UNIVERSITY OF COPENHAGEN

Department of Basic Animal and Veterinary Sciences
Faculty of Life Sciences

Development and metabolic function can be "programmed" by nutrition in pre- and early postnatal life - Evidence from a sheep model

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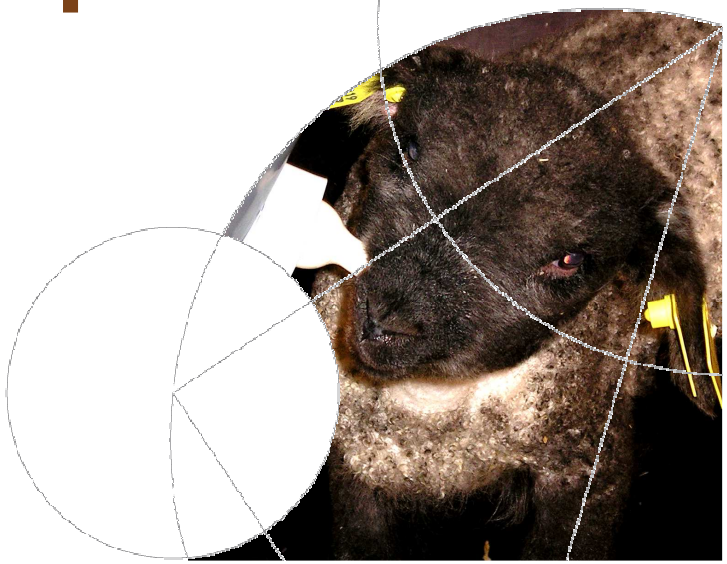
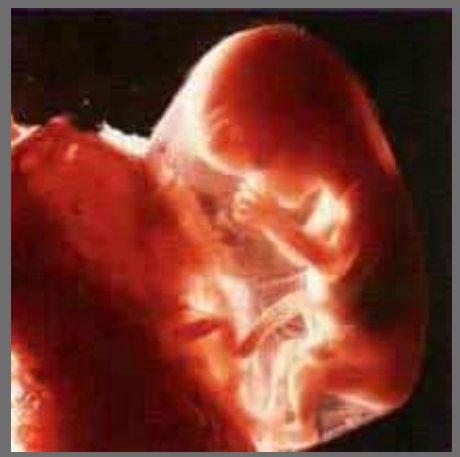


Winter School 7 December 2010

Prevalence of Type II Diabetes and Impaired Glucose Tolerance in Men Aged 59-70 Years



Barker, 1998

Early programming of postnatal health:

Fetal undernutrition:

- Programmes postnatal development and metabolic function
  - Altered phenotypic manifestation of the genome
    - Epigenetic (other?) modifications
  - Age dependent manifestation

Postnatal diet:

- Phenotypic manifestation of fetal programming
  - Mismatch in pre- and postnatal dietary exposure
  - Worst case scenario ?

Aim of our research:

- Improve understanding:
  - Mechanisms underlying fetal programming
  - Phenotypical manifestation of fetal programming
  - Postnatal dietary impact on this manifestation

- In order to:
  - Biological markers to identify adversely programmed individuals early in life
  - Develop intervention strategies (dietary?)

Animal model for human: Sheep

Fetal programming:
The sheep as an experimental model

Advantages:

- Size (50-75 kg)
- Litter size: Singletons and twins (triplets)
- Long gestation period (time windows; ~147 d)
- Off-spring at birth: comparable to the human
- Interventions possible without induction of abortion
    - Fetal intervention studies (catheterisations)

Fetal programming:
The sheep as an experimental model

Disadvantage:

- Sheep are ruminants with distinctive digestive function
  - Large fermentation chambers in forestomachs
  - Little glucose absorption, instead Short Chain Fatty Acids
  - Tolerate rather low levels of fat in the diet
- Makes postnatal dietary interventions difficult to study
  - But special tricks can be applied
  - Suckling
    - Esophageal groove reflex
    - By-pass rumen (liquid feed)

Metabolic programming: The sheep as an experimental model

Disadvantages:

- Sheep are ruminants with distinctive digestive function
  - Large fermentation chambers in forestomachs
- Rumen microbes:
  - Fibre digestion (cellulase)
  - Essential amino acid synthesis
  - Ferment CHO to SCFA
- Nutrient composition: feed differs from inflow to small intestine
  - Little glucose absorption, instead SCFA
  - Tolerate rather low levels of fat in the diet
- Makes postnatal dietary interventions difficult to study
  - But special tricks can be applied

Experimental model (Copenhagen-LIFE):

Pregnant sheep expecting twins

Late gestation (last 6 weeks)	Low diet ~ 50%		Norm diet ~ 100%

Off-spring:

Postnatal nutrition (0 – 6 mo.)	Low/CONV Green hay	Low/HCHF Cream+maize	Norm/CONV Green hay	Norm/HCHF Cream + maize
Postnatal nutrition (6 – 24 mo.)	All sheep fed same moderate grass-based diet All sheep (females only) managed as 1 flock			

Registrations **Challenges** **Samplings**	**0 + 3 + 8 wks + 6 + 12 + 24 mo.**

Adolescent lambs

- 6 months of age (around puberty)
- By the end of the differential feeding treatment
- Metabolic and endocrine challenge tests:
  - Glucose tolerance test
  - Insulin tolerance test
  - Adrenalin tolerance test
  - Fasting (2 d)
- Slaughter -> tissue sampling

