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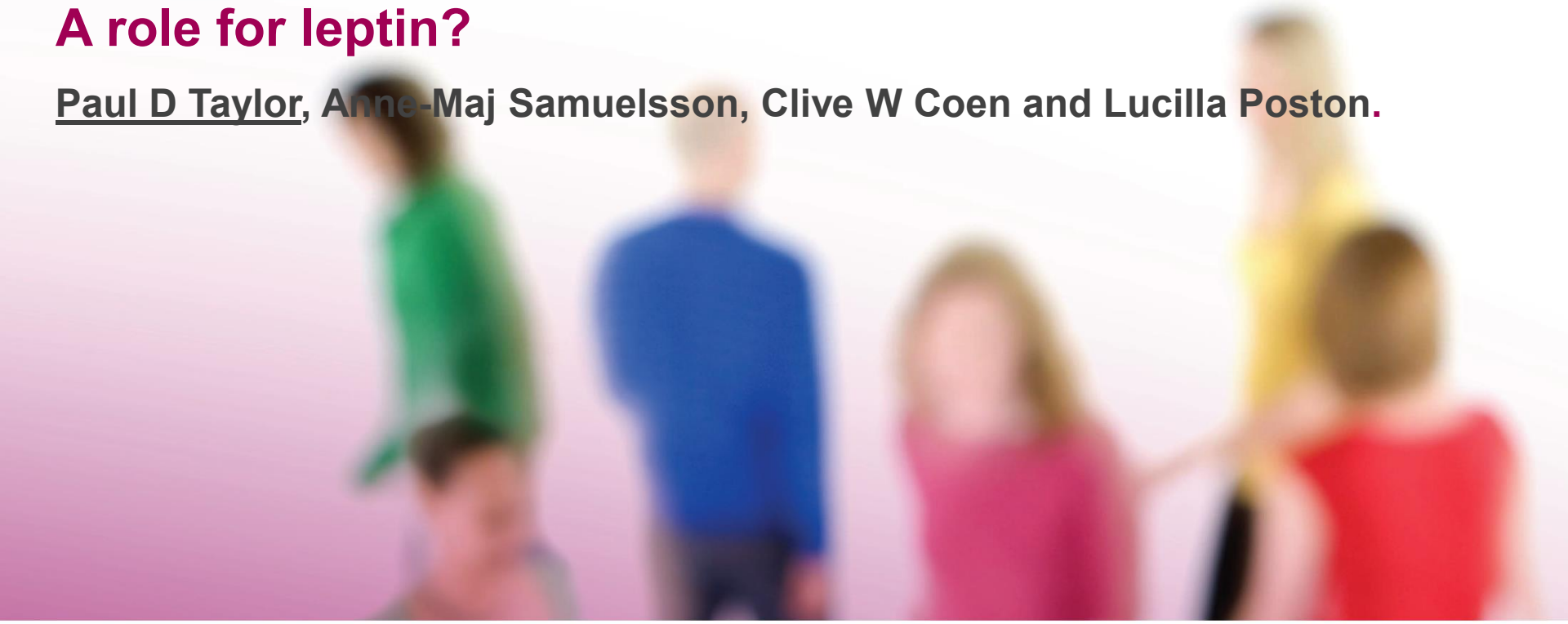
Maternal Obesity and the Developmental Programming of Offspring Appetite and obesity: A role for leptin?

Paul D Taylor, Anne-Maj Samuelsson, Clive W Coen and Lucilla Poston.



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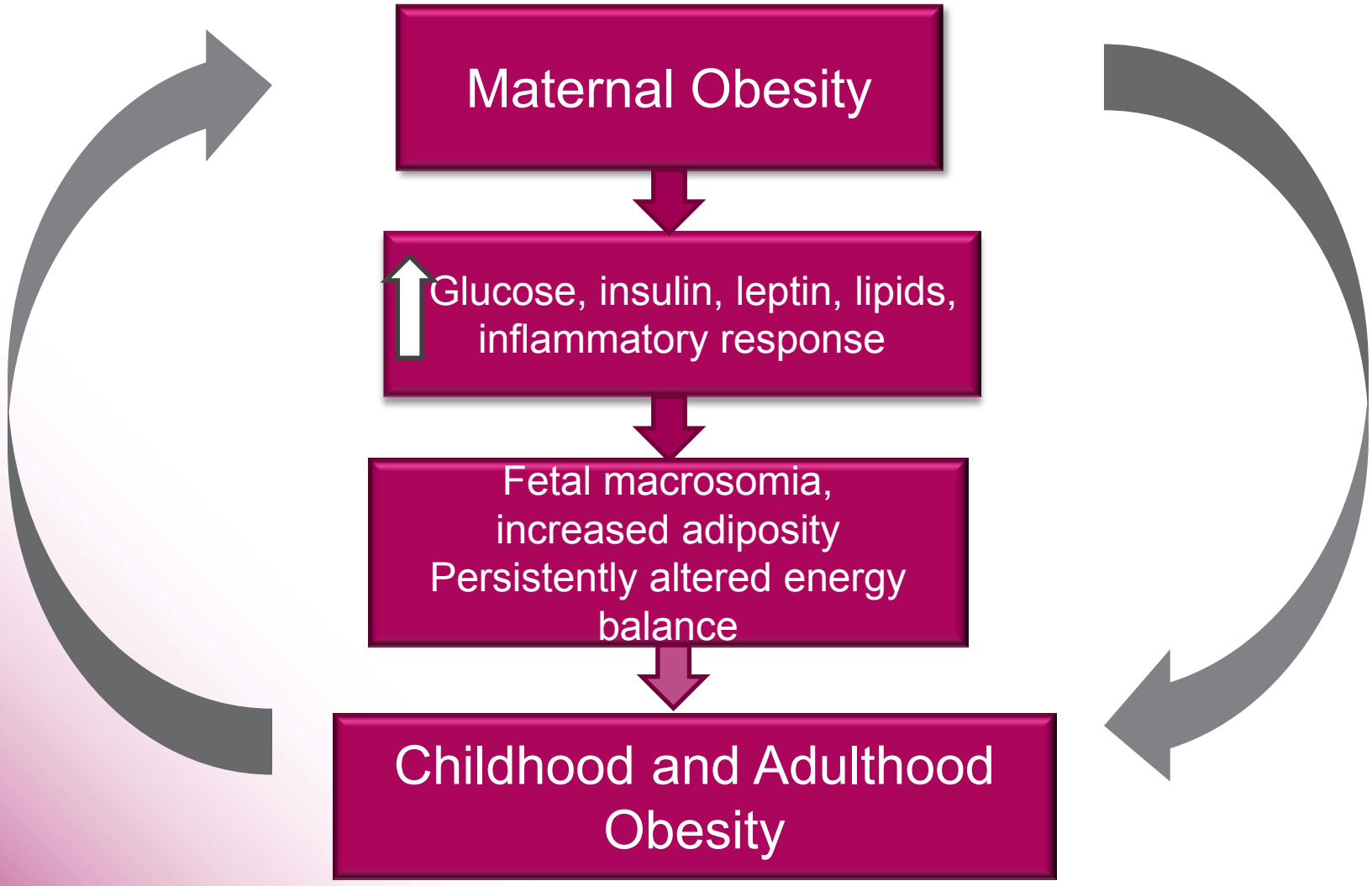
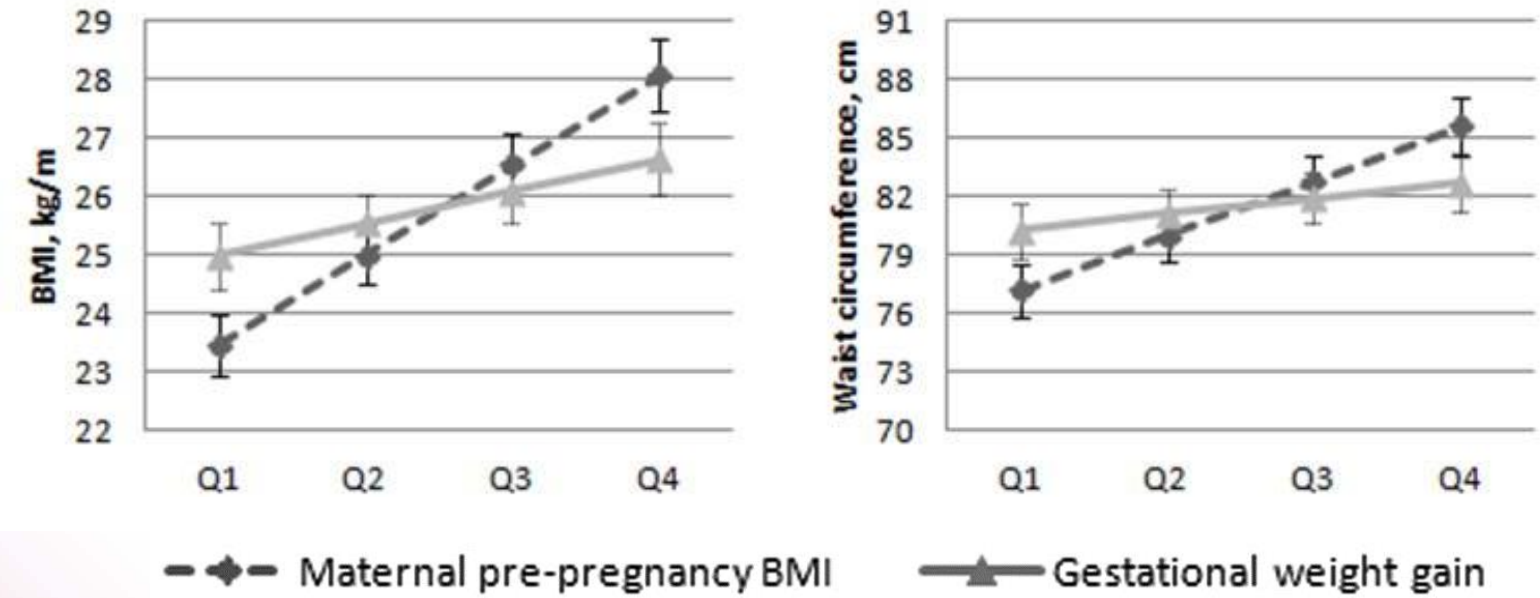
Transgenerational 'Acceleration ' of Obesity?



