Obesity During Sheep Pregnancy: Implications for the Following Generations

Dr. Stephen P. Ford, PhD
Professor and Rochelle Chair, Department of Animal Science, University of Wyoming, Laramie and Director, Center for the Study of Fetal Programming

Center for the Study of Fetal Programming

- University of Wyoming and the University of Texas Health Sciences Center

- Established at the University of Wyoming in 2001 to evaluate the impacts of maternal undernutrition and overnutrition/obesity on offspring health and growth efficiency.
- Utilized beef cattle and sheep as target species

A working definition of Developmental Programming:

The response to a specific challenge to the mammalian organism during a critical developmental time window that alters the trajectory of development qualitatively and/or quantitatively with resulting effects on health that are persistent throughout life.

Advantages of the Sheep as a Model for Human Pregnancy Studies

- The fetal sheep has a metabolism similar to the human fetus as shown be a large number of studies world wide.
- The importance and relevance of all the metabolic studies is that the fetal sheep is, like the fetal human, dependent on glucose as its major source of energy.
- The sheep is a monotocous precocial specie of similar size and weight to humans, and with a similar maternal to fetal weight ratio at term.
- The temporal pattern of fetal tissue and organ development during gestation is similar to the human.
- The fetus is large enough to be catheterized and instrumented

Facility used for nutritional studies at the Laramie Research and Extension Center

Epigenetic Changes

Recent evidence suggests that environmentally induced epigenetic changes in gene expression are one of the most important mechanisms mediating the observed alterations in offspring phenotype.

Environmentally-induced changes in gene expression may provide a mechanism whereby parents can pass on important information about the environment they will experience in postnatal life.

YOUR PHENOTYPE IS MORE IMPORTANT TO YOUR LIFE LONG HEALTH THAN YOUR GENOTYPE.

Rational for the Development of a Model of Maternal Obesity

- Obesity during pregnancy is increasing
- High prepregnancy BMI is associated with fetal overgrowth & newborn adiposity
- Fetal overgrowth is associated with…
  - offspring obesity, insulin resistance, type II diabetes, hypertension and coronary heart disease in adolescence & later life

Obesity

1. Body mass index (BMI) equal to or greater than 30
2. BMI = weight (kg) / height ^{2} (m^2).
3. Recognized as a systemic disease.

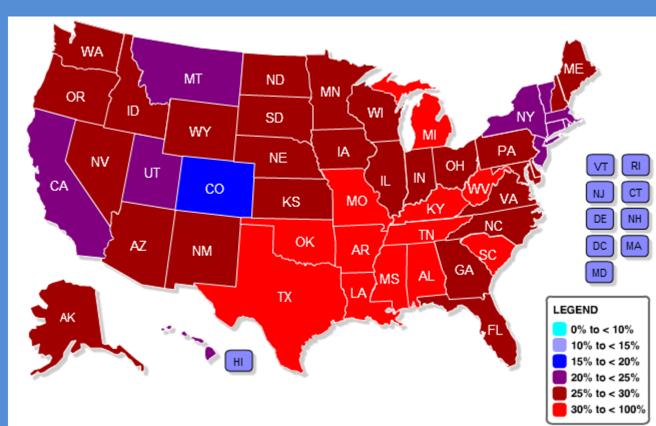
Obesity

Obesity ---- serious problem in the United States and many areas around the world.

Thirty-eight states had adult obesity rates above 25 percent, based on a report from the Trust for America's Health (TFAH, 2013).

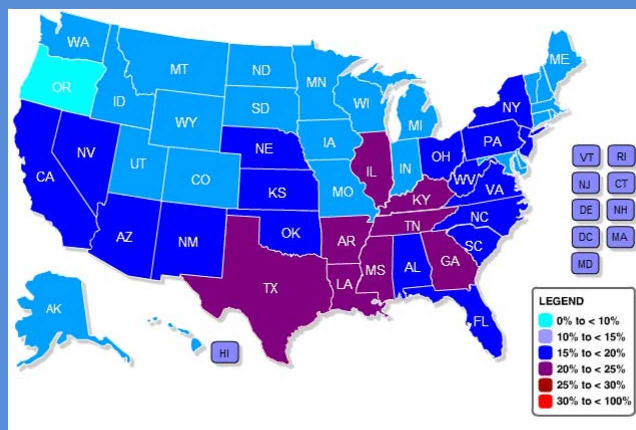
NOTE: only one state (Mississippi) was above 25% five years ago!!!

ADULT OBESITY RATES



TRUST FOR AMERICA'S HEALTH 2013 http://www.healthyamericans.org/report/88/

CHILDHOOD OBESITY RATES



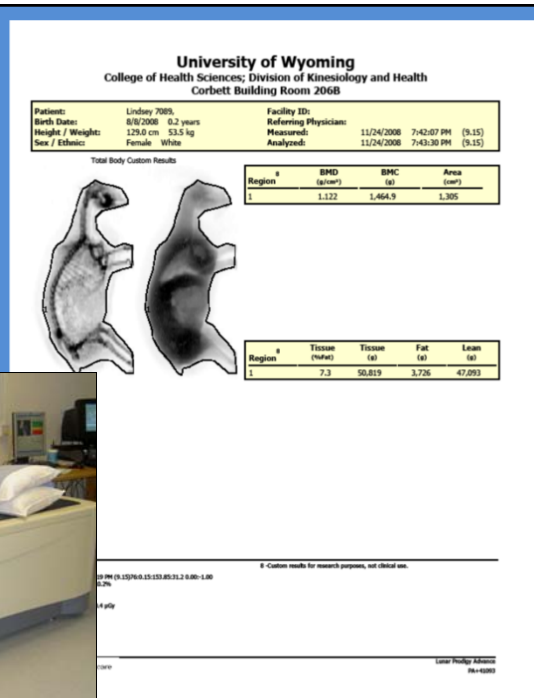
TRUST FOR AMERICA'S HEALTH 2013 http://www.healthyamericans.org/report/88/