Young adult sheep

- 24 months of age (only females)
- After 1½ years of identical management
  - Grass based diet (grazing or silage)
  - Moderate feeding level
- Metabolic and endocrine challenge tests:
  - Glucose+arginine tolerance test
  - Insulin tolerance test
  - Adrenalin tolerance test
  - Fasting (3 d)
- Slaughter -> tissue sampling

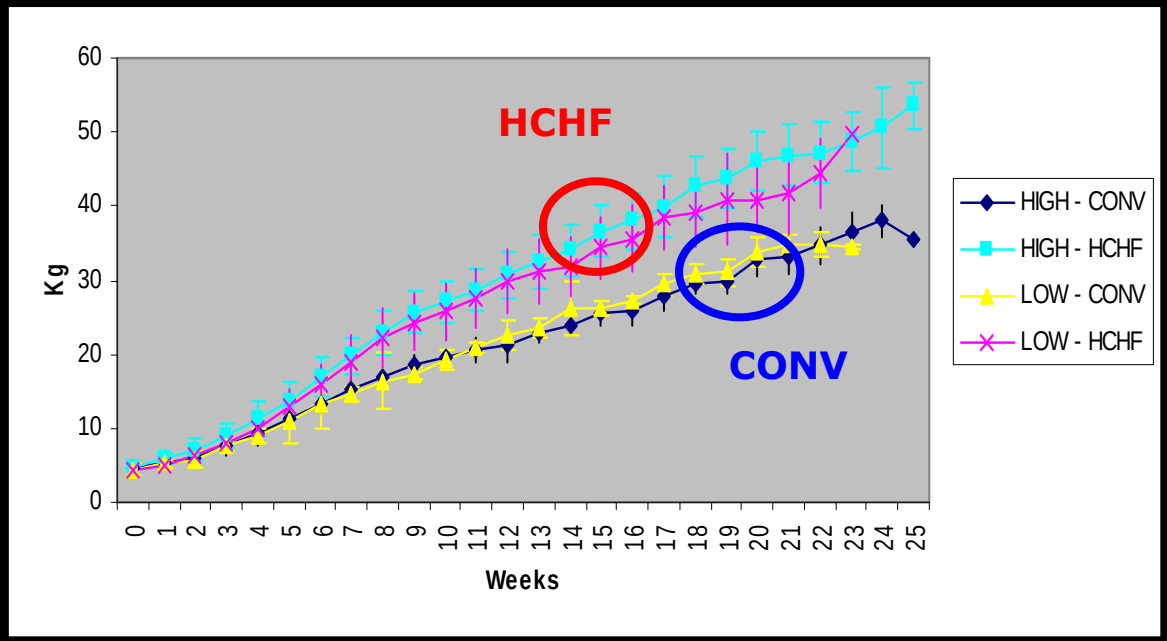
Postnatal energy and protein intake

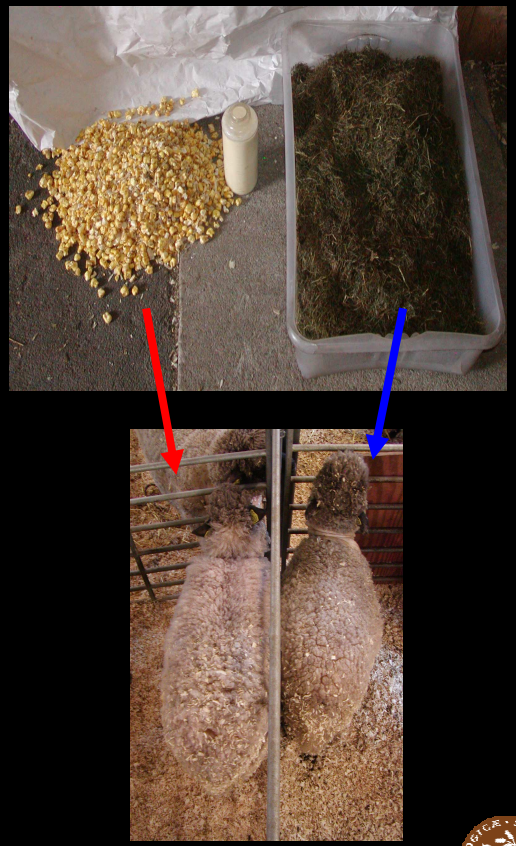
Maternal diet in late gestation	Norm		Low	
Postnatal diet (0-6 mo)	**HCHF**	**CONV**	**HCHF**	**CONV**
Digestible energy intake (MJ DE/d)				
0 - 8 weeks	**11.0**	**6.1**	**11.2**	**5.5**
8 weeks - 6 month	**16.6**	**12.8**	**14.5**	**12.5**
Digestible crude protein intake (g/d)				
0 - 8 weeks	**76.6**	**84.0**	**75.7**	**76.7**
8 weeks - 6 month	**72.0**	**195**	**63.3**	**191**

Growth and development

LG-UN:
- **Reduced birth weight (0.4 kg ~ 10%)**

Postnatal growth:
- **Determined by postnatal diet**
- **But: growth stops earlier in LG-UN**