Maternal pre-pregnancy BMI and gestational weight gain Risk of offspring obesity: Hochner 2012 Jerusalem study



- Adjusted means of offspring obesity outcomes at age 32 by quartiles of maternal pp BMI and GWG
- Greater mppBMI, independently of GWG, was significantly associated with higher offspring BMI, and WC.
- The observed associations were independent of characteristics reflecting the pre- peri- and post-natal environment, including current measures of SES and lifestyle.
- A similar associations of mppBMI and GWG for DBP and SBP was observed, but this disappeared after controlling for offspring current BMI.

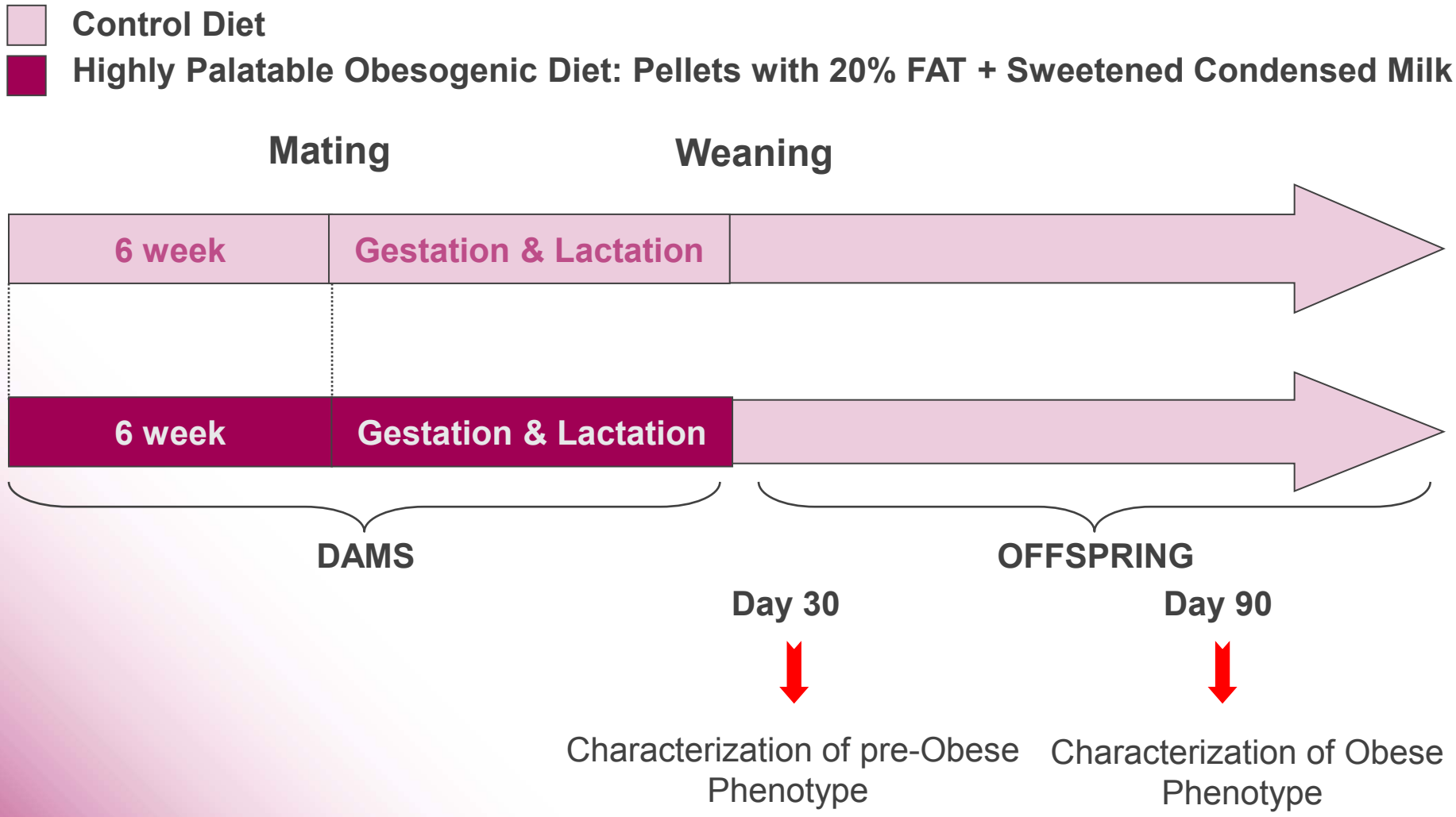
Why use animal models to study mechanisms of Developmental Programming?

- Mice, rats, and humans share all but 1% of each other's genes
- Reduce possible genetic influence
- Relatively quick life cycle
- Environment can be tightly controlled
- Enable different diets to be tested
- Investigate critical periods in development

"Future studies that explore mechanisms underlying the intergenerational cycle of obesity are warranted to identify potentially novel targets for cardiometabolic risk-reduction interventions".

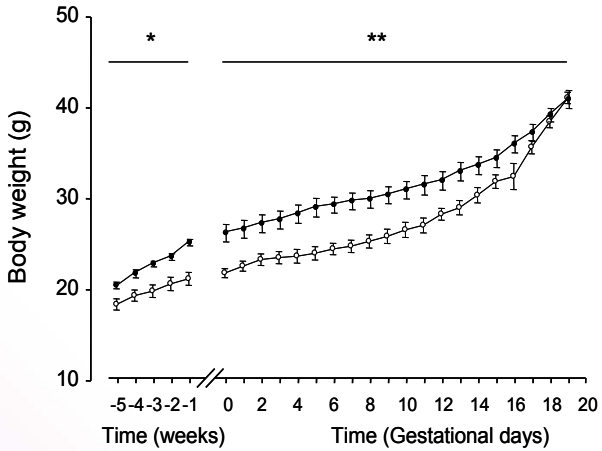
Hochner et al 2012

Breeding Offspring of Obese Rats and Mice (OffOb)



Maternal Characteristics

A



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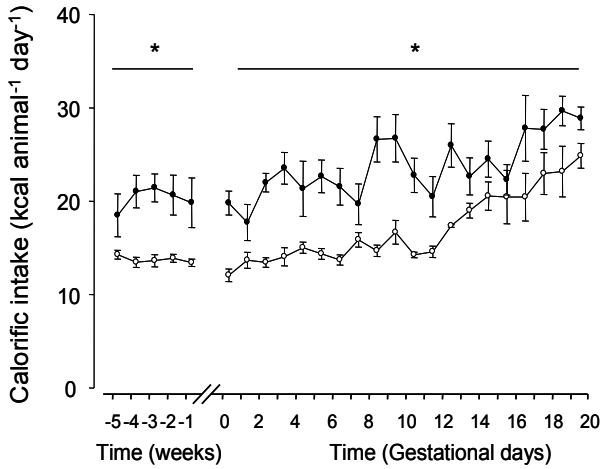

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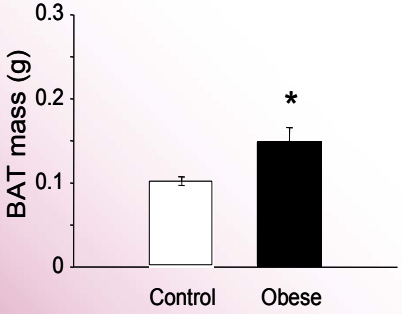

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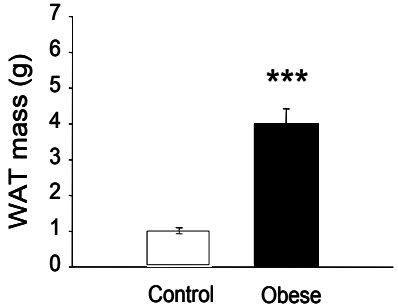

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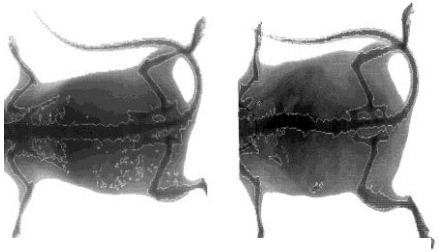
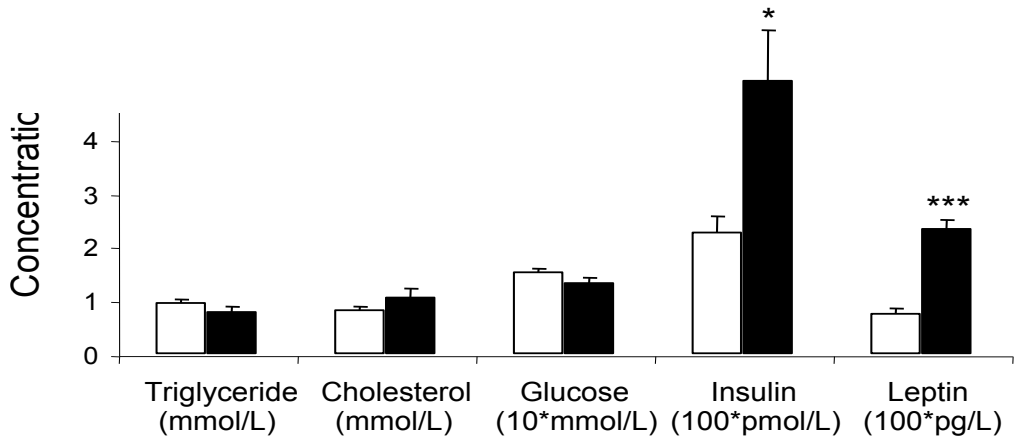