Overnourished/Obese Pregnancy: Methods

- **Ewes carrying singleton fetuses were fed a high energy, highly palatable pelleted diet (88.2% DM, 13.5% CP, 4.05% fat) from 60 days before conception through gestation.**
  - **100% NRC recommendations (Control, C)**
  - **150% of the Control diet (Obese, OB)**
- **Mid-gestation glucose tolerance test (GTT)**
- **Mid-gestation and late gestation necropsy**
- **Lambing**

GrowSafe System

DEXA:
Dual Energy X-ray Absorptiometry



University of Wyoming
College of Health Sciences; Division of Kinesiology and Health
Corbett Building Room 206B

Patient:	Lindsey 7089,	Facility ID:	
Birth Date:	8/8/2008 0.2 years	Referring Physician:	
Height / Weight:	129.0 cm 53.5 kg	Measured:	11/24/2008 7:42:07 PM (9.15)
Sex / Ethnic:	Female White	Analyzed:	11/24/2008 7:43:30 PM (9.15)

Total Body Custom Results

Region	BMD (g/cm²)	BMC (g)	Area (cm²)
1	1.122	1,464.9	1,305

Region	Tissue (%Fat)	Tissue (g)	Fat (g)	Lean (g)
1	7.3	50,819	3,726	47,093

8 -Custom results for research purposes, not clinical use.

Lunar Prodigy Advance

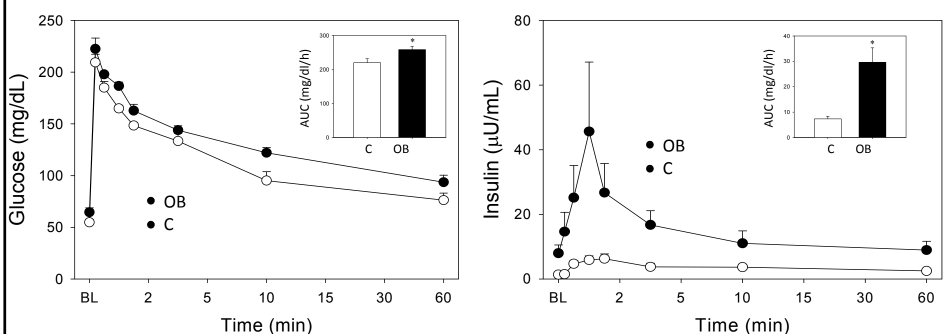
Monthly Increase in Body Weight and Body Condition Score of OB and C Ewes

Treatment	-60days	-30 days	0 days	+35 days	+75 days	+135 days
Weight (% increase) :						
Control	$68.3 \pm 2.9kg^{a}$	$+ 3.5\%^{a}$	$+ 3.9\%^{a}$	$+ 4.4\%^{a}$	$+ 5.7\%^{a}$	$+10.9\%^{b}$
Over-fed	$71.6 \pm 3.2kg^{a}$	$+ 23.7\%^{b}$	$+ 29.6\%^{c}$	$+ 39.5\%^{c,d}$	$+ 48.7\%^{d}$	$+67.3\%^{e}$
Body Condition Score:						
Control	4.9 ± 0.4^{a}	4.7 ± 0.4^{a}	4.8 ± 0.3^{a}	5.0 ± 0.4^{a}	4.9 ± 0.4^{a}	5.2 ± 0.5^{a}
Over-fed	5.0 ± 0.3^{a}	6.4 ± 0.3^{b}	7.2 ± 0.2^{bc}	7.8 ± 0.2^{c}	8.0 ± 0.2^{c}	8.7 ± 0.2^{d}

^{abcde}Means $\pm$ SEM within a row with no common superscript differ ($P < 0.05$).
n=6 for each group

Control and Obese Overfed Ewe at Midgestation

Concentration of Glucose and Insulin in Maternal Blood on D75 0f Gestation



Mean + SEM; n = 6 in each group; *Area Under the Curve differs by dietary treatment (P<0.01)

Day 75 maternal and fetal hormonal profile. (*P<0.05; **P<0.001)

