

PROGRAMMED SYNDROME OF HYPERNATREMIC HYPERTENSION IN OVINE TWIN LAMBS

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Health Epidemics: Hypertension and Obesity

- Hypertension: 29% of U.S. population
- Obesity:- U.S. adults 65% overweight, 31% obese
Childhood obesity 20%
- Etiology? -Food availability, high fat diets, reduced energy expenditure
 - Predisposition to Obesity, Obesity associated HTN
- ? Result of "gestational programming"
 - In utero stress: nutrient, oxygen, steroids
 - Postnatal environment: nutrition

Barker Hypothesis Gestational Programming

- Small for Gestational Age (SGA) and/or Low Birth Weight (LBW) human newborns
 - Paradoxical increased risk of obesity and hypertension as adult
- "Thrifty Phenotype"

Gestational Programming

"...a process whereby a stimulus or stress, at a critical period of development, has lasting or lifelong significance..."

- Varying effects dependent upon developmental period of fetus/neonate, species
- Altered cell number, organ structure, hormonal axes, gene expression
- Possible survival advantage for species

Mutations vs Programming

Genetic mutations: Long epochs,
irreversible

Programmed phenotypes: Respond to
acute environmental stresses

Environmental Stresses on Survival

Famine: Nutrient reduction

Drought: Water reduction

Secondary Nutrient Reduction:

Associated Famine

Dehydration-anorexia

Result in LBW infants with reduced
nutrient availability as offspring
Benefits of "Thrifty Phenotype"

“Inadvertent Thrifty Phenotype”

- IUGR:
 - Maternal hypertension, autoimmune
 - Substance abuse, cigarette smoking
- Twins and higher order:
 - Natural and IVF
- Enhanced Survival

High fat, high calorie diet



Human Twin Births

- Natural model of low birth weight
 - Smaller than singletons at birth
 - Reduced growth throughout gestation
 - Relative prematurity
- Since 1980, twin birth ↑74% in the US
 - 3.2% of births in 2001

Gestational Programming in Human Twins

- Reduced adult height in twins
 - Significant inverse association of height and coronary heart disease (CHD) for twins and singletons
- Human female twins: LBW twins have ↑systolic BP at 18-34 yrs
- Prepubertal twins: ↑nocturnal BP

Animal Models of Gestational Programming

- Model of LBW simulated by maternal nutrient restriction
- Early gestation growth restriction has greater programming effects
- Rats
 - Offspring of maternal low protein during preimplantation have ↑BP and ↓GFR
 - Offspring of maternal low protein during pregnancy have ↑BP
- Ovine
 - Nutrient restriction day 0 to 70, lambs ↑BP
- Twins?

Experimental Designs

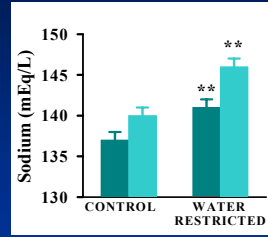
- Drought: Maternal water restriction
- Famine: Maternal nutrient restriction and twin gestation
- Rats:
 - altricial species: postnatal effects, cross foster, short gestation
- Sheep:
 - precocial species: similar to human litter size and maturation, invasive studies

Simulated Drought: Pregnant Rat Water Restriction

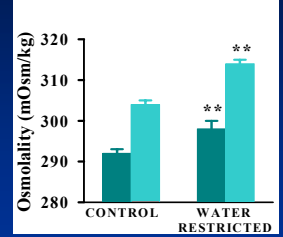
- **Pregnant rats:**
 - Water-restricted ↑ plasma Na (~6 mEq/L)
 - Pregnancy: 10 day to term (21 d)
 - Lactation: Birth to day 21 postpartum
 - Controls: ad libitum water and food
- **Offspring:** Ad lib nursing to water restricted dams
- **21 d of age:** Weaned to ad libitum food and water
- **1 d and 12 wk old**