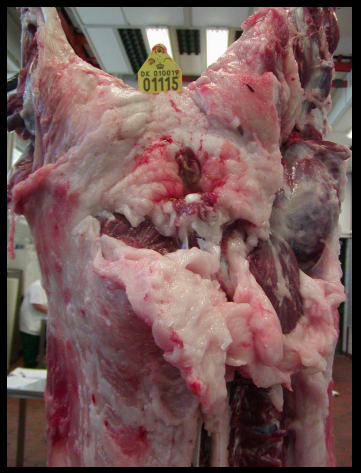




Adolescent lambs (6 mo.):

HCHF **CONV**

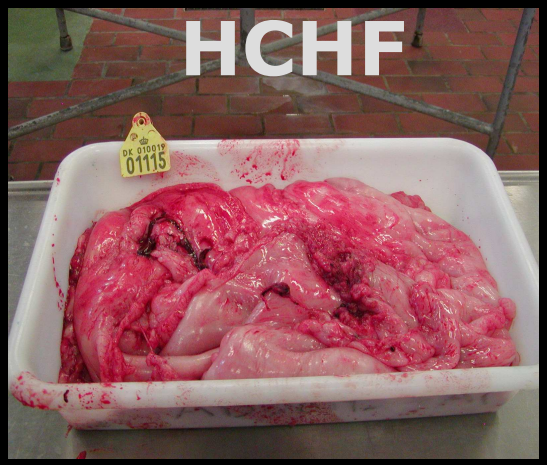
Carcass

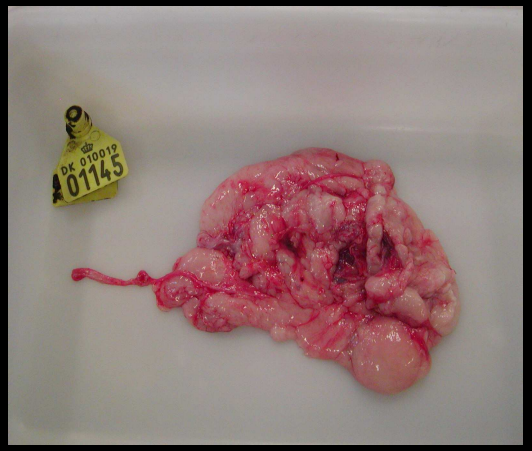


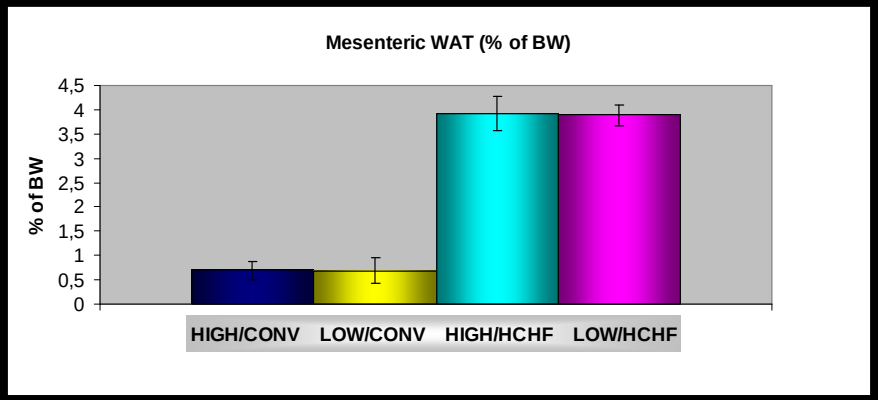
"BMI" in HCHF:
~ 30 (obese)