Control Dam Obese Dam

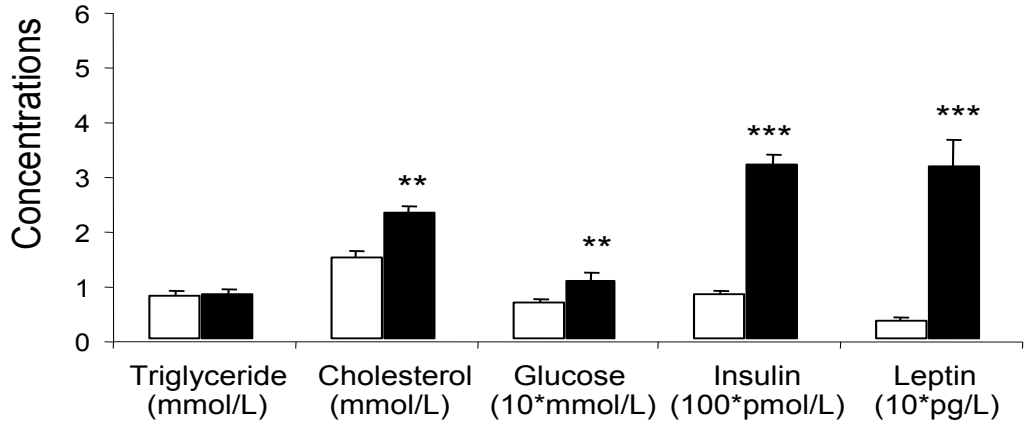
	CONTROL	OBESE
Lean mass (g)	20.5	18.8
Fat mass (g)	3.6	9.8
% Fat	14.8	34.4

Maternal Characteristics

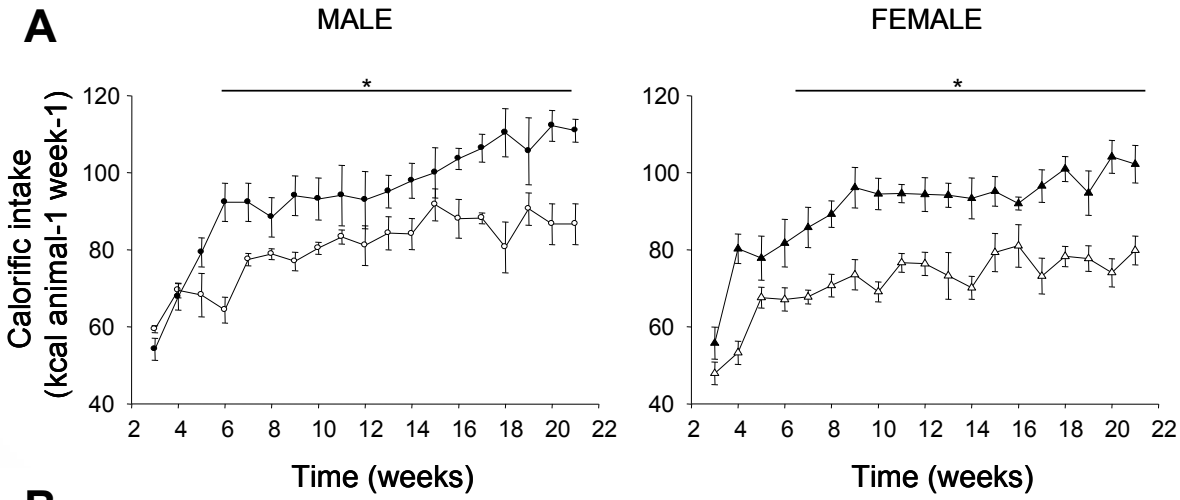
Late gestation

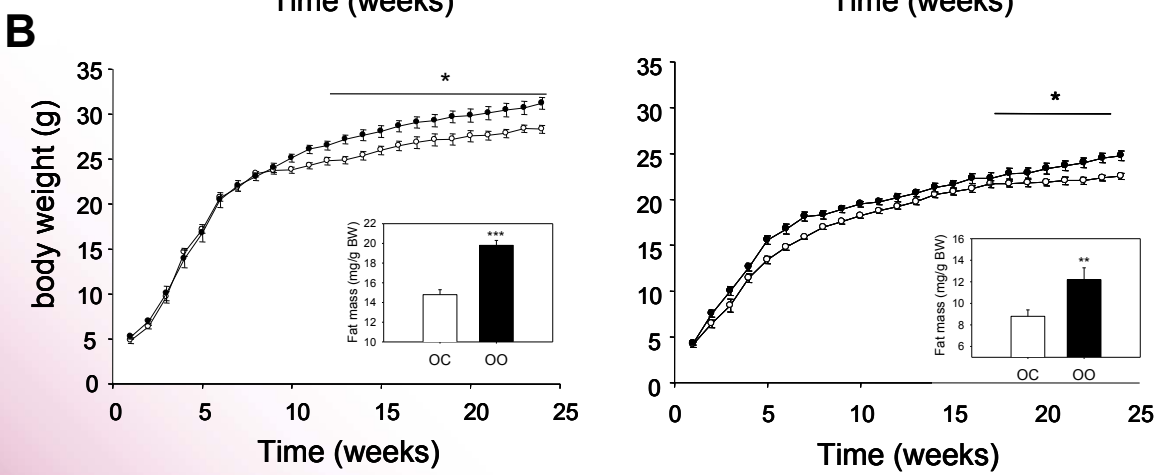


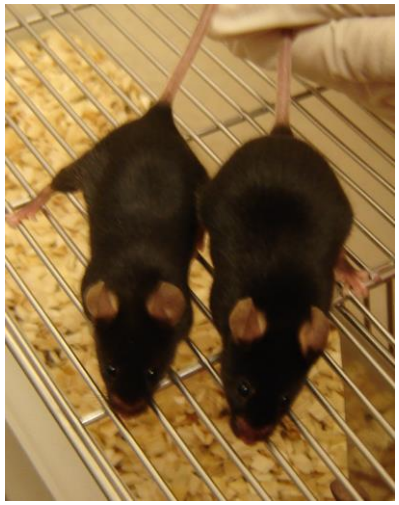
Weaning



Offspring Phenotype: hyperphagia and adult obesity







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Summary (1): Maternal Obesity in rodents

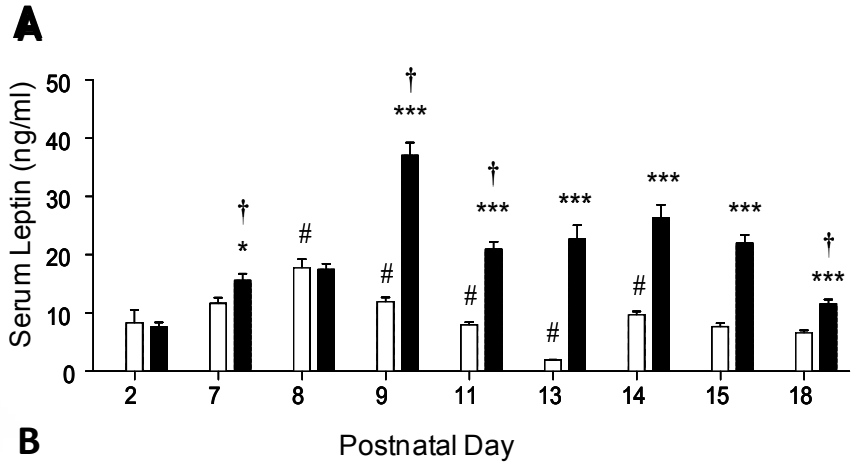
Maternal Phenotype

- High-fat high-sugar diet leads to hypercalorific intake & maternal obesity.
- Hyperleptinaemia, hyperinsulinaemia in pregnancy
- Further hyperglycaemia, dyslipidaemia during suckling.