Maternal Response to Water Restriction

Sodium



Osmolality

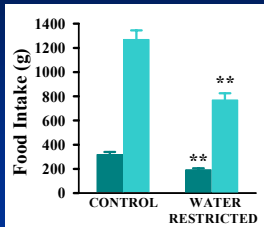


■ Pregnancy ■ Lactation

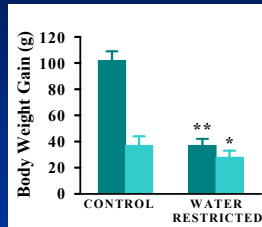
* P < 0.001 Control versus Water-Restricted

Maternal Response to Water Restriction

Food Intake



Body Weight Gain

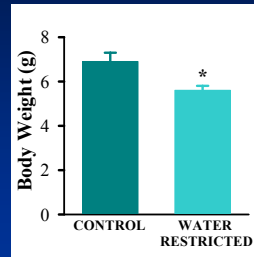


■ Pregnancy ■ Lactation

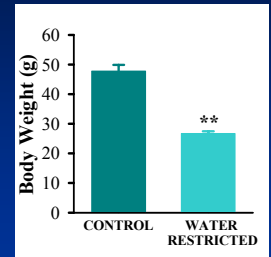
P < 0.001 Control versus Water-Restricted

Rat Offspring Body Weights

1 day



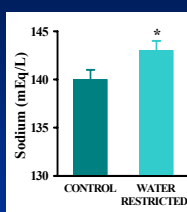
12 weeks



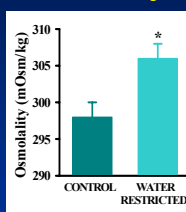
* P < 0.01 Control versus Maternal Water-Restricted Offspring

12 Week Old Male Offspring

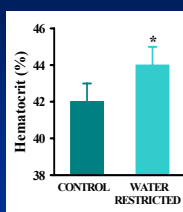
Sodium



Osmolality



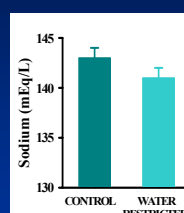
Hematocrit



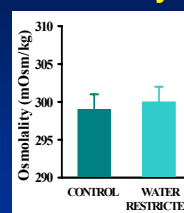
* P < 0.01 Control versus Maternal Water-Restricted Offspring

12 Week Old Female Offspring

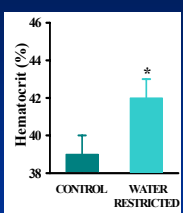
Sodium



Osmolality



Hematocrit



* P < 0.01 Control versus Maternal Water-Restricted Offspring

Simulated Drought: Pregnant Ewe Water Restriction

Pregnant Ewe 110 d – term (145 d):

Water-restriction

- ↑ maternal plasma Na (8-10 mEq/L)

Controls: ad libitum water

All ewes ad libitum food and water following delivery

Lambs: ad libitum nursing
Studied at 3 wks

Body Weights of Offspring

	Control Singleton	Prenatal-Dehy Singleton
Birth Weight (kg)	5.0 ± 0.2	4.1 ± 0.3 ^s
Weight at 3 wks (kg)	12.1 ± 1.3	11.0 ± 1.0

Mean ± SE; significant from Control Singleton (s)

Blood Values of Offspring 3 wks

	Control Singleton	Prenatal-Dehy Singleton
Osmolality (mOsm/kg)	293 ± 2	303 ± 2 ^s
Sodium (mEq/L)	140 ± 1	144 ± 1 ^s
Hematocrit (%)	24 ± 1	26 ± 1 ^s
AVP (pg/ml)	1.6 ± 0.2	1.5 ± 0.2

Mean ± SE; significant from Control Singleton (s)

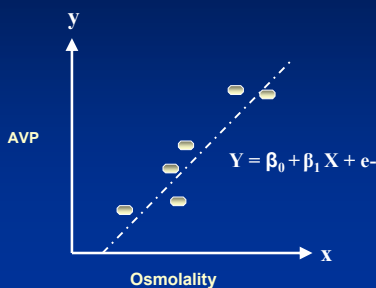
Arterial Pressure of Offspring 3 wks

	Control Singleton	Prenatal-Dehy Singleton
Systolic MAP (mmHg)	80 ± 3	91 ± 3 ^s
Diastolic MAP (mmHg)	52 ± 2	60 ± 1 ^s

Mean ± SE; significant from Control Singleton (s)