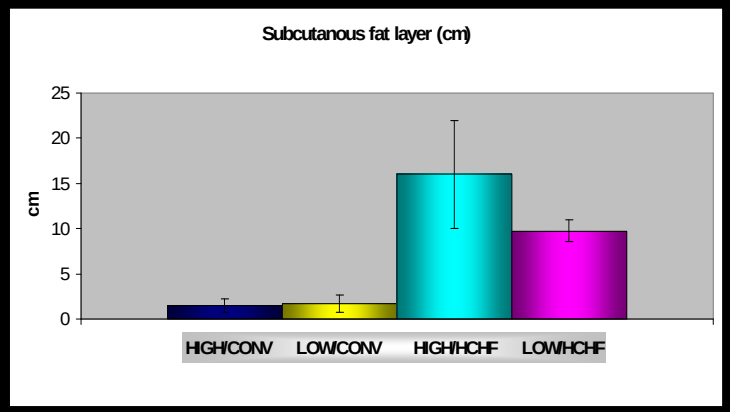
Subcutaneous fat

Mesenteric and subcutaneous fat: postnatal diet



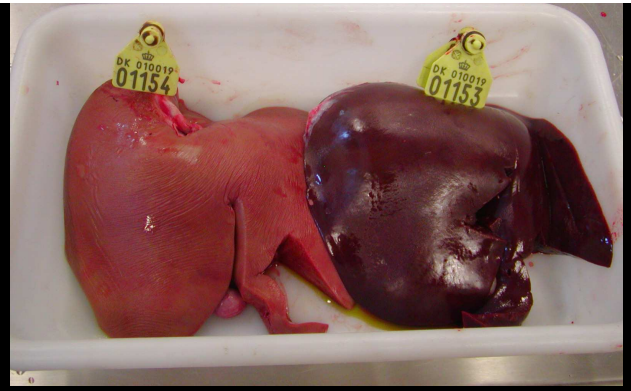


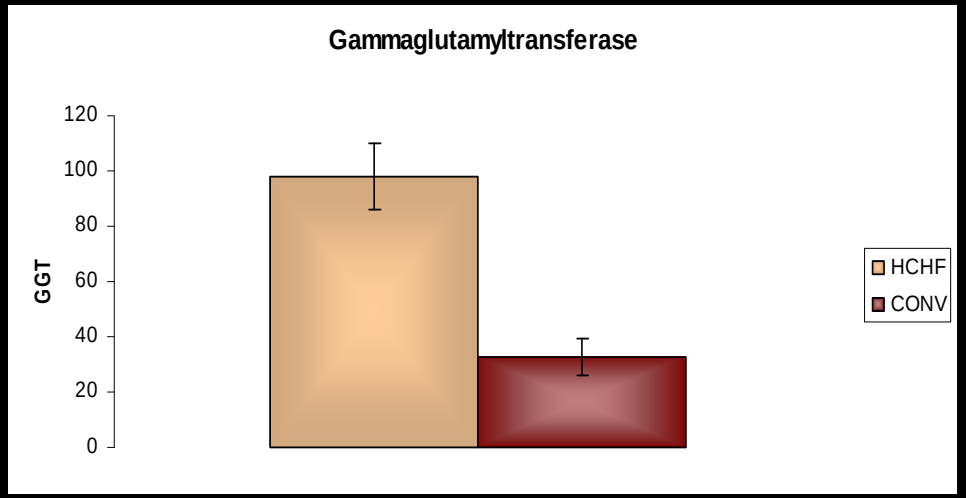


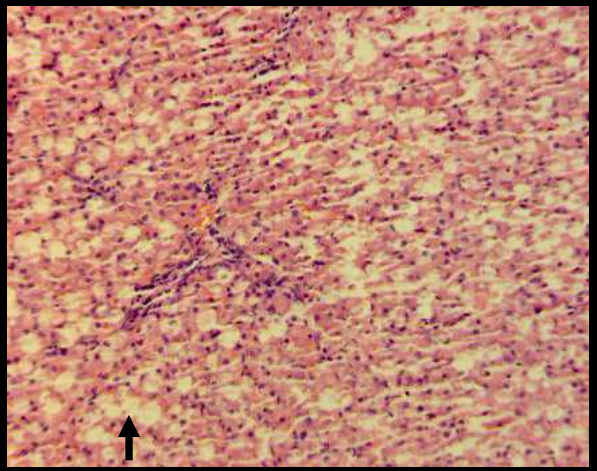


Liver function and morphology (H&E) (6 mo.):

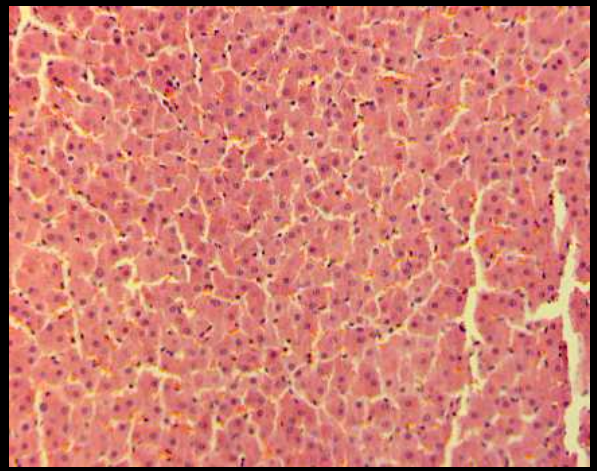
Left: HCHF right: CONV







HCHF



CONV

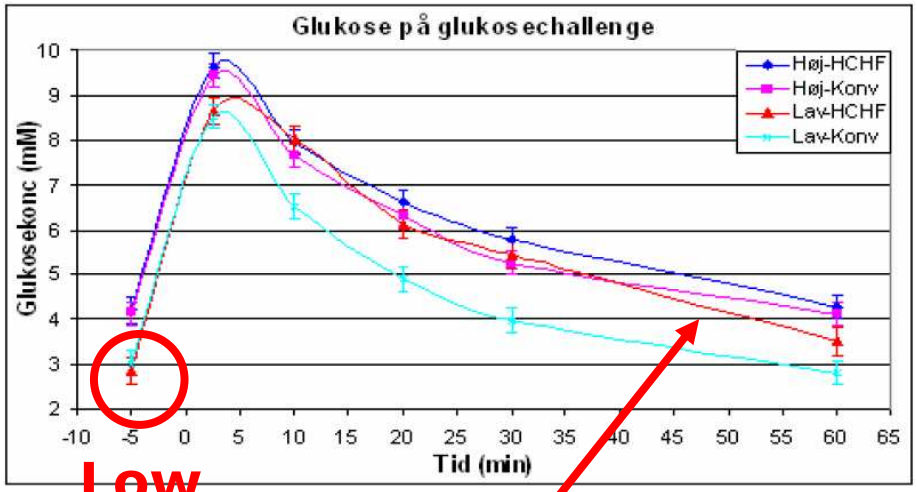
Impact of pre- and postnatal diet: Organ weight – age 6 and 24 mo

	SHEEP DIET		LAMB DIET			
			6 month (♂+♀)		24 month (♀)	
Organ weight	HIGH	LOW	CONV	HCHF	CONV	HCHF
kidneys (g)	113	101	129	> 99.3	110	108
Liver (g)	529	475	577	607	467	475
Adren_gland (g)	3.52	3.68	2.75	2.49	3.97	4.27
Thyroid (g)	2.97	< 3.03	4.12	> 2.92	3.02	3.65
Heart (g)	228	186	161	203	208	235
LD (g)	680	587	420	581	651	657
Biceps_fem (g)	339	300	275	290	329	450
Renal_WAT (g)	772	636	123	1129	562	432
Abdom_fat (g)	1529	1108	277	1803	1157	1549
Subc_fat (mm)	4.21	2.83	1.60	13.2	0.31	0.38

Impact of pre- and postnatal diet: Organ weight (%) – age 6 and 24 mo.