Offspring Phenotype

- Increased birthweight & weight at weaning (milk content)
- Hyperphagic and hypertensive from weaning
- Abdominal obesity, hyperleptinaemia, dyslipidaemia, hyperinsulinaemia and reduced muscle mass by 3 months.
- Hyperglycaemia (type 2 DM) and fatty liver disease (NAFLD) by 6 months

Neonatal serum leptin and insulin in OffOb



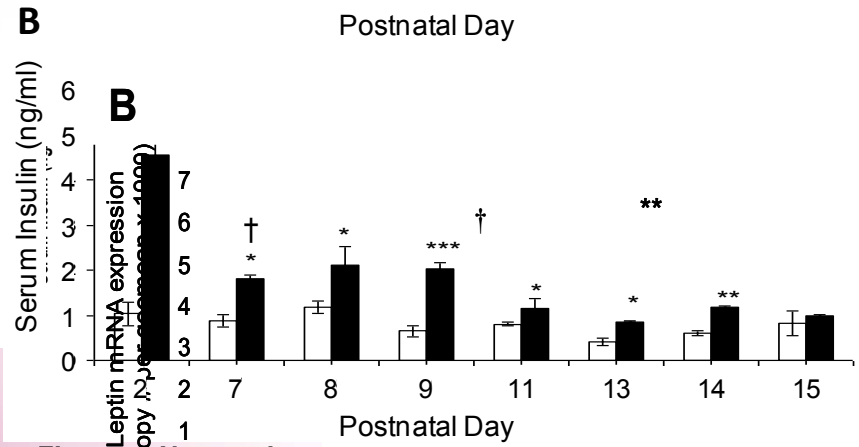


Figure 1. Neonatal serum leptin and insulin concentrations in offspring of control and obese dams. Serum leptin (A) and insulin (B) were measured in offspring of control dams (open bars) and obese dams (closed bars) over the suckling period. * p<0.05, ** p<0.01 and *** p<0.01 *versus* offspring of control dams for the same period (n = 3-6). For longitudinal comparisons, a significant difference (p<0.05) from the preceding period is indicated by **#** for offspring of control dams and by **†** for offspring of obese dams.

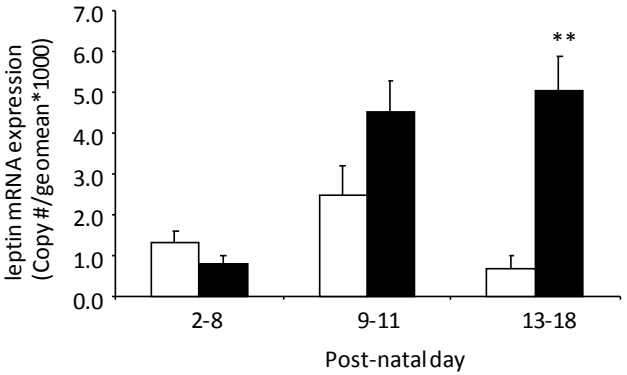
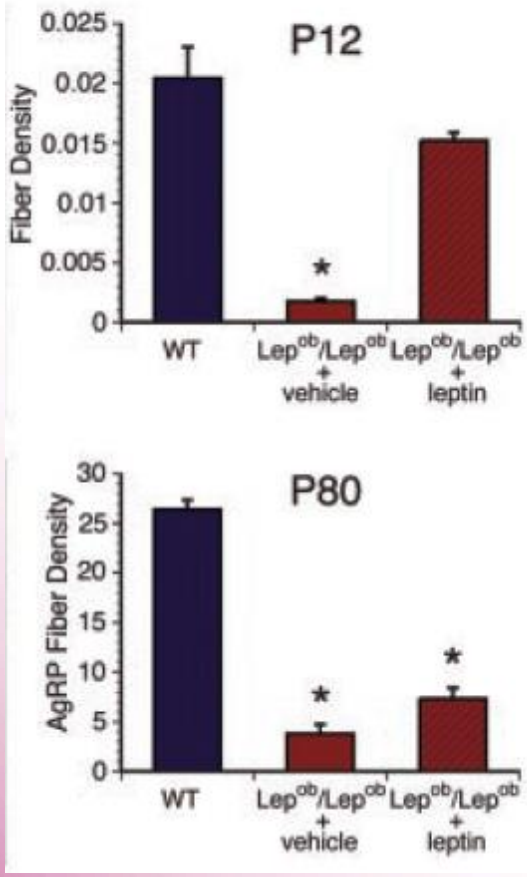


Fig 2. Leptin mRNA expression in adipose tissue from in offspring from control (open bars) and obese dams (closed bars). Day 2-8 (n=5-10), 9-11 (n=3-4), 13-18 (n=4-8) mRNA expressed as copy number divided by the geometric mean of 2 reference genes (28S, Actin beta), multiplied by 1000. Days 13-18, ***P*<0.005 vs control.

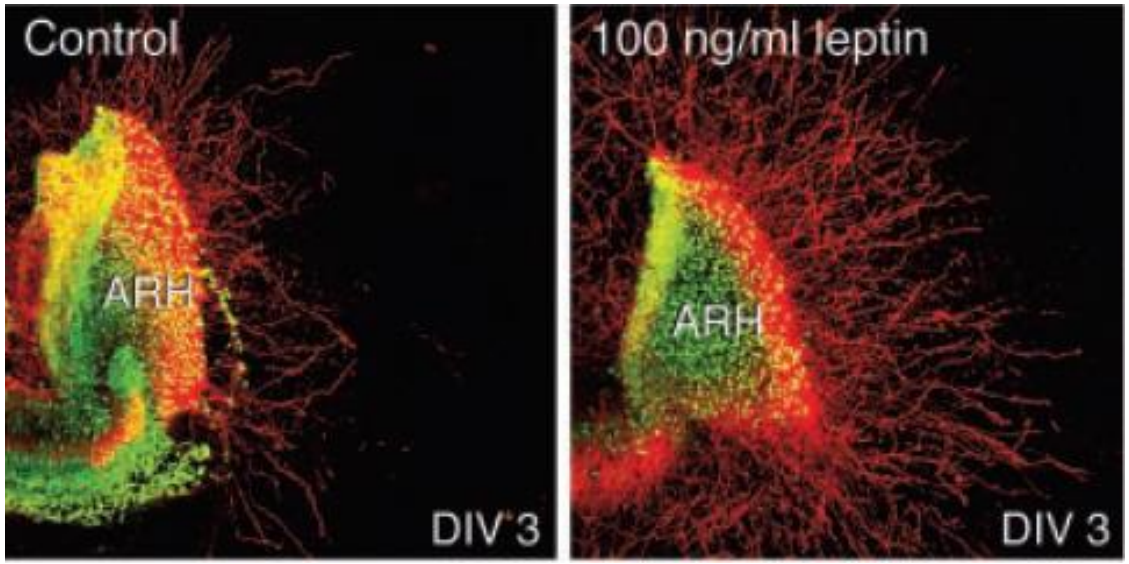
Leptin in Developmental Programming

- Postnatal leptin surge in rodents (Ahima 1998) – a key developmental signal for the hypothalamus
- Leptin in early life modulates the formation of neural energy-regulation circuits in the hypothalamus (Bouret et al 2004).
- Maternal undernutrition can influences the timing and amplitude of the leptin surge (Delahaye et al 2008; Yura et al 2005; Stocker et al 2007; Attig et al 2008).
- Genetic models of obesity?

Leptin in the neuro-development of the brain

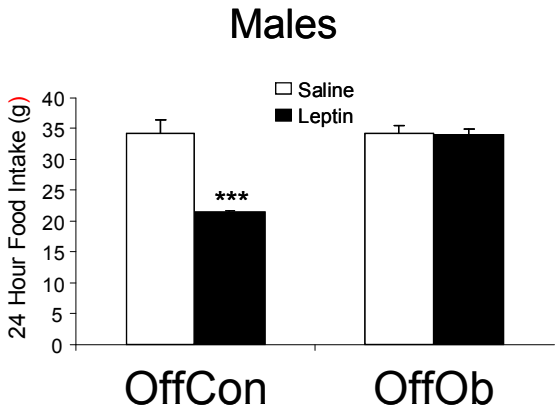


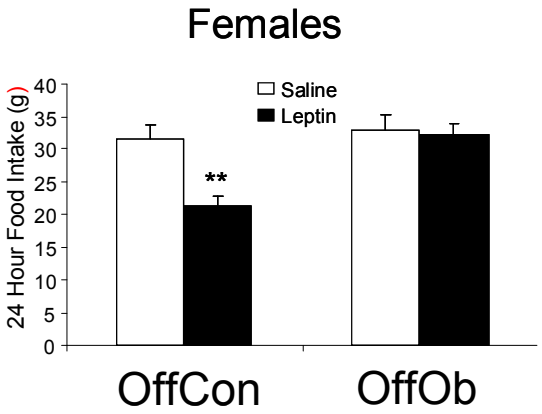
Exogenous leptin rescues arcuate nucleus development in neonatal but *not* adult Lep^{ob}/Lep^{ob} mice (Bouret et al, *Science 2004)*