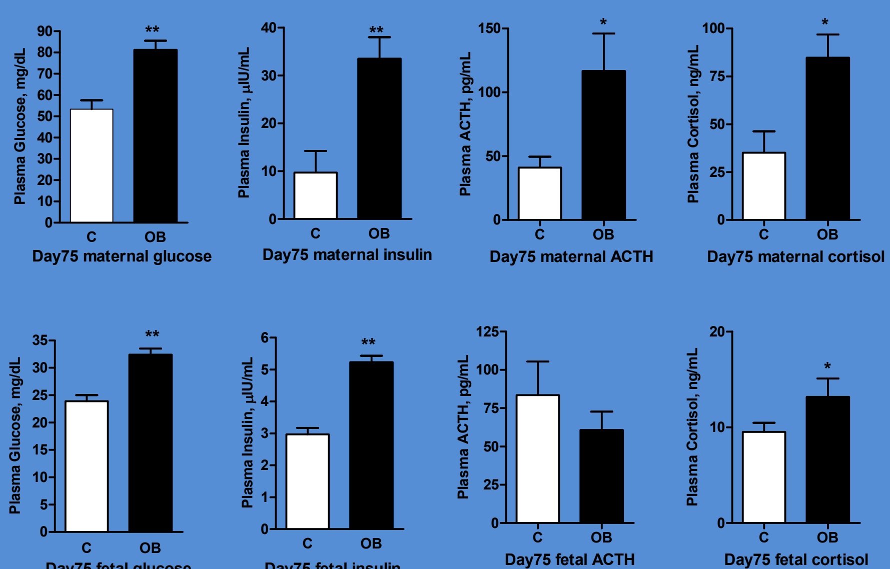
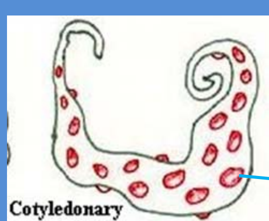


Table 1. Selected fetal organ measurements in control (C), maternal obese (OB) dietary groups at mid- gestation.

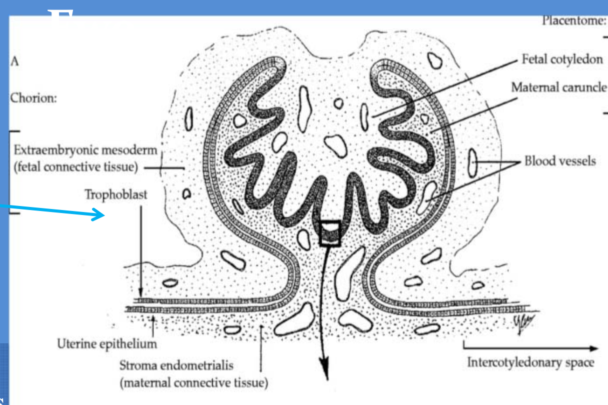
Item	Day 75	
	C	OB
Fetal wt, g	268 ± 12[a]	374 ± 10[b]
Right Ventricle wt, g	0.50 ± 0.03[a]	0.64 ± 0.03[b]
RV thickness, cm	1.62 ± 0.05[a]	2.46 ± 0.05[b]
Left ventricle wt, g	0.82 ± 0.03[a]	0.99 ± 0.04[b]
LV thickness, cm	2.44 ± 0.06[a]	3.48 ± 0.06[b]
Total kidney wt, g	2.47 ± 0.11[a]	3.09 ± 0.11[b]
Pancreas wt, g	0.24 ± 0.02[a]	0.47 ± 0.03[b]
Liver wt, g	14.53 ± 0.52[a]	17.27 ± 0.52[b]
Perirenal fat wt, g	1.02 ± 0.08[a]	1.36 ± 0.08[b]

[a,b]Means ± SEM within a gestational age and measurement with different superscripts differ (P<0.05; n=6).

Introduction-Sheep Placenta



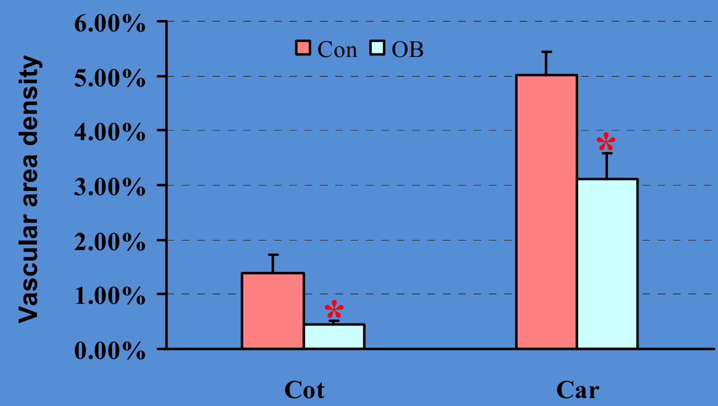
Sheep placenta



Placentome

A ruminants placenta is covered with **placentomes** composed of maternal caruncular (CAR) and fetal cotyledonary (COT) tissues, where maternal:fetal nutrient , gas and waste exchange takes place.

Placental Vascularity Decline at Midgestation in OB Vs. C Ewes



Vascularity in both CAR and COT tissues of OB and C ewes at d 75 of gestation

*(P<0.01, n=10)

Ovine Placentomes

•In association with the midpregnancy decline in placental vascularity, we also observed:

- Decreased COT mRNA and protein expression of angiogenic factors in OB ewes vs. C ewes.