	SHEEP DIET		LAMB DIET			
			6 month (♂ + ♀)		24 month (♀)	
% of body weight	Norm	Low	CONV	HCHF	CONV	HCHF
Kidneys	0.207	0.207	0.324 >	0.210	0.206	0.191
Liver	0.97	1.01	1.45	1.34 ↘	0.87	0.83
Adrenal glands	0.0064 <	0.0076	0.0069	0.0056	0.0075	0.0077
Thyroids	0.005 <	0.006	0.010	0.006	0.006	0.007
Heart	0.419	0.389	0.405	0.443	0.391	0.403
Longissimus Dorsii	1.24	1.20	1.05	1.25	1.20 ↗	1.15 ↘
Biceps femoris	0.604	0.637	0.686	0.626	0.618	0.826
Renal fat	1.39	1.31	0.309	2.43	0.984 ↗	0.717 ↘
Mesenteric fat	2.72	2.34	0.69	3.89	2.08 ↗	2.47 ↘

Glucose tolerance test - 6 mo.: Worst case = glucose intolerant

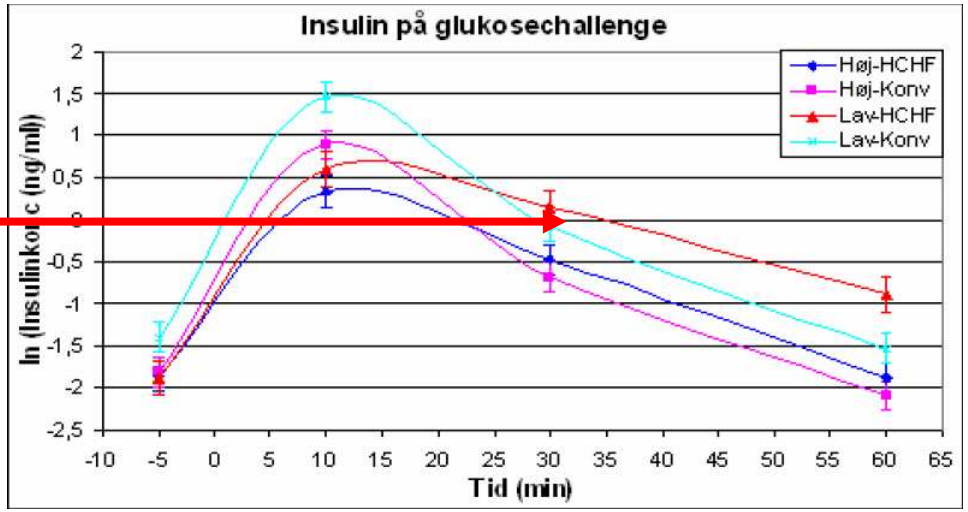


Low

Low/HCHF (interaction):
- Glucose intolerant
- Sustained insulin release
- Insulin clearance reduced

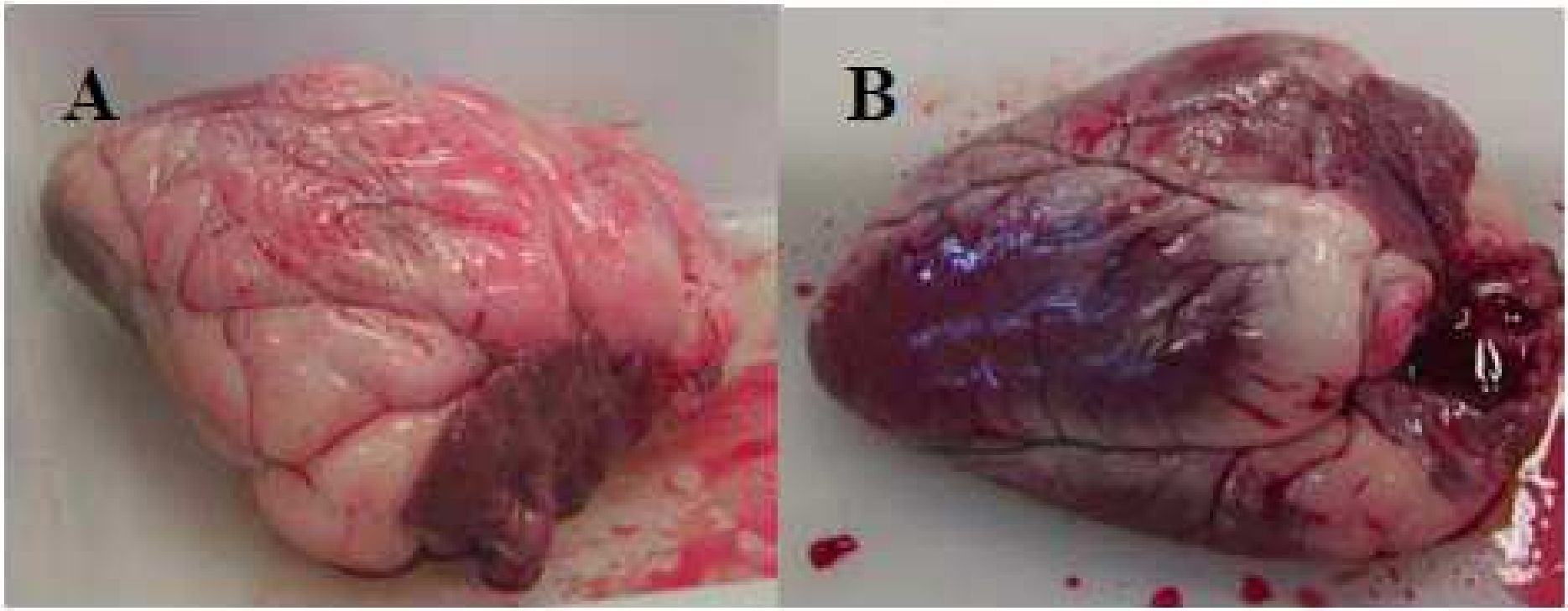
First time we see impact of prenatal nutrition on glucose tolerance in adolescent lambs

