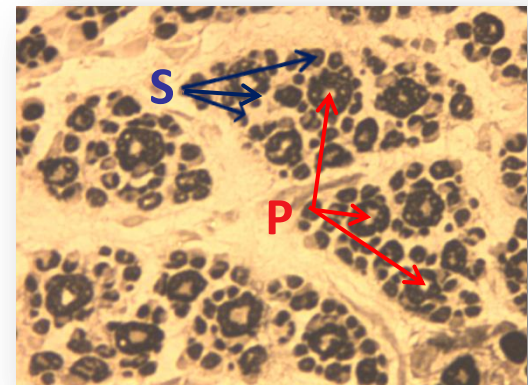
- Improved placental-fetal blood flow = improved fetal development
- Increased litter size without impact of birth weight

Primary fibers serve as a scaffold for the formation of secondary fibers

Greater number primary fibers = greater number of secondary fibers



Wu et al. 2006



Bérard and Bee 2010

Hypothesis



Based on the association between dietary arginine supply, the extent of placental vascularization, the fetal nutrient supply and muscle development 2 working hypothesis were formulated:

- **Hypothesis 1:**
 - *Supplementing L-arginine to an early gestational diet of the dams would promote hyperplasia leading to an increased number of myofibers in their offspring at birth.*
- **Hypothesis 2:**
 - *L-arginine is especially efficient in piglets suffering from IUGR.*

Experimental design



Animals:

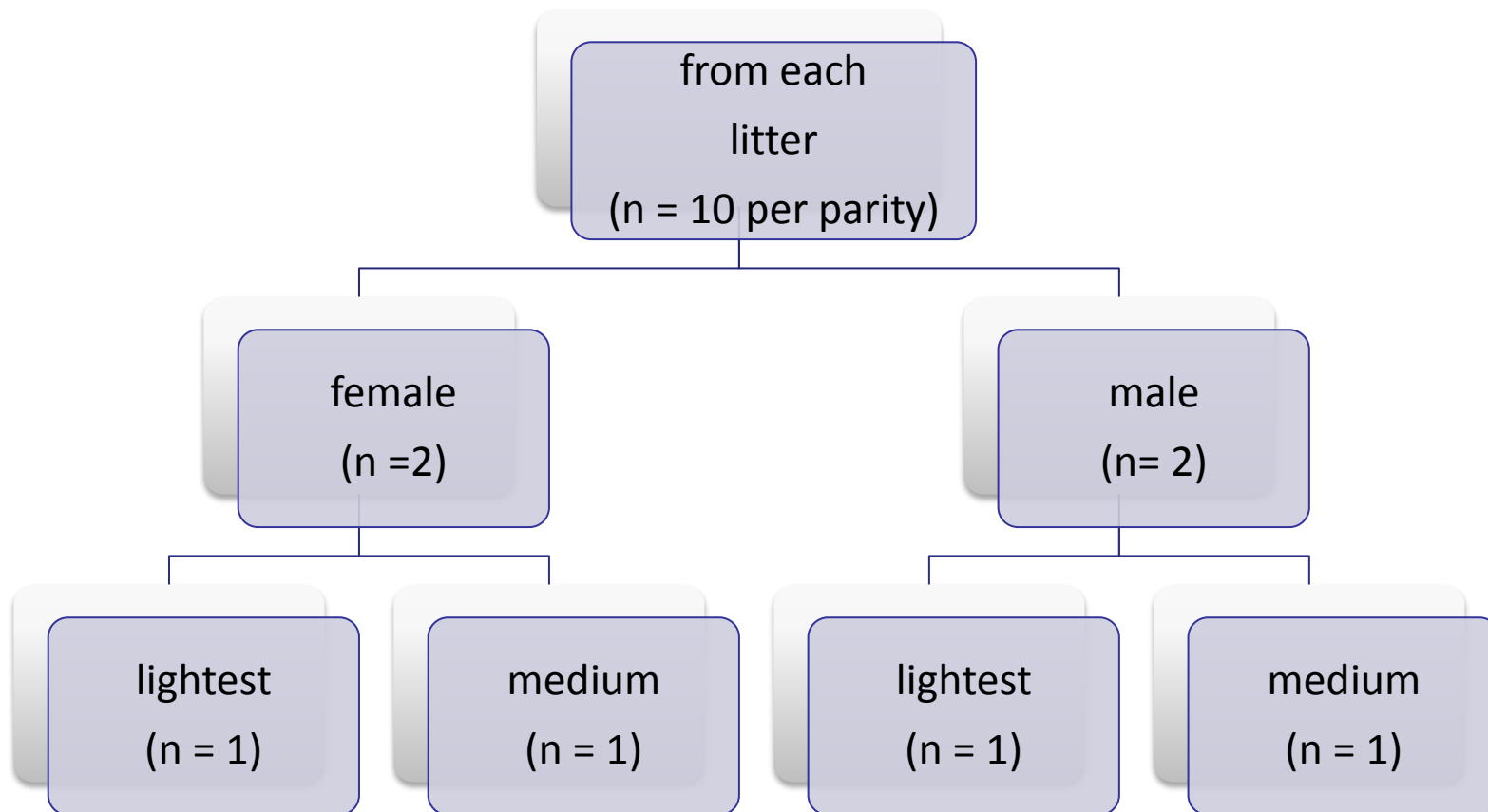
- Intact sows (**I**; litter size: > 15; naturally **crowded**)
- OL sows (**OL**; unilaterally oviduct ligated; **uncrowded**)