- Decreased COT mRNA and Protein expression of Glucose and Amino Acid Transporters in OB vs. C ewes

Our Hypothesis

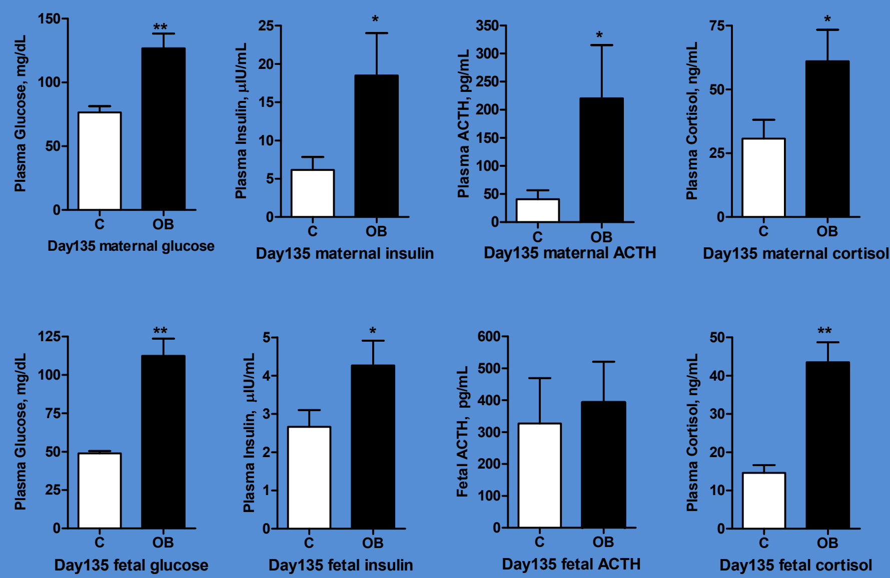
- The decreased COT vascularity in OB ewes reduced nutrient delivery to the fetuses and slowed fetal growth rate, thereby protecting the fetus from overgrowth in late gestation
- Since angiogenesis facilitates the increase in placentomal vascularity throughout pregnancy, changes in angiogenic factor expression may play an important role in reducing COT vascularity

Table 2. Selected fetal organ measurements in control (C) and maternal obese (OB) dietary groups at late gestation.

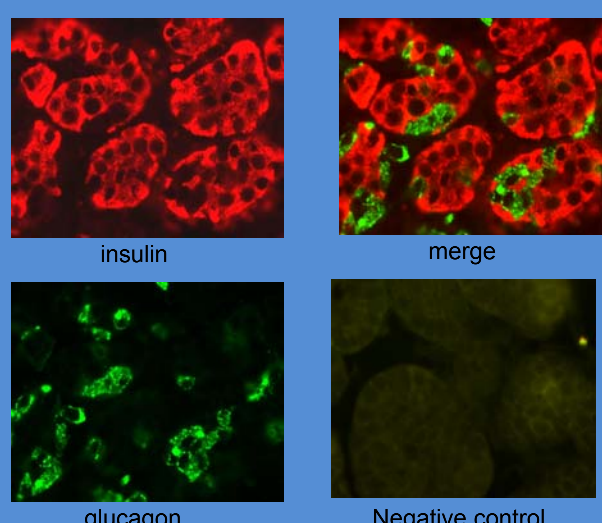
Item	Day 135	
	C	OB
Fetal wt, kg	5.05 ± 0.28[a]	5.02 ± 0.25[a]
Right Ventricle wt, g	7.77 ± 0.55[a]	9.87 ± 0.57[b]
RV thickness, cm	4.97 ± 0.18[a]	6.07 ± 0.19[b]
Left ventricle wt, g	10.84 ± 0.72[a]	12.64 ± 0.72[b]
LV thickness, cm	6.74 ± 0.23[a]	8.38 ± 0.24[b]
Total kidney wt, g	21.57 ± 0.97[a]	23.89 ± 1.02[a]
Pancreas wt, g	4.55 ± 0.30[a]	2.98 ± 0.27[b]
Liver wt, g	102.38 ± 6.89[a]	119.54 ± 7.23[a]
Total Perirenal fat wt, g	24.20 ± 1.31[a]	31.40 ± 1.37[b]

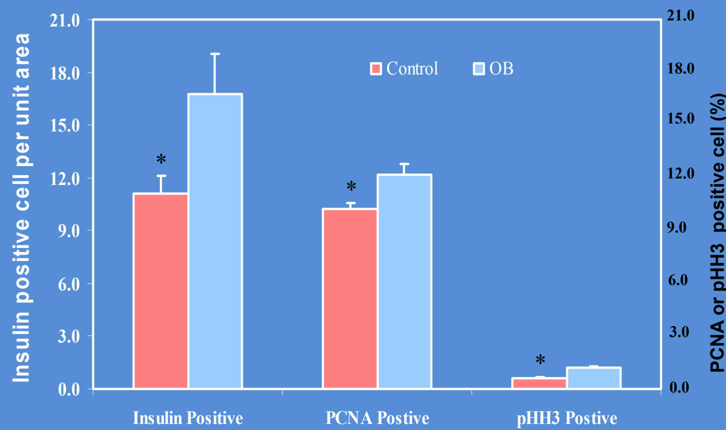
[a,b]Means ± SEM within a gestational age and measurement with different superscripts differ (*P*<0.05; n=6).

Day 135 maternal and fetal hormonal profile. (*P<0.05; **P<0.001)



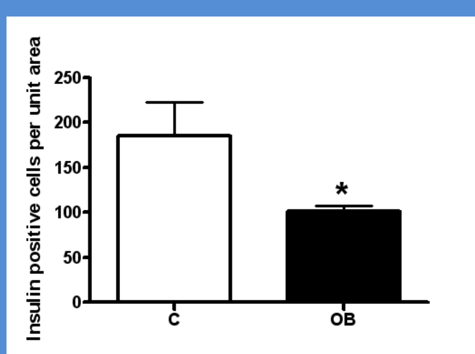
Fetal pancreas dual fluorescent stained for glucagon (green) and insulin (red)