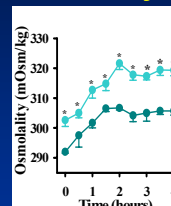
Plasma Osmolality Threshold for AVP Secretion

IV infusion of hypertonic (0.83M) NaCl

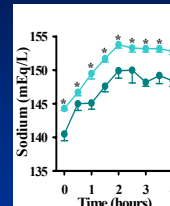


Hypertonic Saline Infusion

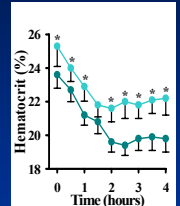
Osmolality



Sodium



Hematocrit



Control singleton

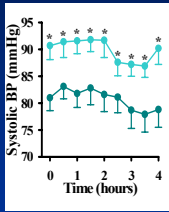


Prenatal-Dehy singleton

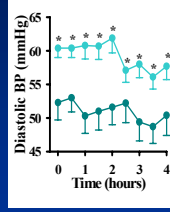


Hypertonic Saline Infusion: Blood Pressures

Systolic



Diastolic

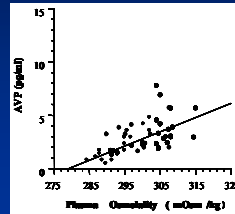


Control singleton

Prenatal-Dehy singleton

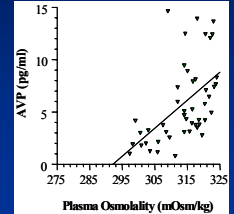
Threshold for AVP Secretion in the Offspring

Control Singleton



Threshold: 280 mOsm

Prenatal-Dehy Singleton



290 mOsm, ↑ slope

Summary - Water Restriction

Prenatal-dehydrated rat pups:

Males:

↑pOsm, pNa, hematocrit

Females:

↑hematocrit

Prenatal-dehydrated lamb singleton:

↑pOsm, pNa, hematocrit

↑Arterial blood pressure

Simulated Famine: Nutrient Restriction

- Rats: Maternal nutrient restriction
 - Pregnancy and/or lactation
 - Effect of rapid newborn catch-up growth

Study Design: Nutrient Restriction

- **Rat Dams:** 50% food restriction
 - Pregnancy (day 10 - 21)
- **SGA Offspring:**
 - Nursed by ad libitum fed dams
- **Pups Studied:** 1 day, 3 weeks, 9 months

Study Design Food Restriction during Pregnancy

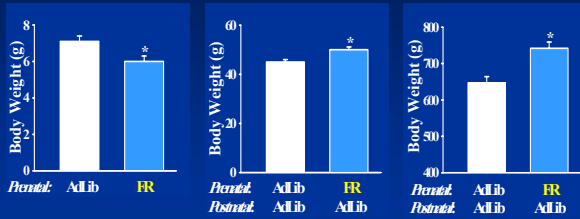
Offspring	Maternal Feeding	
	Pregnancy	Lactation
Control (Pre-AdLib/Post-AdLib)	ad libitum	ad libitum
Pre-FR/Post-AdLib	50% FR	ad libitum