All sows (n = 10) were at the beginning of the experiment in their fifth parity

Diets:

- Control (**C**; 100 g/d alanine from d 14 to 28 of gestation)
- Arginine (**Arg**; 25 g/d arginine from d 14 to 28 of gestation)

Selection criteria at farrowing



Data and sample collection and analysis

Collection of data and samples at birth

- Litter size
- BtW of all piglets born



From the selected newborn piglets

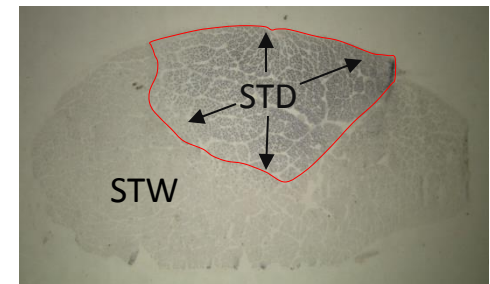
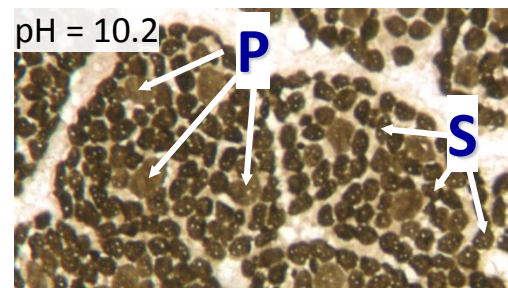
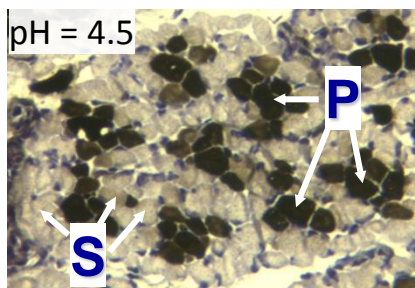
- Weight of the heart, kidney, liver, lung and spleen
- Weight of the brain
- Weight of the semitendinosus and psoas major



Histological analyses in the semitendinosus

(mATPase staining after pre-incubation at pH 4.5 and 10.2)