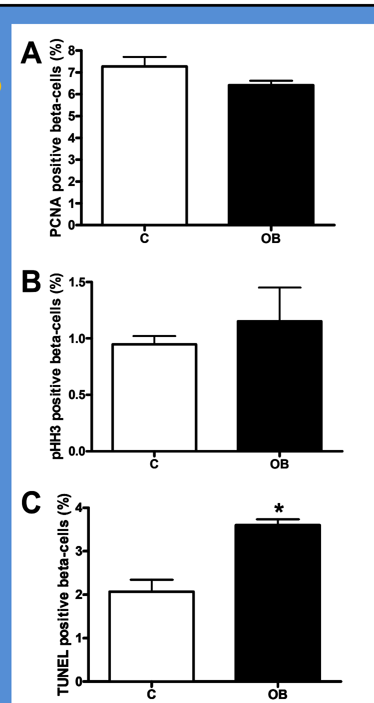




Numbers of insulin positive cells, and the percentage of insulin positive cells staining positively for PCNA and pHH3 in the pancreas of control and obese ewes at 75 days of gestation. , *Means ± SEM differ ($P<0.01$; n=5)

Pancreatic Data From Day 135 C and OB Fetuses





*Means ± SEM differ ($P<0.05$); n=5/group

Day 75 fetuses of C & OB ewes DEXA scan

	Control (n=7)	Obese (n=6)
Maternal % Fat	17.7 ± 1.3[a]	28.6 ± 1.6[b]
Fetal compartment % Fat	10.6 ± 1.6[a]	20.5 ± 3.1[b]

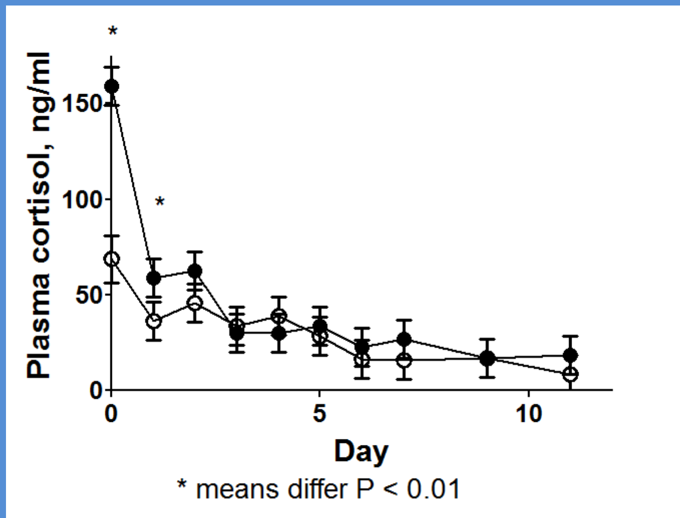
Means ± SEM (within row a,b means differ P < 0.05)

Newborn lambs of C & OB ewes

	Control (n=5)	Obese (n=5)
Birth weight, kg	5.31 ± 0.49	6.28 ± 0.54
CRL,cm	58.2 ± 1.1[c]	53.9 ± 1.1[d]
% Fat	5.66 ± 0.75[a]	13.22 ± 0.71[b]

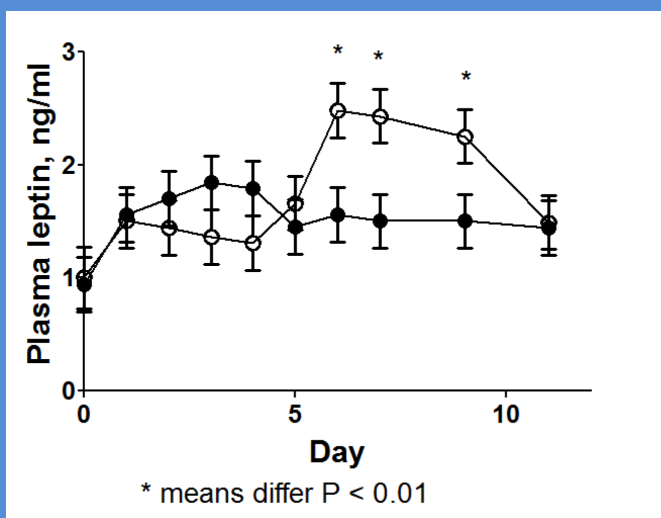
Means ± SEM (within row a,b means differ P < 0.01; c,d means differ P < 0.05)

F1 Offspring – Plasma Cortisol



C offspring (open circles, n=5)
OB offspring (closed circles , n=5)

F1 Offspring – Plasma Leptin



C offspring (open circles, n=5)
OB offspring (closed circles, n=5)