Body Weight of Male Offspring

1 Day

3 Week

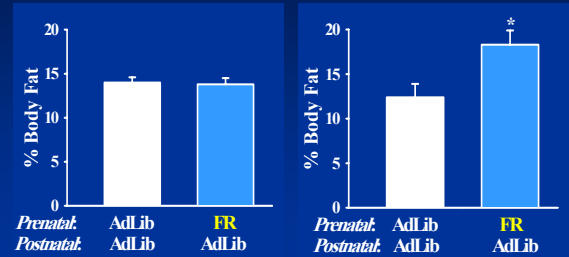
9 Month



Male Percent Body Fat

3 Week

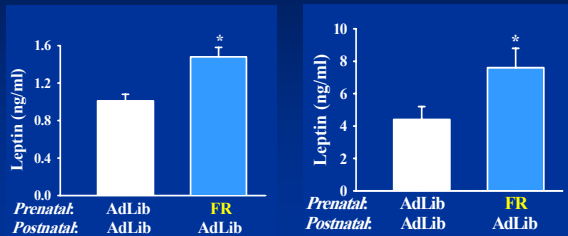
9 Month



Male Plasma Leptin

3 Week

9 Month



“Delayed Catch-up Growth” Food Restriction during Pregnancy and Lactation

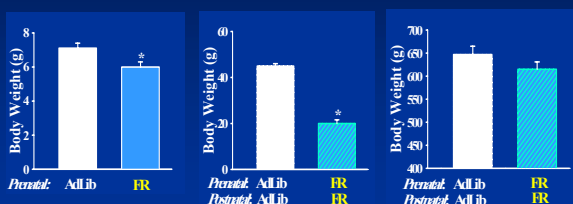
Offspring	Maternal Feeding	
	Pregnancy	Lactation
Control (Pre-AdLib/Post-AdLib)	ad libitum	ad libitum
Pre-FR/Post-FR	50% FR	50% FR

Body Weight of Male Offspring

1 Day

3 Week

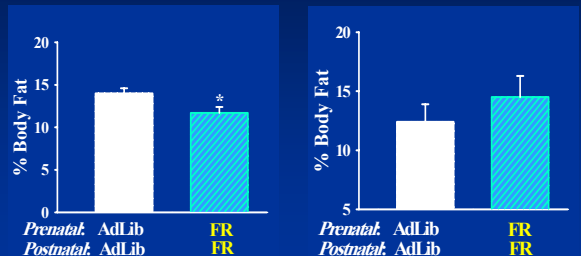
9 Month



Male Percent Body Fat

3 Week

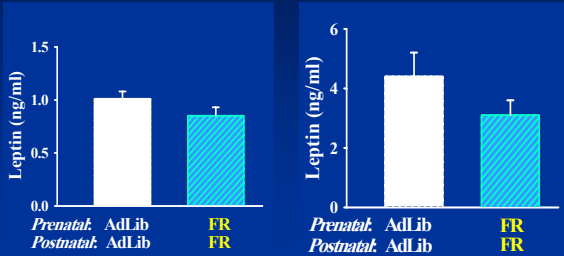
9 Month



Male Plasma Leptin

3 Week

9 Month



Summary

Pregnancy food restriction: Low birth weight

Rapid catch-up growth: NI nutrition in lactation

Catch-up growth beyond controls

↑ body fat, ↑ plasma Leptin

Delayed catch-up growth: Lactation restriction

Catch-up to control body weight

Normal body fat, plasma leptin

Nutrient Restriction

- Sheep: Model of human twins
 - Primarily singleton gestations
- Similarity to human
 - Twin litters and twin nursing

Study Design

- Time dated pregnant ewes
 - 6 twins, 6 singletons
 - Spontaneous labor and delivery
- Lambs
 - Ad libitum singleton or twin nursing
 - 15 day of age
 - Bladder, femoral arterial and venous catheters
 - Studied at 3 weeks of age

Body Weights of Twin Offspring

	Singleton	Twin
Birth Weight (kg)	5.0 ± 0.2	3.5 ± 0.1 ^s
Weight at 3 wks (kg)	12.1 ± 1.3	5.8 ± 0.7 ^s

Mean ± SE; significant from Singleton (s)

Basal Blood Values at 3 Weeks

	Singletons	Twins
Osmolality (mOsm/kg)	294.3 ± 1.4	296.2 ± 1.3
Sodium (mEq/L)	140.1 ± 0.6	143.2 ± 0.6 **
Potassium (mEq/L)	4.2 ± 0.2	4.1 ± 0.2
Chloride (mEq/L)	111.5 ± 1.2	110.6 ± 1.2
AVP (pg/ml)	1.6 ± 0.2	1.5 ± 0.2
Hct (%)	25 ± 1	25 ± 1
pH	7.43 ± 0.01	7.43 ± 0.01

Basal Cardiovascular Values at 3 Weeks

	Singletons	Twins
Systolic Pressure (mmHg)	87 ± 2	94 ± 2 *
Diastolic Pressure (mmHg)	53 ± 1	58 ± 2 *
Mean Pressure (mmHg)	68 ± 1	74 ± 2 *
Heart Rate (bpm)	168 ± 8	169 ± 8