- CSA
- Number of P- and S-fibers
- S/P ratio
- TNF



RT-PCR analysis



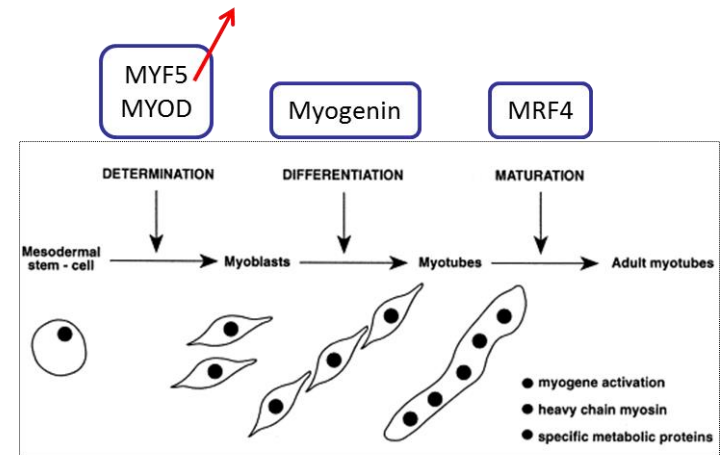
Transcript expression of

- myogenic factor 5 (***MYF5***)
- myogenic differentiation factor (***MYOD***)
- myogenin (***MYOG***)
- muscle-specific regulatory factor 4 (***MRF4***)

- myostatin (***MSTN***)

- AMP-activated protein kinase catalytic subunit alpha-2 (***PRKAA2***)

- insulin growth factor 2 (***IGF2***)
- insulin growth factor binding protein 5 (***IGFBP5***)



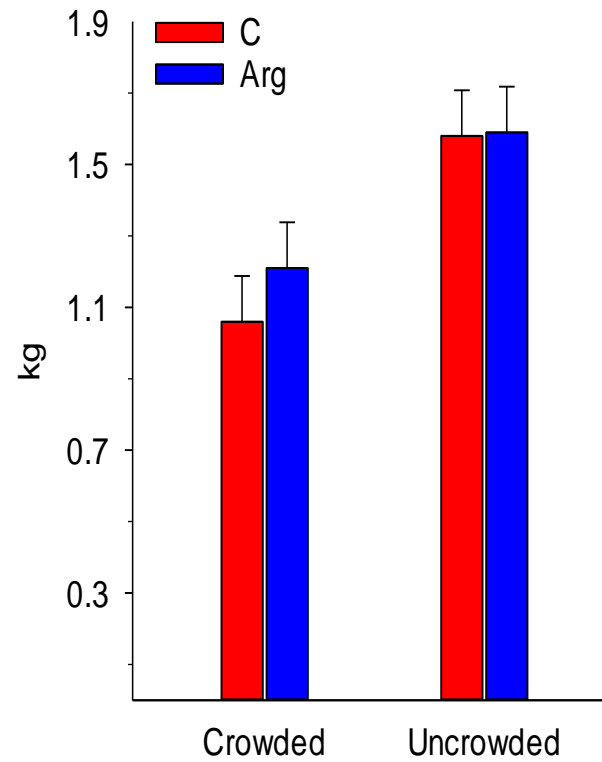
www.sciencedirect.com



| Trait | Crowded | | Uncrowded | | SEM | IUC | <i>P</i> -values | |
|------------------|---------|------|-----------|------|-------|------|------------------|------------|
| | C | Arg | C | Arg | | | DIET | IUC x DIET |
| Litter size, n | | | | | | | | |
| Total born | 15.5 | 15.3 | 10.5 | 11.9 | 1.71 | 0.26 | 0.72 | 0.66 |
| Born alive | 13.1 | 14.5 | 9.1 | 10.3 | 1.49 | 0.23 | 0.46 | 0.96 |
| Birth weight, kg | | | | | | | | |
| Total born | 1.22 | 1.48 | 1.57 | 1.51 | 0.068 | 0.28 | 0.21 | 0.13 |
| Born alive | 1.24 | 1.50 | 1.59 | 1.53 | 0.068 | 0.28 | 0.22 | 0.13 |
| STD | 0.24 | 0.25 | 0.18 | 0.14 | 0.056 | 0.41 | 0.87 | 0.63 |