Neonatal Leptin Surge

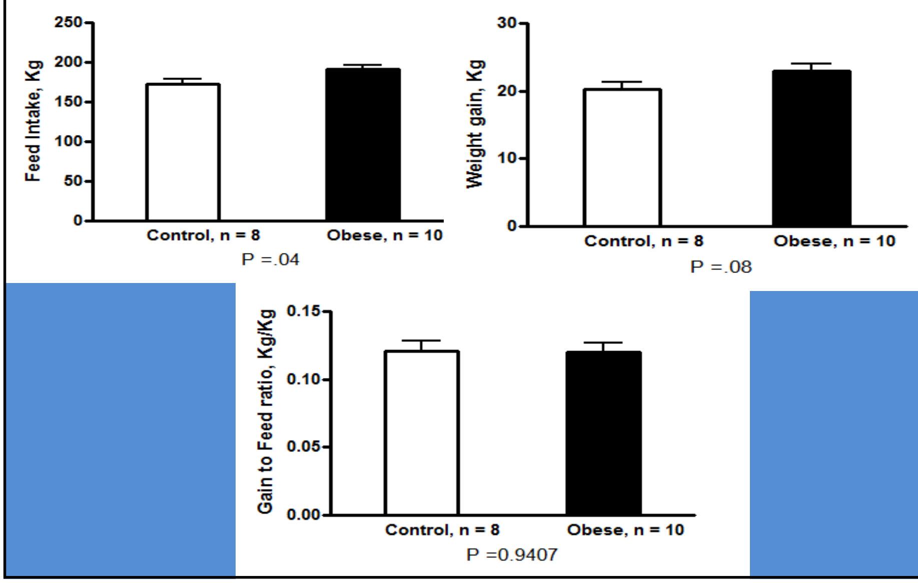
•A neonatal leptin surge has been shown in the rodent to be responsible for programming the hypothalamic appetite control center, which regulates appetitive behavior in later life

•Recent studies in obese rodents demonstrated that administration of leptin to offspring during the early postnatal period can reduce appetite and prevent the development of obesity and the metabolic syndrome in their offspring.

Methods: Adult Male and Female Offspring of C and OB ewes

- Male and female lambs born to C & OB ewes
  - Managed together
  - Fed only to requirements
  - 19-22months (approx. 20's in human years)
- Fed *ad libitum* 11 weeks
  - GTT
  - DEXA
  - Feeding behavior, GrowSafe

Results from Mature F1 Offspring After the Ad Lib Feeding Trial



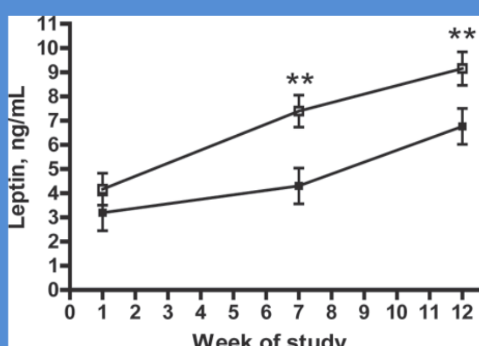
DEXA Evaluation of Mature F1 Offspring Before and After the *Ad Lib* Feeding Trial

	Control	Obese	P-value
Initial DEXA Scan	n = 8	n = 10	
Fat (%)	5.42± .79	5.20 ± 0.69	0.83
Lean (%)	88.03 ± 0.69	88.46 ± 0.61	0.65
Bone mineral density	1.20 ± 0.02	1.20 ± 0.02	0.91
Weight of fat tissue (g)	3432 ± 706	3180 ± 390	0.48
Final DEXA Scan			
Fat (%)	16.53 ± 1.21	20.75 ± 1.12	0.02
Lean (%)	78.42 ± 1.24	73.05 ± 1.15	0.02
Bone mineral density	1.24 ± 0.02	1.22 ± 0.02	0.40
Weight of fat tissue (g)	13694 ± 1320	16898 ± 1230	0.09

GTT Results of Mature F1 Offspring Before and After the Ad Lib Feeding Trial

	Control	Obese	P-value
Initial GTT Results	n=8	n=10	
Insulin Sensitivity (SI), x10^{-4}mIU^{-1}·L·min^{-1}	3.12 ±0.44	1.84 ±0.39	0.046
Insulin independent glucose clearance (Sg), x10^{-2}min^{-1}	0.027 ±0.003	0.016 ±0.003	0.028
Acute insulin response to glucose (AIRg), mIU·L^{-1} ·min	313.6 ±67.1	367.2 ±60.0	0.560
Disposition index	823.1 ±144.2	648.6 ±127.2	0.380
Final GTT Results			
Insulin Sensitivity (SI), x10^{-4}mIU^{-1}·L·min^{-1}	3.38 ±0.19	0.93 ±0.18	0.010
Insulin independent glucose Clearance (Sg), x10^{-2}min^{-1}	0.068 ±0.014	0.022±0.014	0.035
Acute insulin response to glucose (AIRg), mIU·L^{-1} ·min	1082.4 ±144.7	660.0 ±136.4	0.048
Disposition index	1305.0 ±146.1	656.4 ±137.8	0.005

Leptin concentrations of Mature F1 Offspring Throughout the Ad Lib Feeding Trial



■; OBF1 n = 8
□; CONF1 n = 10

Plasma concentrations of leptin at week 1, 7, and 12 during an ad lib feeding challenge from 19 to 22 months of age in F1 offspring (**differ P < 0.025)

These data clearly demonstrate that maternal overnutrition/obesity lead to:

1) **Decreased fetal pancreatic growth and ß-cell numbers by late gestation.**

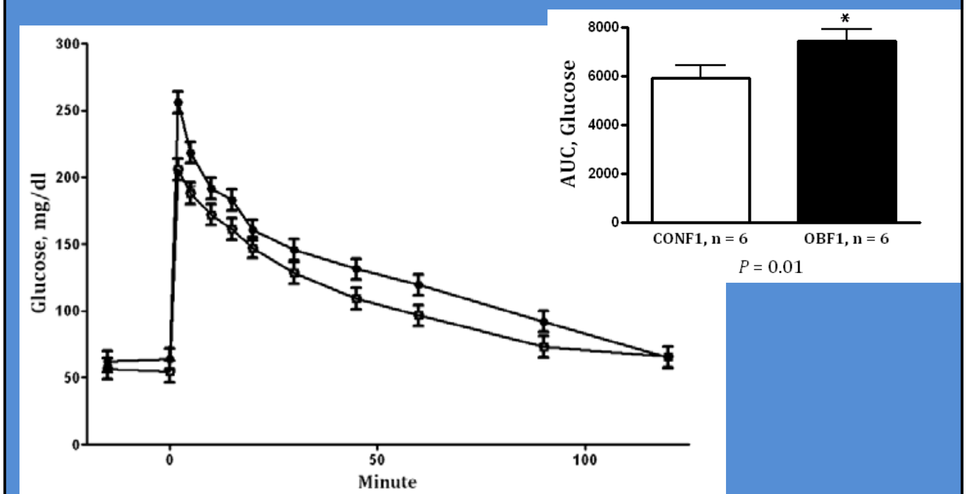
2) **Alterations in appetite, as well as glucose/insulin dynamics and adiposity in postnatal life, which are exacerbated by an *ad libitum* feeding challenge.**

- **Additional studies are needed to determine exactly when these metabolic alterations occur, how they change as offspring age, and how postnatal nutrition can either exacerbate or inhibit these phenotypic changes from emerging.**

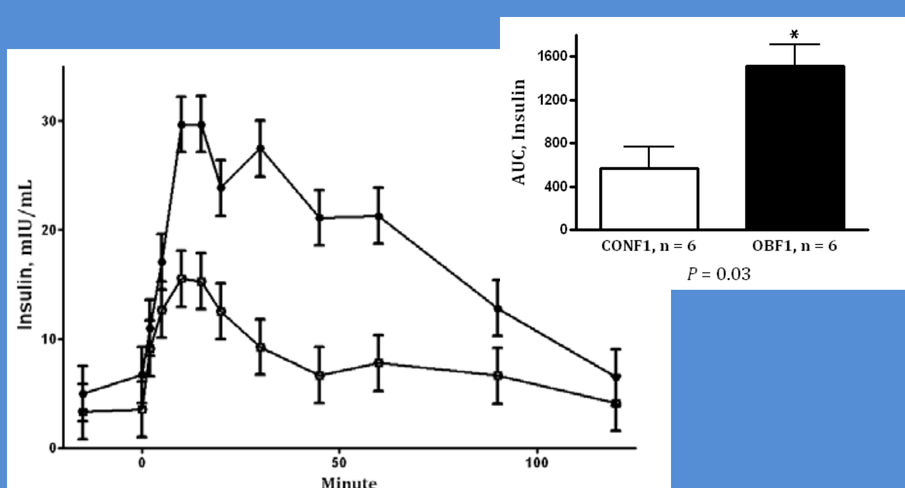
Methods: F1 offspring pregnancy study

- Three year old ewes born to C or OB ewes
  - Managed together
  - Fed only to requirements
  - Bred to a single ram
- Fed only to requirements during gestation
  - GTT at day 75 and 135
  - Lambs weighed at birth
  - DEXA of Lambs at birth and daily blood samples taken

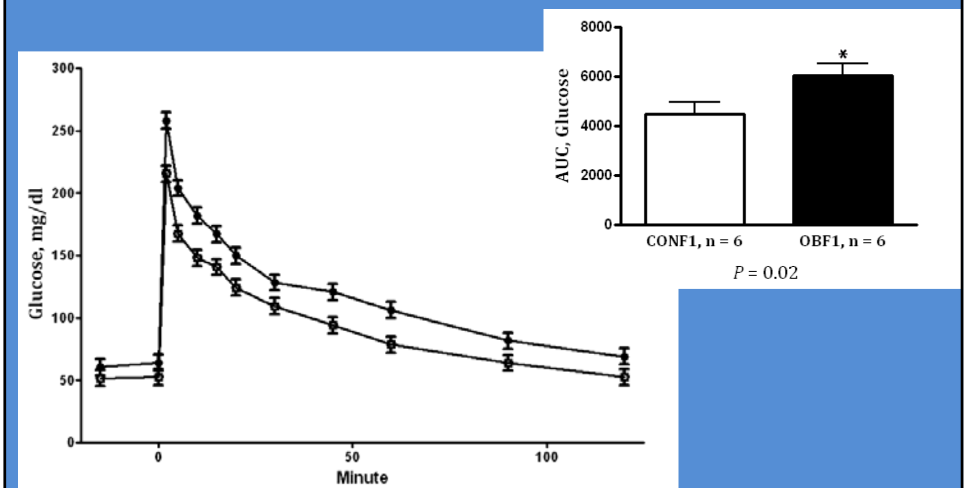
Glucose Concentrations of OBF1 and CONF1 Ewes on Day 75 of Gestation