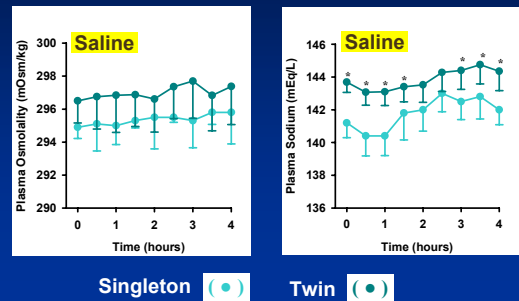
Basal Urine Values at 3 Weeks

	Singletons	Twins
GFR (ml/min/kg)	4.1 ± 0.9	2.5 ± 0.3 *
Volume (ml/min/kg)	0.086 ± 0.009	0.084 ± 0.007
Osmolality (mOsm/kg H ₂ O)	359 ± 34	279 ± 28 *
Osmolar excretion (μOsm/ml/kg)	29.3 ± 3.9	20.3 ± 1.7 *
Osmolar clearance (μOsm/min/kg)	0.089 ± 0.009	0.054 ± 0.006 *

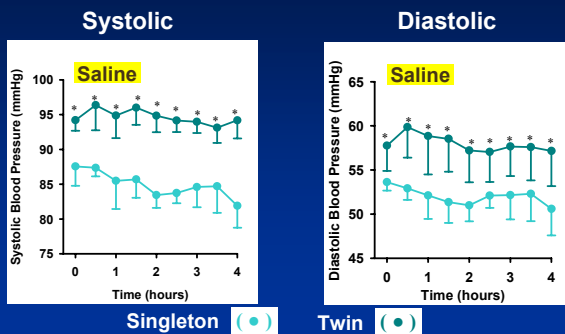
Study Design: Response to Hypotonic Saline Infusion

- Studied in Neonatal Slings
- ³H-Inulin infusion for GFR
- IV infusion of 0.075M NaCl at 0.15 ml/kg/hr
- Blood samples: pH, blood gases, osmolality, electrolytes, hct
- Urine samples: volume, osmolality, electrolytes, GFR

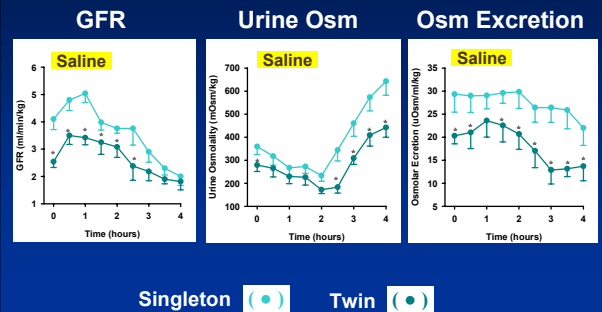
Plasma Osmolality and Sodium Responses during Hypotonic Saline Infusion



Blood Pressure during Hypotonic Saline



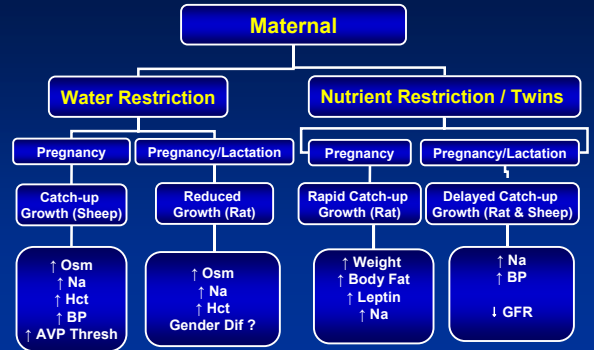
Urinary Responses during Hypotonic Saline



Syndrome of Hypernatremic Hypertension in Twin Offspring

- ↑ plasma Na: ↓ osmolar excretion
- ↓ GFR (35%): ↓ glomeruli in twin lambs
- Systemic hypertension:
 - ? Na dependent, ? Renal-vascular

Effects of Drought vs Famine



Gestational Programming: Questions for the Ob/Gyn

- Maternal nutrition and hydration
- Low birth weight infants: Role of catch up growth, nutrition and lactation counseling
- IVF: Consequences of twins and higher order gestations
 - Response to Western, high fat diets
- Offspring pregnancy: generational impact