Results

Birth weight of selected piglets



DIET, $P = 0.07$

IUC, $P < 0.01$

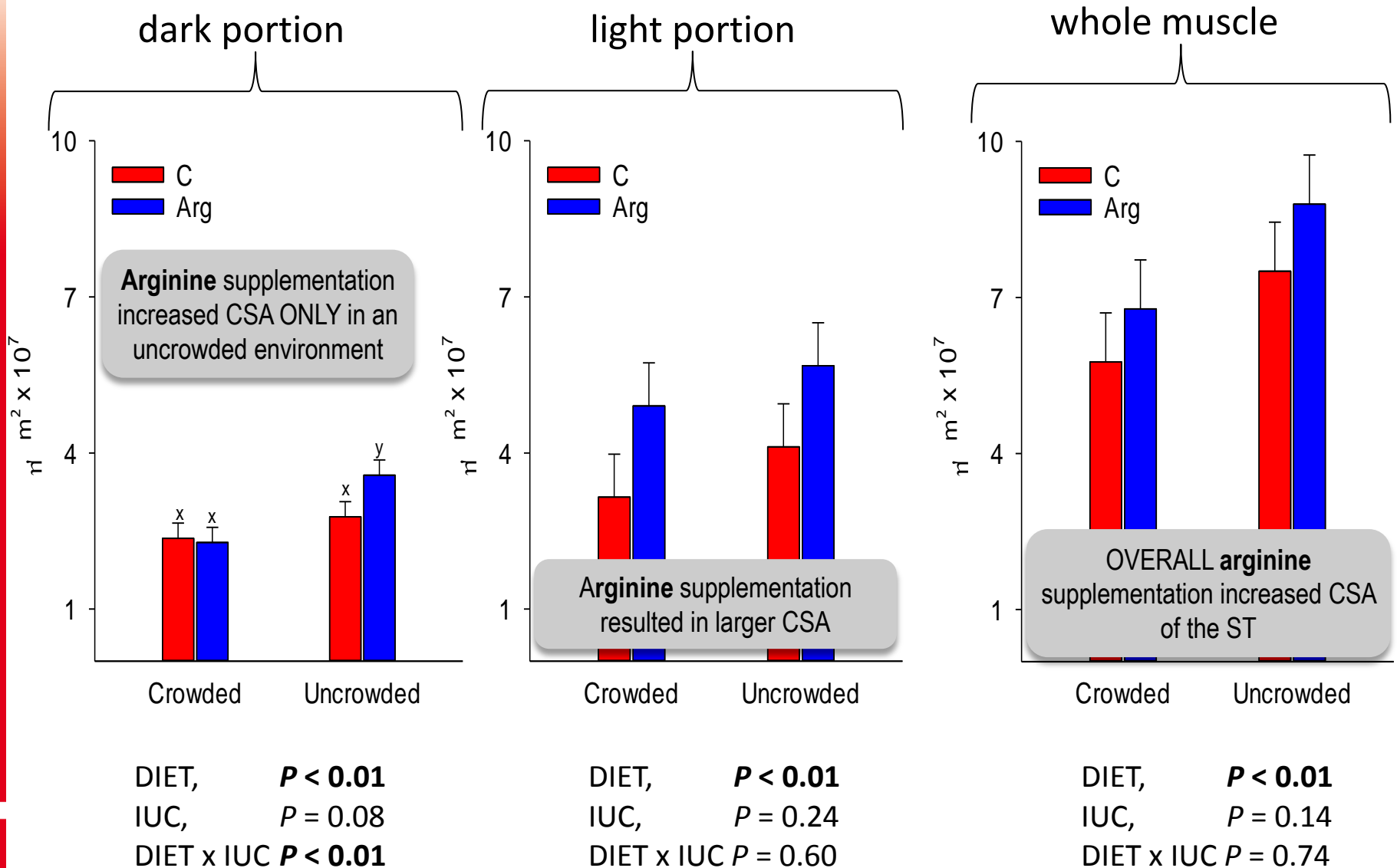
DIET x IUC $P = 0.14$



| Trait expressed in g/100 g birth weight | Crowded | | Uncrowded | | SEM | IUC | <i>P</i> -values | |
|--|-------------------|-------------------|--------------------|--------------------|------|------|------------------|------------|
| | C | Arg | C | Arg | | | DIET | IUC x DIET |
| Heart | 0.70 | 0.67 | 0.67 | 0.65 | 0.02 | 0.49 | 0.03 | 0.72 |
| Liver | 2.21 | 2.47 | 2.78 | 2.77 | 0.18 | 0.13 | 0.19 | 0.14 |
| Spleen | 0.10 ^b | 0.09 ^a | 0.09 ^{ab} | 0.09 ^{ab} | 0.01 | 0.67 | 0.18 | 0.02 |
| Lung | 1.47 | 1.39 | 1.31 | 1.27 | 0.11 | 0.36 | 0.25 | 0.76 |
| Kidney | 0.74 | 0.72 | 0.80 | 0.82 | 0.04 | 0.27 | 0.76 | 0.40 |
| Brain | 2.87 | 2.53 | 2.47 | 2.50 | 0.32 | 0.62 | 0.17 | 0.11 |
| Brain:liver weight ratio | 1.36 | 1.12 | 0.94 | 0.92 | 0.22 | 0.27 | 0.08 | 0.18 |
| Semitendinosus | 2.09 | 2.08 | 2.19 | 2.28 | 0.11 | 0.33 | 0.42 | 0.34 |
| Psoas major | 2.06 | 2.14 | 2.31 | 2.39 | 0.14 | 0.28 | 0.28 | 0.99 |