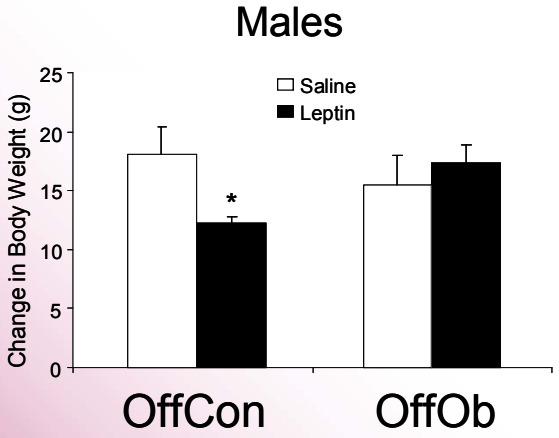
Leptin Challenge in Day 30 Offspring

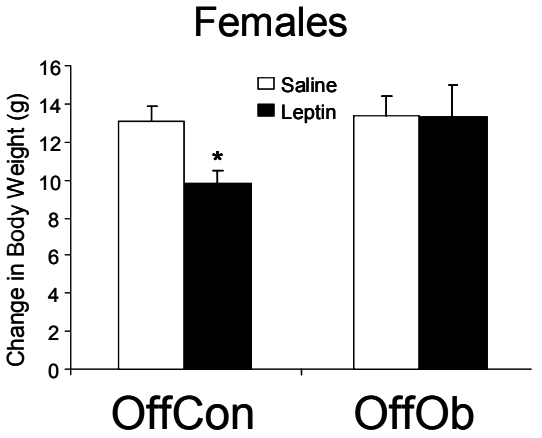
24 hr Food Intake



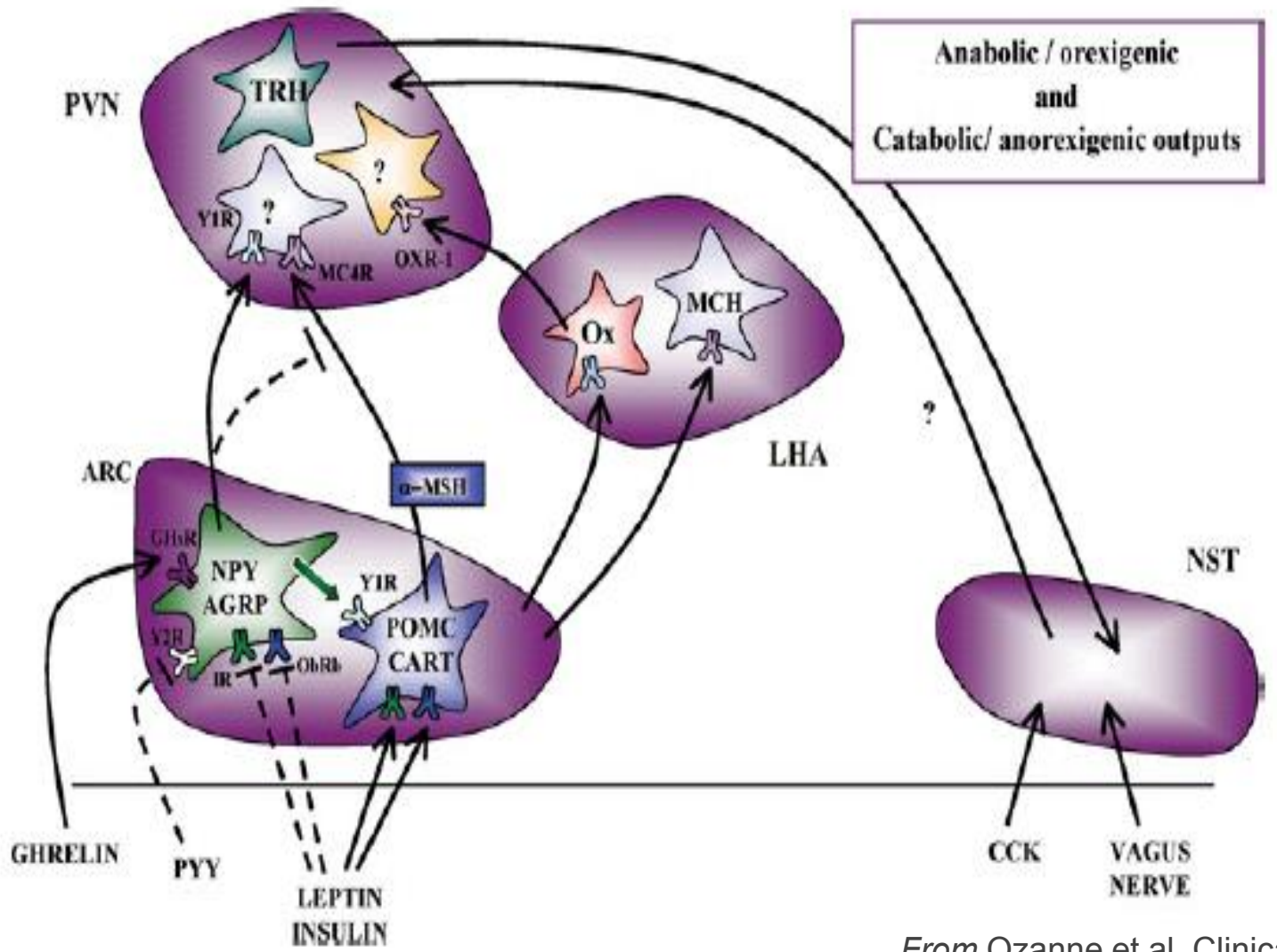


Δ Body Weight





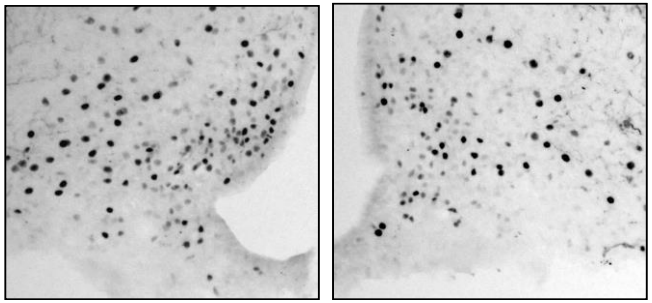
A role for the melanocortin system in programming of appetite?



From Ozanne et al, Clinical Science 2005

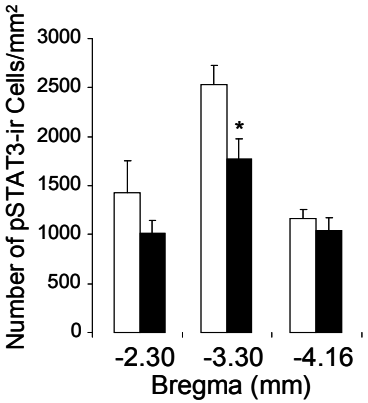
Leptin Signalling in Day 30 Offspring

ARC pSTAT3-ir Cells
~3.30 mm Caudal to Bregma



OffCon OffOb

ARC pSTAT3-ir Cells

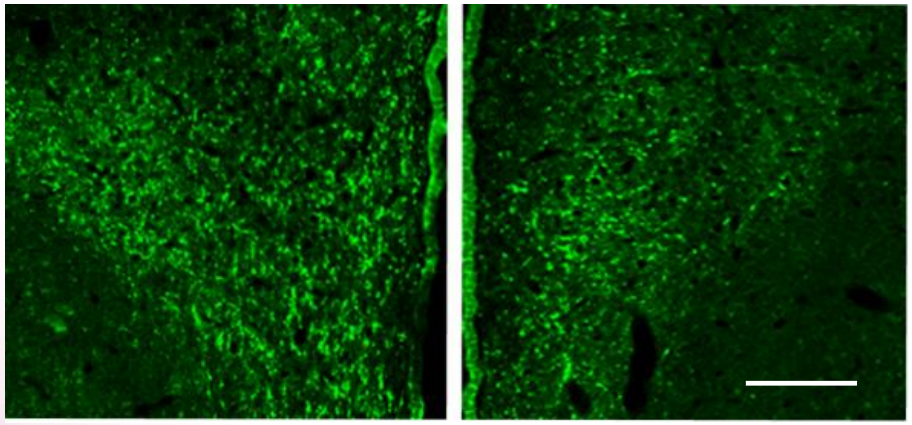


VMH pSTAT3-ir Cells

	Bregma	Group	Mean	SEM	n	p
VMH	-2.30 mm	OffCon	339	36	6	
	"	OffOb	304	71	4	NS
VMHdl	-3.30 mm	OffCon	1146	121	8	
	"	OffOb	1152	288	4	NS
VMHvl	-3.30 mm	OffCon	765	50	8	
	"	OffOb	789	185	5	NS

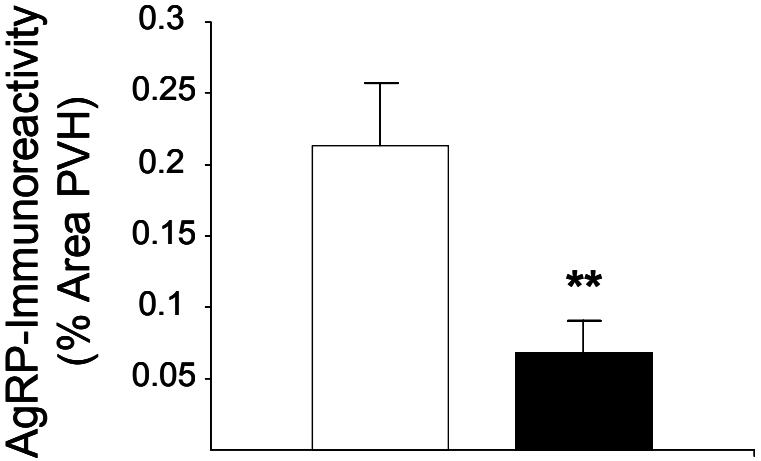
AgRP Immunoreactivity in Female PVH at Day 30

AgRP Fluorescence (Bregma -1.80 mm)