Low/HCHF



Cardiac fat infiltration – 6 mo

HCHF **CONV**



Conclusion 1 (adolescence): Pre- and postnatal dietary interactions

LG-UN alters adipose tissue dynamics

- Adipogenesis/maturation of adipocytes delayed/reduced?
- Altered ECM turnover ?
- Increased propensity to adiposity and insulin insensitivity
  - Increased visceral : subcutaneous fat ratio
  - Dietary mismatch (worst case scenario)
- Underlying regulation ?

Conclusion 2 (adolescence): Pre- and postnatal dietary interactions

Hepatic function:

- HCHF lambs showed indications of:
  - Hepatic lipidosis (H&E stainings)
  - Liver function negatively affected
    - Higher gamma glutamyl transferase levels
    - Lower gluconeogenetic capacity (not shown)
  - More lipid based and ketogenic metabolism during fasting (not shown)

- Appeared to be exaggerated in lambs exposed to prenatal undernutrition (Low)
  - Additive effect

Conclusion 3 (adolescence): Pre- and postnatal dietary interactions

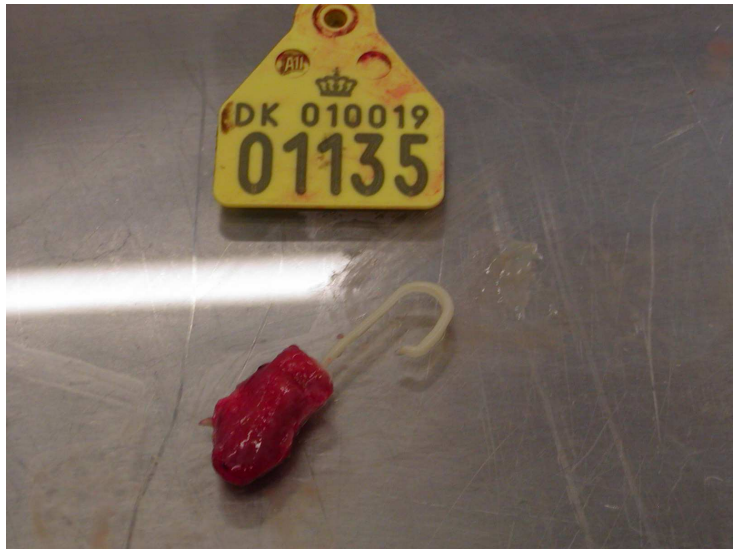
Glucose-insulin homeostasis

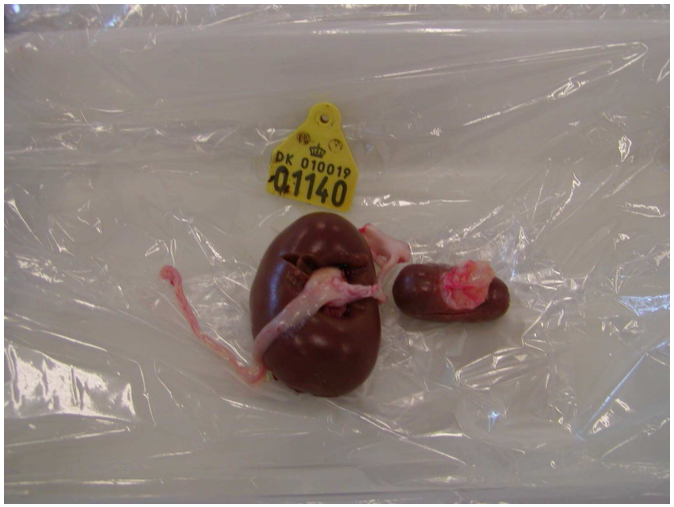
- Worst case scenario: Mismatch in nutrion (Low -> HCHF)
  - Lowered glucose tolerance
  - Lowered insulin clearance
  - Higher total insulin secretion ?
    - Increased stress on pancreas?
  - Increased disposition for visceral fat deposition
    - Particularly on HCHF diet (dietary mismatch)