Acknowledgements

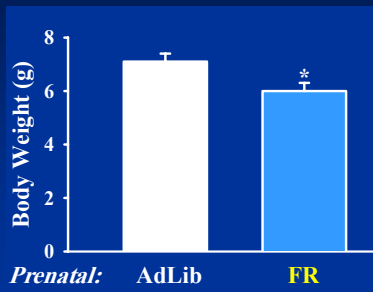
- National Institutes of Health
- American Heart Association
- March of Dimes
- AGOS

Stop

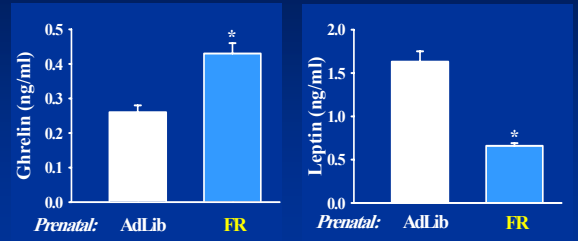
Programming Effects of Maternal Osmotic Stress

- **Human:** Mothers with moderate to severe emesis
 - Infants: ↑ salt preference at 16 wks of age
- **Rats:** Acute extracellular dehydration in pregnancy
 - Offspring: ↑ salt appetite and blood pressure

Body Weight 1 Day Old Male



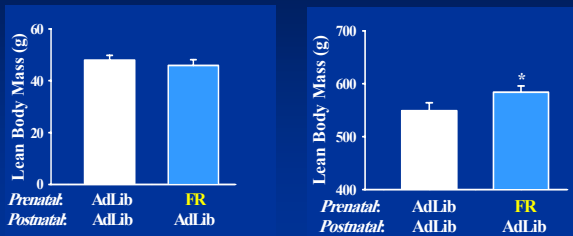
Plasma Ghrelin and Leptin 1 Day Old Male



Lean Body Mass

3 Week

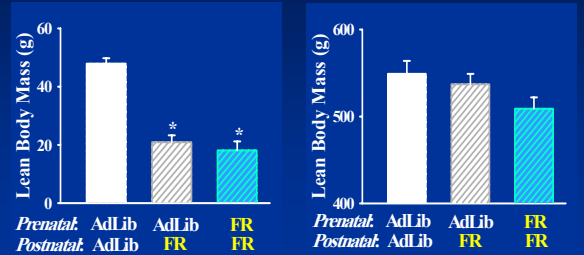
9 Month



Lean Body Mass

3 Week

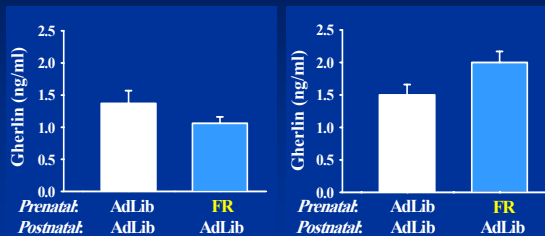
9 Month



Plasma Ghrelin: Appetite Stimulant

3 Week

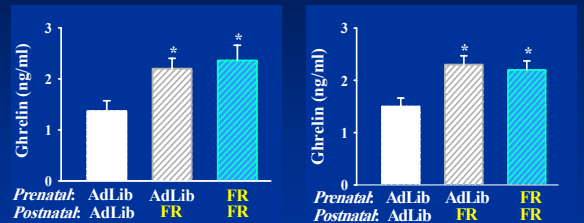
9 Month



Plasma Ghrelin: Appetite Stimulant

3 Week

9 Month



In Utero Programming of Obesity

➤ Maternal Rats

- 30-50% food restriction during pregnancy
- Newborn pups: growth restricted
- Offspring at 12 weeks: Rapid catch-up growth exceeding control

➤ Mechanism(s): ?

- Increased appetite
- Efficient metabolism
- Reduced energy expenditure

Summary

➤ Pregnancy food restriction:

➤ At Day 1

- Low birth weight
- Decreased plasma Leptin

➤ At 3 wks

- Rapid catch-up growth

➤ At 9 months:

- Catch-up growth beyond Controls
- ↑ body fat
- ↑ plasma Leptin