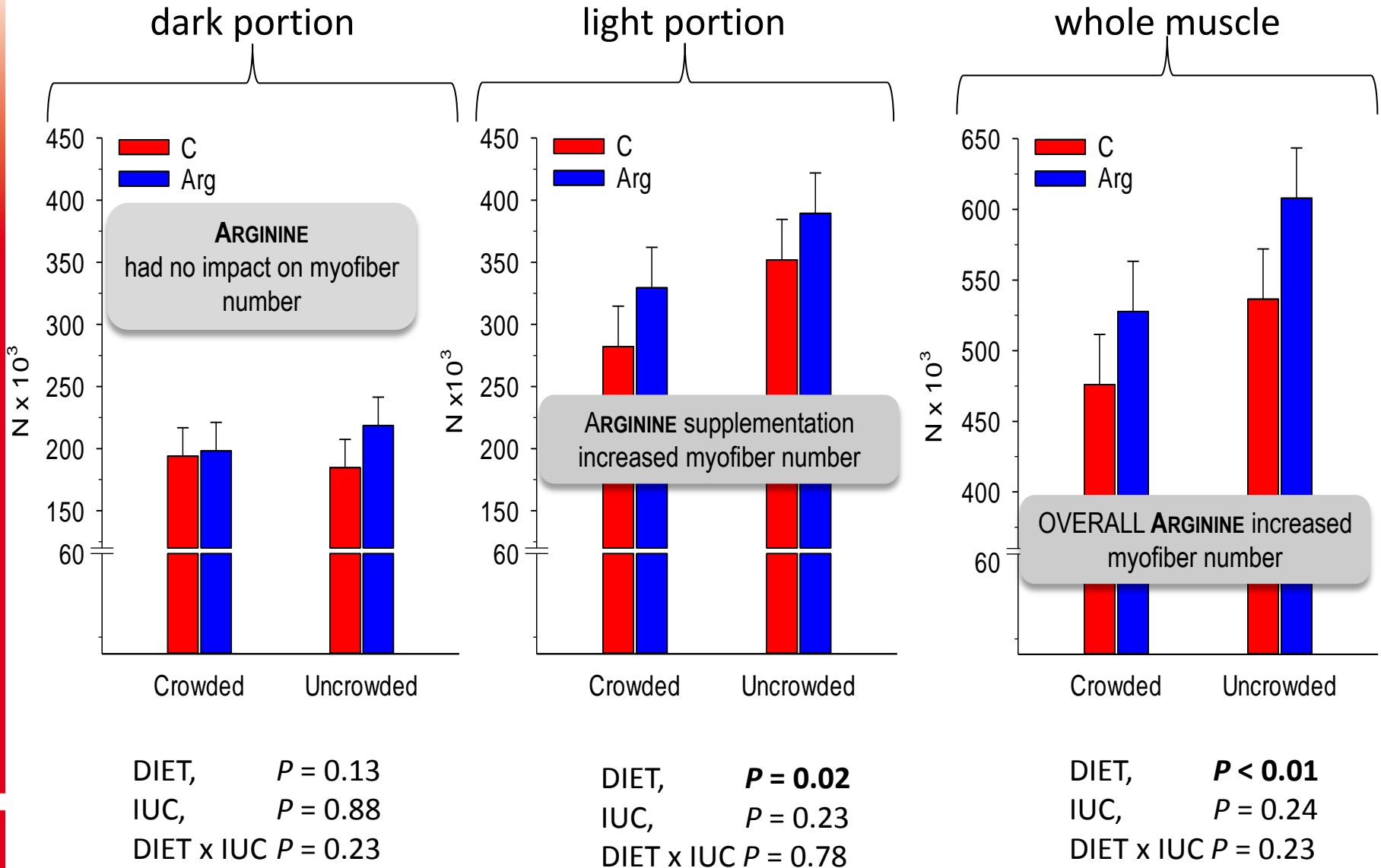
Results

Muscle area



Results

Myofiber number



Results

Gene expression of myogenesis-related genes

| Trait | Crowded | | Uncrowded | | SEM | IUC | <i>P</i> -values | |
|--------|-------------------|-------------------|-------------------|-------------------|-------|-------|------------------|------------|
| | C | Arg | C | Arg | | | DIET | IUC x DIET |
| IGF2 | 2.36 | 1.55 | 0.91 | 1.70 | 0.555 | 0.487 | 0.967 | 0.950 |
| IGFBP5 | 5.63 | 5.65 | 2.32 | 4.62 | 2.190 | 0.489 | 0.233 | 0.297 |
| MSTN | 1.60 | 1.64 | 0.46 | 0.98 | 1.754 | 0.351 | 0.232 | 0.110 |
| MYF5 | 0.35 | 0.42 | 0.24 | 0.28 | 1.694 | 0.631 | 0.531 | 0.159 |
| MYF6 | 0.58 | 0.65 | 1.18 | 0.98 | 0.576 | 0.623 | 0.859 | 0.631 |
| MYOD1 | 2.36 | 1.84 | 0.68 | 0.48 | 2.143 | 0.223 | 0.340 | 0.418 |
| MYOG | 0.94 | 0.70 | 1.32 | 1.11 | 0.255 | 0.333 | 0.168 | 0.417 |
| PRKAA2 | 0.72 ^b | 1.04 ^b | 0.26 ^a | 0.15 ^a | 0.194 | 0.024 | 0.264 | 0.091 |

PRKAA2 (inhibitor of muscle protein synthesis)
greater expression related to

- lower BtW
- lower muscle weight

Answer to hypothesis

Supplementing L-arginine early in gestation

- reduces the negative impacts of IUGR,
 - increased hyperplasia, birth weight and STM area.
- as muscle area increased more than TNF → prenatal myofiber hypertrophy
- not especially efficient in L-BtW piglets