Adult sheep (ewes) - 2 years of age

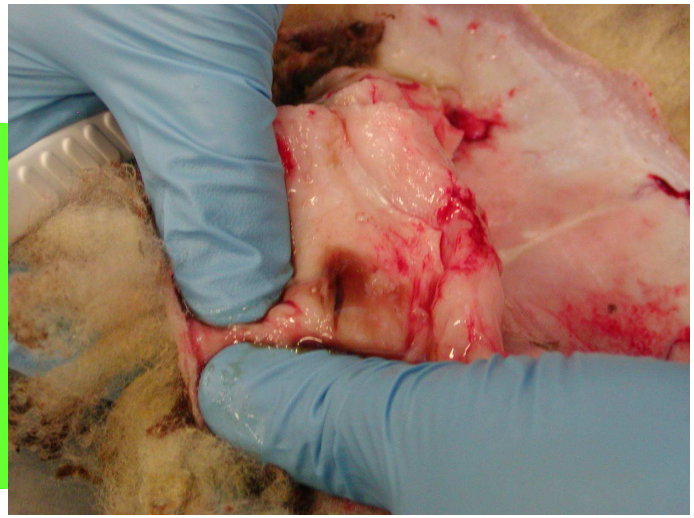
- Differentially fed in late gestation
  - Norm vs. Low
- Differentially fed from 0-6 mo. of age
  - HCHF vs. CONV
- Managed as one flock and identically fed from 6 mo. to 2 years of age on a moderate diet (grass based)

Abnormalities in LOW sheep – 2 years

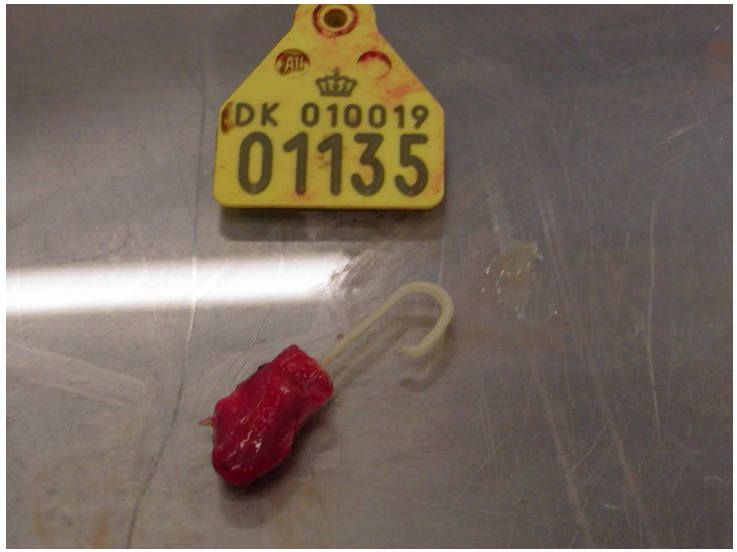


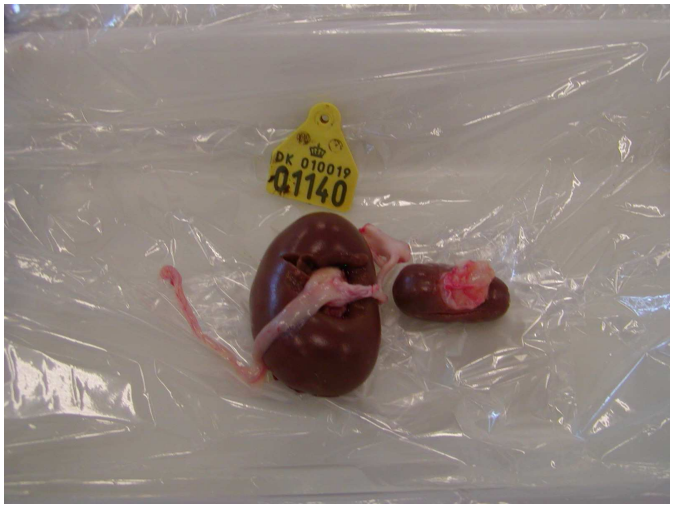


Thyroid:
- Accumulation of cheratin (?)
  Seen in more than half of LOW
  At 6 mo and 24 mo of age
- Not seen in any HIGH
- Functional endocrine tissue mass ? ⬇