OffCon OffOb

AgRP Fluorescence

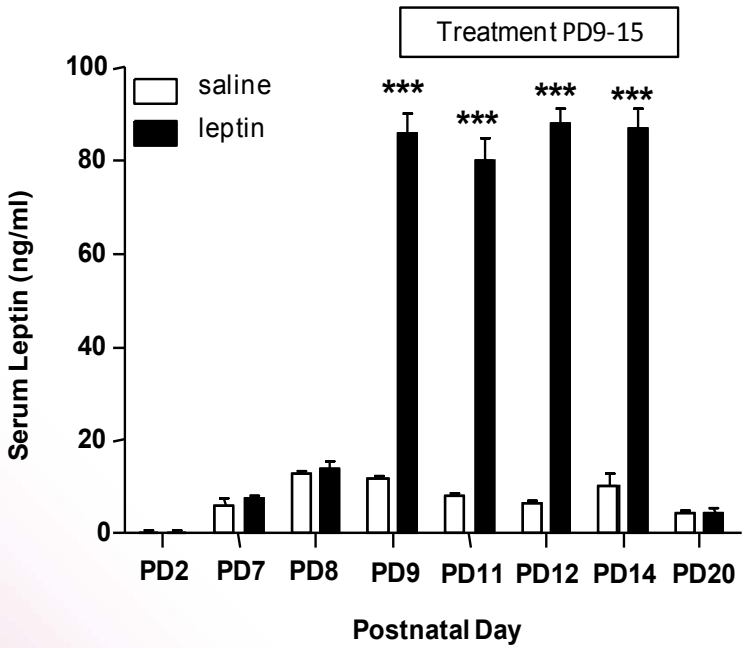


Bregma -1.80 mm

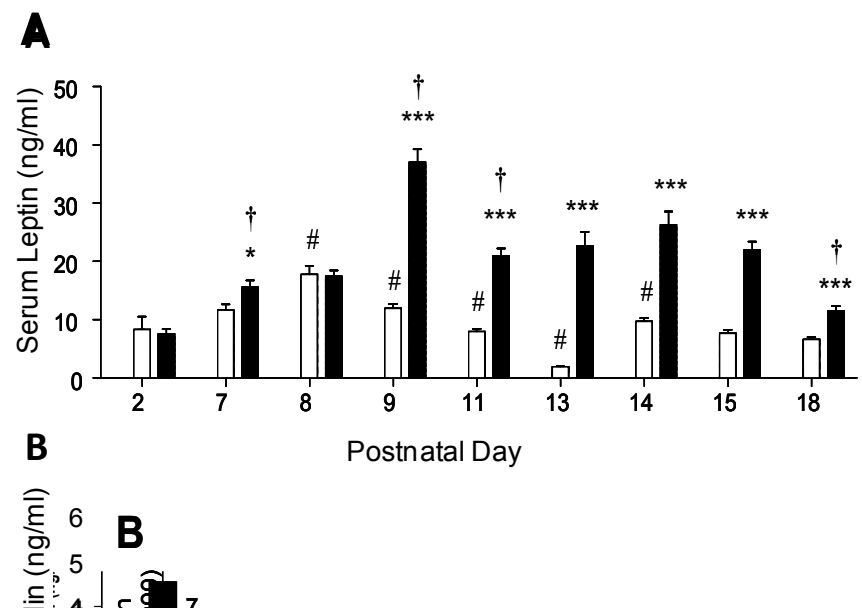
Summary (2)

- **Maternal obesity has adverse consequences for offspring energy balance including leptin resistance and hyperphagia.**
- **We hypothesise that leptin resistance in the ARC is acquired during a critical period due to an amplified and prolonged neonatal leptin surge.**
- **Deficits in leptin-signalling may impair leptin's neurotrophic effects on AgRP projections to the PVH.**
- **Abnormal neuronal development in the appetite regulatory centres of the brain may permanently programme hyperphagia and therefore obesity in adult life.**

Leptin Treatment in naïve rat pups Postnatal Day 9-15



Neonatal serum leptin concentrations achieved leptin (3mg/kg ip) and saline treated pups (male and female combined) ***P<0.001, **P<0.01 *versus* saline using t-test, n=4-7.



Leptin Challenge in L-Tx neonates at 30 days and 5 months

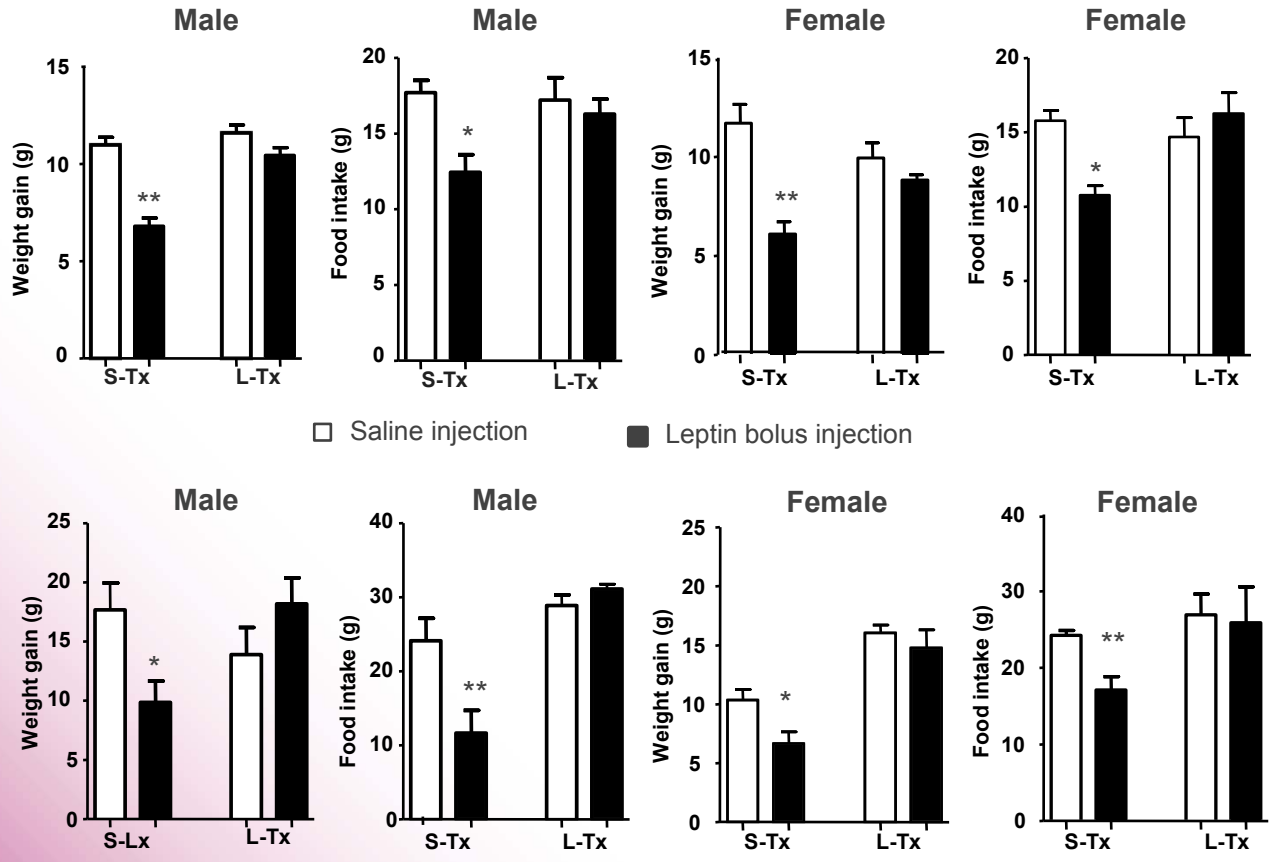


Figure S2. Weight gain and food intake (24hr) following a bolus leptin challenge (10 mg/kg i.p.) in fasted saline-treated (S-Tx) or leptin-treated (L-Tx) male and female rats at (A) 30 days and (B) 5 months of age. ***P<0.001, **P<0.01, *P<0.05 versus saline injection using t-test, n=8. Abbreviation S-Tx-saline treated; L-Tx-leptin treated

Body weight and organ weights 5 and 12 months

Weight	5 months				12 months			
	male		female		male		female	
	S-Tx	L-Tx	S-Tx	L-Tx	S-Tx	L-Tx	S-Tx	L-Tx
Bodyweight (g)	222.9±6.9	238.2±12.1	188.5±10.9	194.9±9.1	600.0±29.6	631.3±23.7	346.8±7.2	402.2±17.2†
Inguinal WAT (mg)	1.32±0.15	2.03±0.35*	1.01±0.15	1.86±0.05†	5.31±0.41	9.25±1.21†	5.23±0.76	12.3±1.35‡
Heart (mg)	0.87±0.02	1.12±0.09*	0.71±0.02	0.82±0.03*	1.61±0.03	1.77±0.03*	1.19±0.03	1.56±0.10†
Liver (mg)	9.08±0.29	8.82±0.29	7.35±0.79	7.57±0.79	16.8±0.42	19.1±1.00	9.16±0.60	10.77±0.05

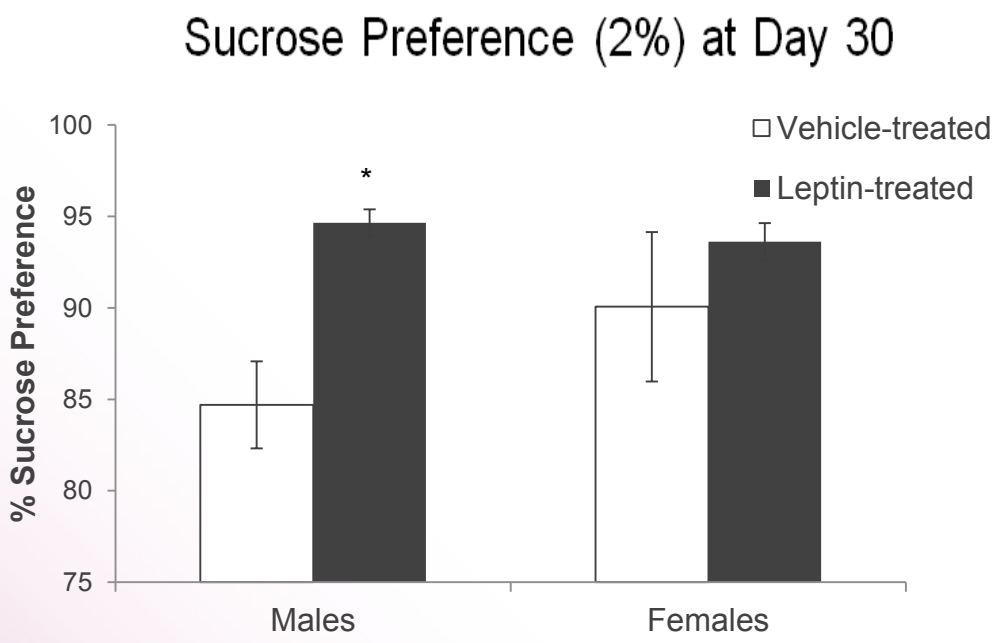
Table S1. Body weight, inguinal WAT, heart –, and liver weight in 5- and 12-month-old male and female rats treated with saline or leptin in neonatal period.