Insulin Concentrations of OBF1 and CONF1 Ewes at Day 75 of Gestation



Glucose concentrations of OBF1 and CONF1 ewes at day 135 of gestation



Insulin Concentrations of OBF1 and CONF1 Ewes at Day 135 of Gestation

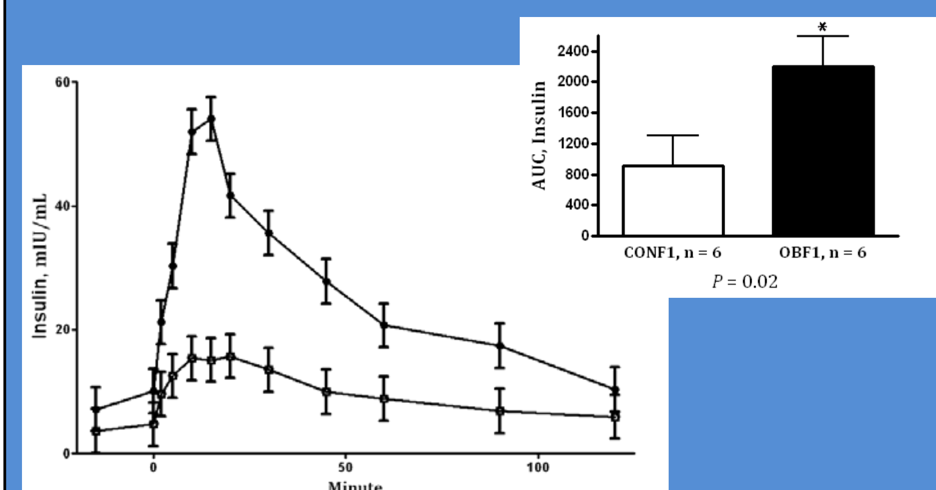
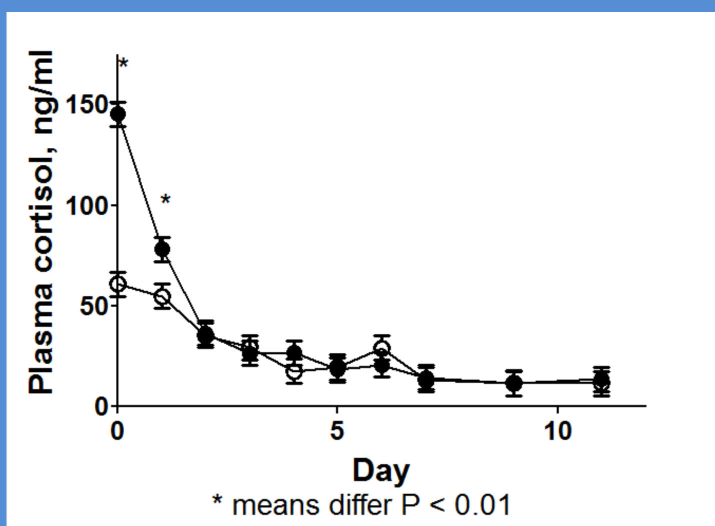


Table 1. Birthweight and % fat determined by DEXA scanning in F1 and F2 newborm lambs from Obese and Control F0 mothers

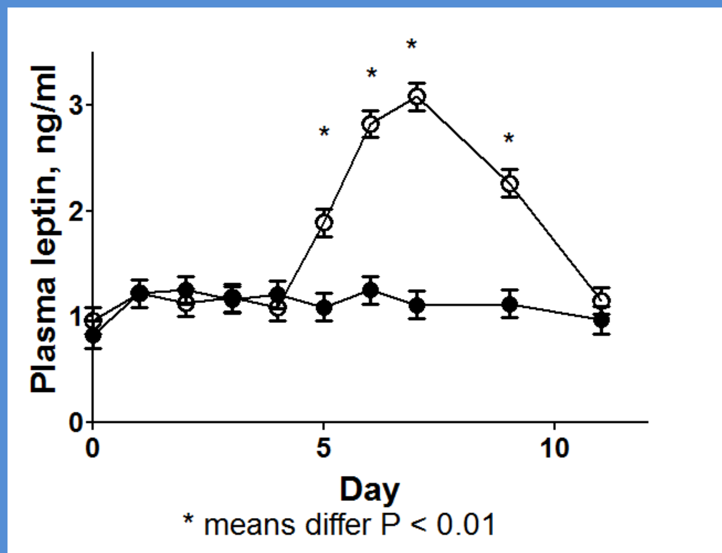
	F1 offspring		F2 offspring	
	Control	Obese	Control	Obese
	n = 5	n = 5	n = 8	n = 8
Birthweight, kg	5.31 ± 0.49	6.28 ± 0.54	5.00 ± 0.20	5.20 ± 0.20
% fat at birth	5.66 ± 0.75[a]	13.22 ± 0.71[b]	5.66 ± 0.75[a]	9.70 ± 0.60[b]
[a,b] means differ P < 0.01				

F2 Offspring – Plasma Cortisol



Offspring of C F1 ewes, Open circles, n=8
Offsring of OB F1 ewes, Closed circles, n=8

F2 Offspring – Plasma Leptin



Offspring of C F1 ewes, Open circles, n=8
Offspring of OB F1 ewes, Closed circles, n=8

Adult OBF2 and CF2 offspring in response to a bout of ad libitum feeding

- OBF2 offspring were insulin resistant and had elevated blood levels of glucose and insulin
- OBF2 offspring ate more feed and gained more weight predominantly as fat
- OBF2 offspring had elevated blood leptin

Conclusion

- **These data provide clear evidence that maternal overnutrition/obesity can alter the phenotype of offspring both in utero and into postnatal life.**
- **Further, these effects are observed at least 2 generations downstream from the original in utero exposure.**

Take-Home Message

- Perhaps most importantly, these data demonstrate that when fed only to requirements, OB offspring exhibit no differences in body weight or body composition from C offspring until allowed to consume unlimited amounts of feed, suggesting that diet per se has a potential in mitigating disease onset, even after programming.

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Timeline of bovine fetal development. The portion in red represents the period of feed restriction in the study