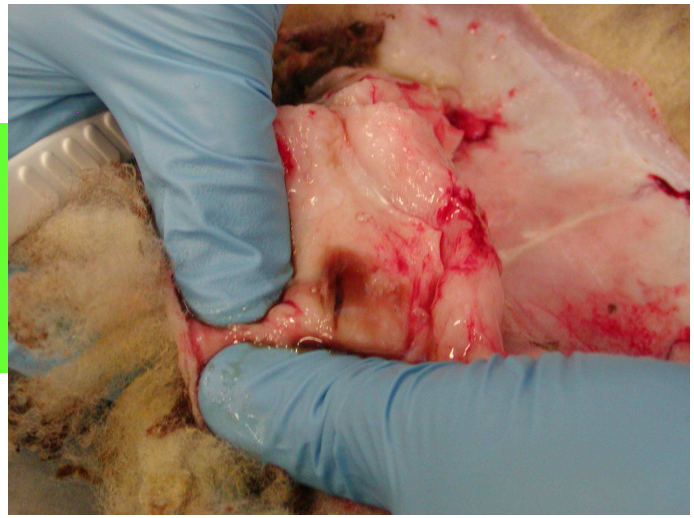


Abnormalities in LG-UN (Low) sheep – 2 years

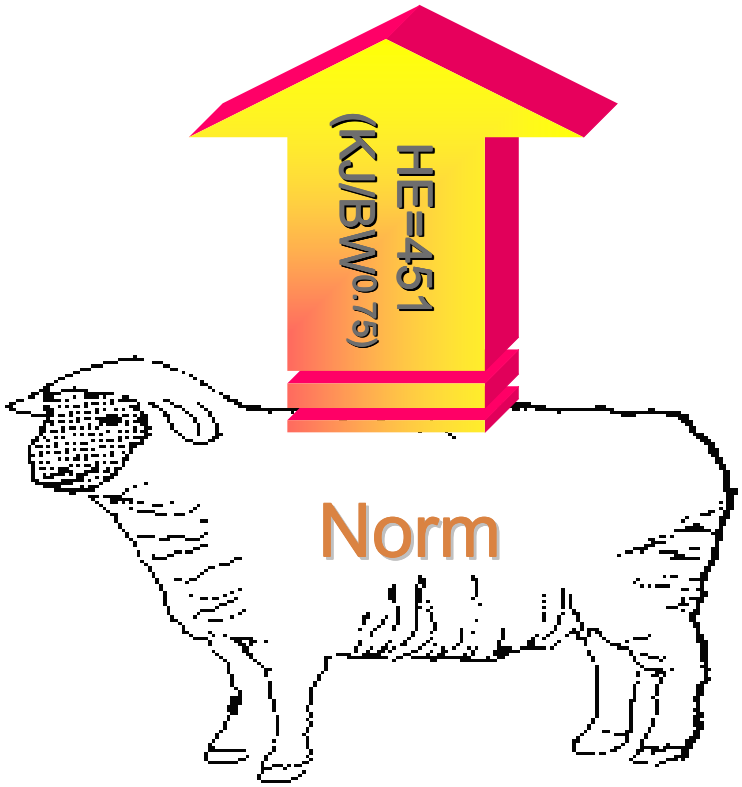


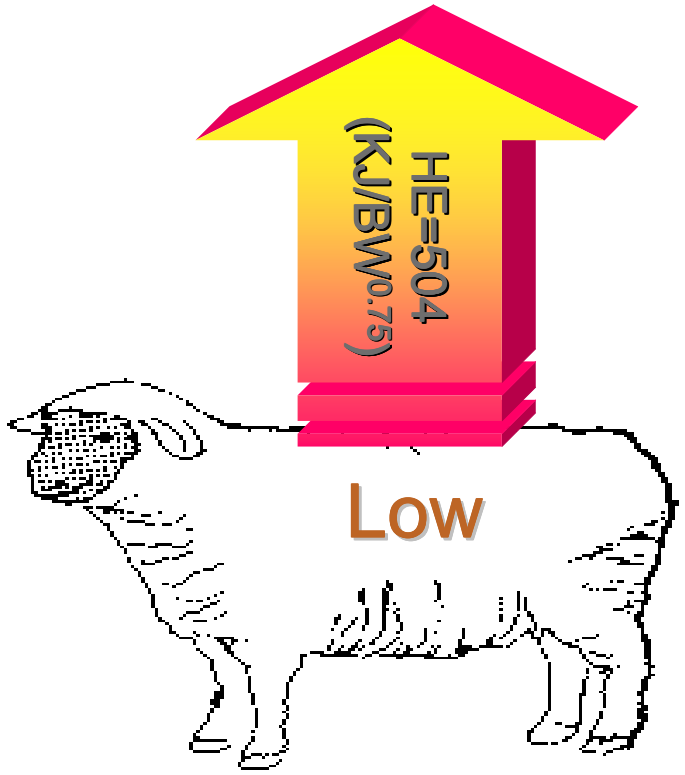


Thyroid:
- Accumulation of cheratin (?)
  Seen in more than half of Low
- Not seen in any Norm



Energy metabolism not more efficient





Smaller but not more energy efficient !!

Kiani et al 2008. Archiv Anim Nutr 62, 117-126

Overall conclusion (1): Pre-postnatal dietary interactions

1. Effects of an unhealthy diet early in *postnatal life:*
    1. Can to a large extent be normalized upon dietary corrections
    2. Except:
        1. Disposition for visceral fat deposition
        2. Metabolic features of m. Longissimus Dorsii
2. But *late gestation undernutrition* programmes for:
    1. Permanent developmental changes
    2. That become increasingly manifested with age
    3. An unhealthy diet early in life exaggerates this

Overall conclusion (2): Pre-postnatal dietary interactions

1. In LG-UN individuals:
    1. Birth weight is reduced (”marker”)
    2. Growth stops earlier (smaller adult body size)
    3. Development of key organs affected (-> anomalities)
        1. Adipose, thyroid, adrenals, mammary (?), kidneys, other?
    4. Metabolic and endocrine function permanently changed
        1. Glucose-insulin axis
        2. Thyroid hormones
        3. Catecholamine sensitivity in adipose tissue
    5. FA composition in hepatic TG and structural lipids programmed -> membrane fluidity
        1. Hormone sensitivity ?
        2. Nutrient transporters etc. ?
    6. Differential effects in different skeletal muscles

Overall conclusion (3): Implications

1. LG-UN associated with increased risk of metabolic diseases later in life
    1. An unhealthy diet early in life increases risk of early onset
        1. Dietary recommendations for SGA babies !!!!
    2. Appropriate markers yet to be identified
        1. Adipose morphology?
2. Early postnatal growth is determined by post-natal feeding
    1. Meat production 👍 (catch-up)
    2. Breeding stock 👎 (epigenetic heritage)
3. The sheep is a powerful animal model

The others:

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