Body weight and organ weights 5 and 12 months

Weight	5 months				12 months			
	male		female		male		female	
	S-Tx	L-Tx	S-Tx	L-Tx	S-Tx	L-Tx	S-Tx	L-Tx
Bodyweight (g)	222.9±6.9	238.2±12.1	188.5±10.9	194.9±9.1	600.0±29.6	631.3±23.7	346.8±7.2	402.2±17.2†
Inguinal WAT (mg)	1.32±0.15	2.03±0.35*	1.01±0.15	1.86±0.05†	5.31±0.41	9.25±1.21†	5.23±0.76	12.3±1.35‡
Heart (mg)	0.87±0.02	1.12±0.09*	0.71±0.02	0.82±0.03*	1.61±0.03	1.77±0.03*	1.19±0.03	1.56±0.10†
Liver (mg)	9.08±0.29	8.82±0.29	7.35±0.79	7.57±0.79	16.8±0.42	19.1±1.00	9.16±0.60	10.77±0.05

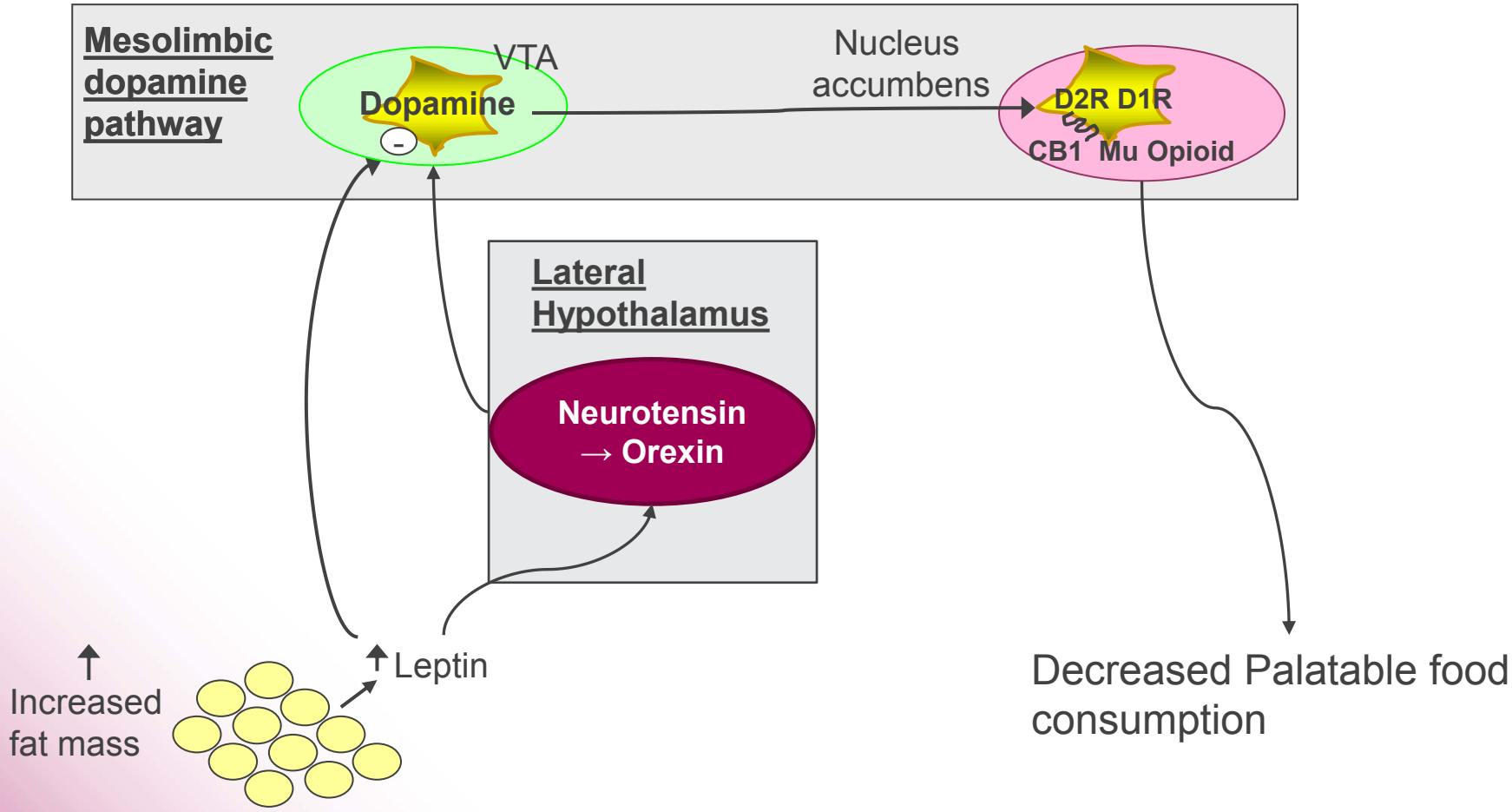
Table S1. Body weight, inguinal WAT, heart –, and liver weight in 5- and 12-month-old male and female rats treated with saline or leptin in neonatal period.

Sugar Preference Tests Performed at Day 30



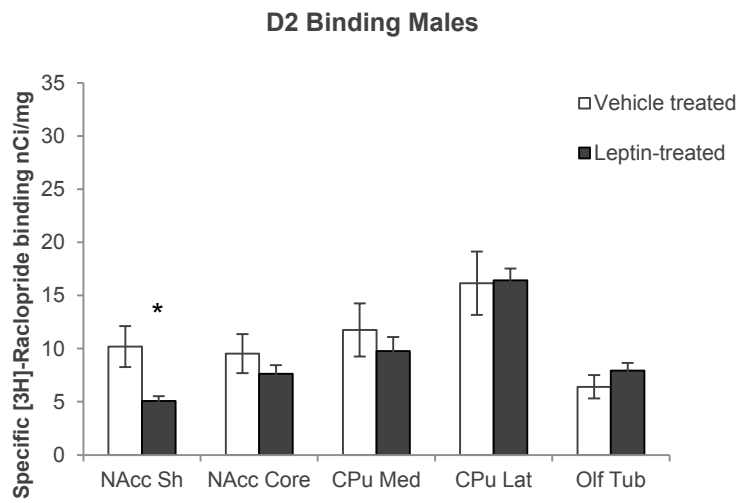
Rats with experimental neonatal hyperleptinaemia have increased sucrose preference at Day 30

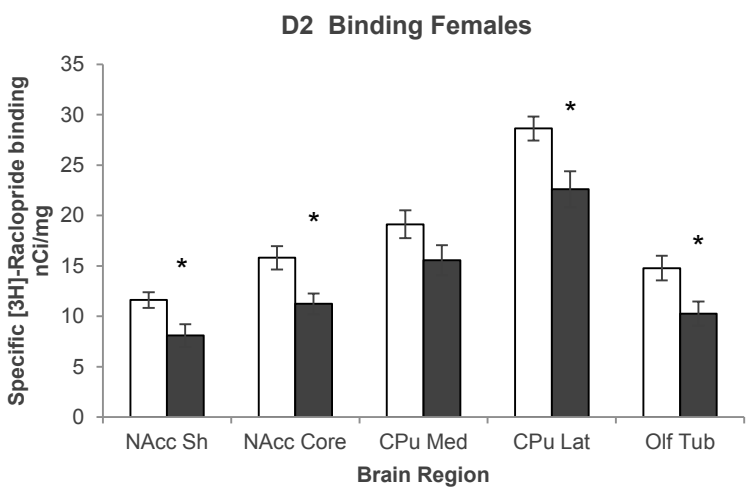
Leptin's Involvement in the Central Control of Mesolimbic Dopamine Pathway

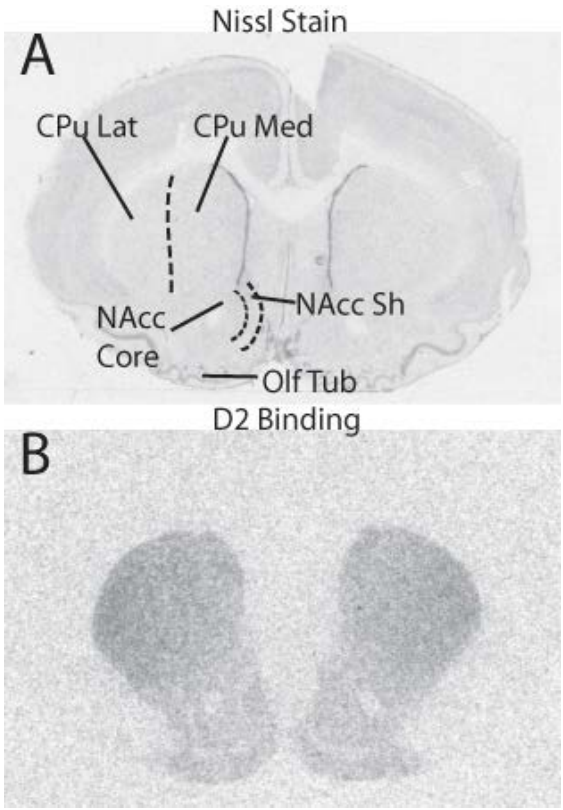


Leinninger & Myers, 2008

D2 receptor binding

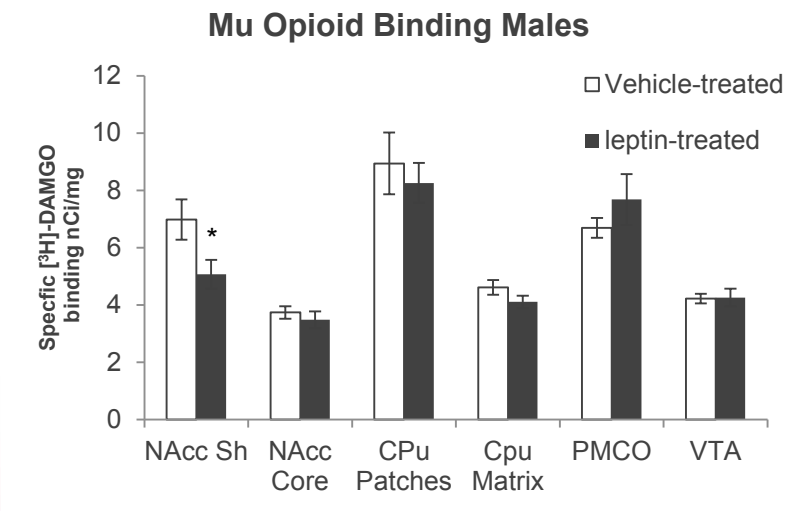


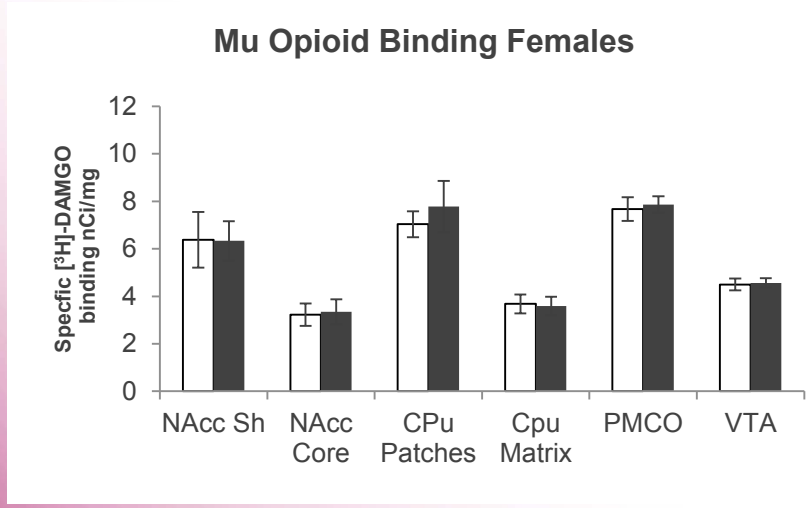


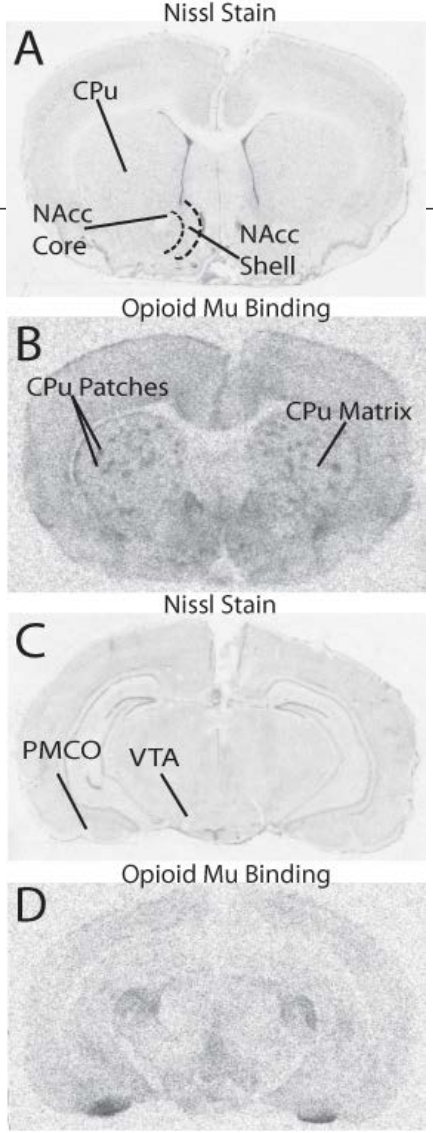


Total binding: [^{3}H]-Raclopride
Non specific binding: [^{3}H]-Raclopride +Butaclamol

Mu Opioid Receptor Binding







*Total Binding: [^{3}H]-DAMGO
*Non Specific Binding: [^{3}H]-DAMGO +Naloxone

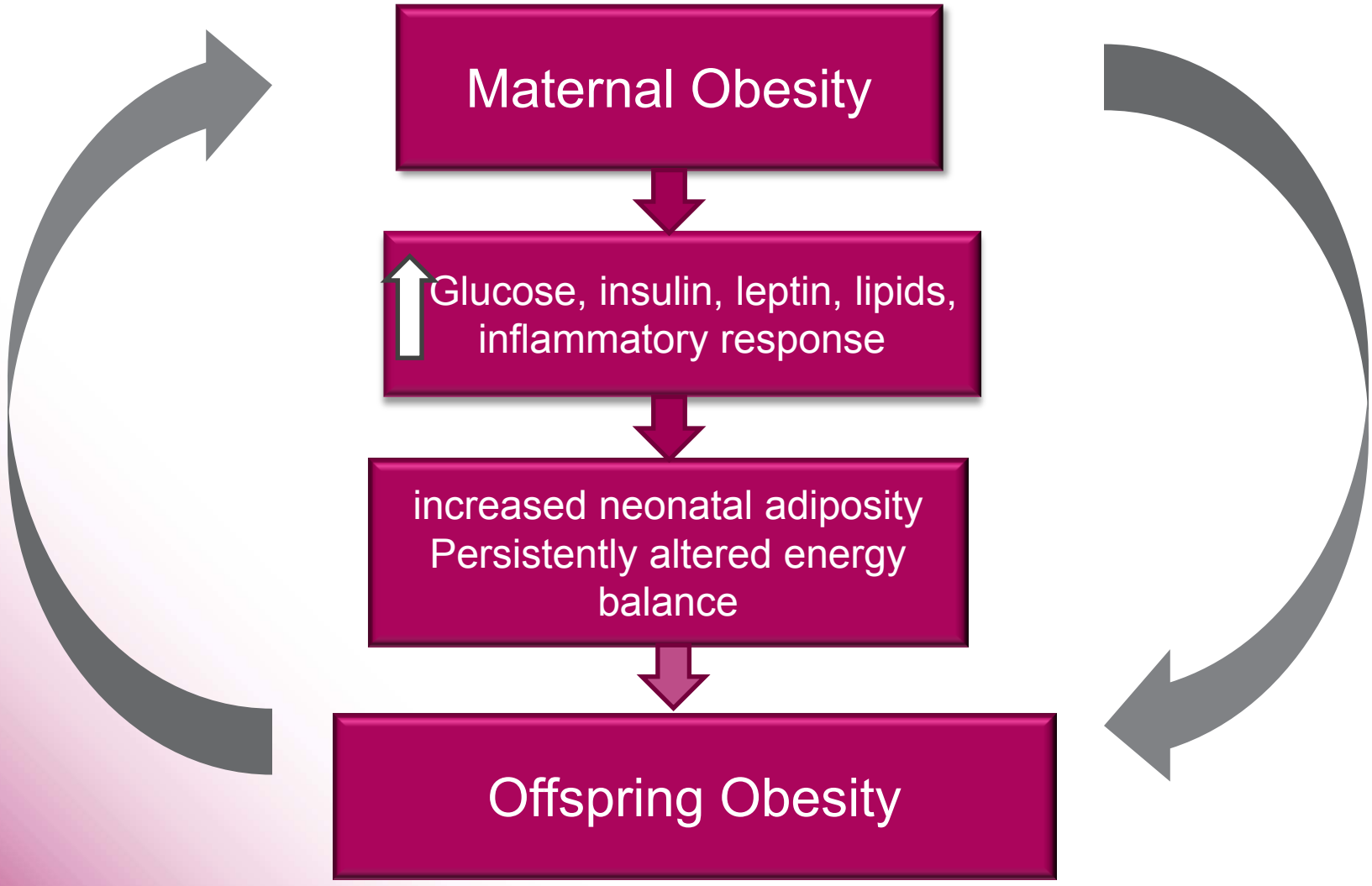
Neonatal Hyperleptinaemia Influences Reward Pathways

- Leptin receptors on dopaminergic neurons in the VTA and also on neurotensin neurons in the lateral hypothalamus influence dopamine VTA neurons (Leinninger & Myers, 2008).
- Leptin resistance at these sites may increase dopaminergic activity in the VTA, thereby leading to down-regulation of receptors in the nucleus accumbens.
- The extent to which these receptor changes underlie the increased sucrose preference remains to be determined.

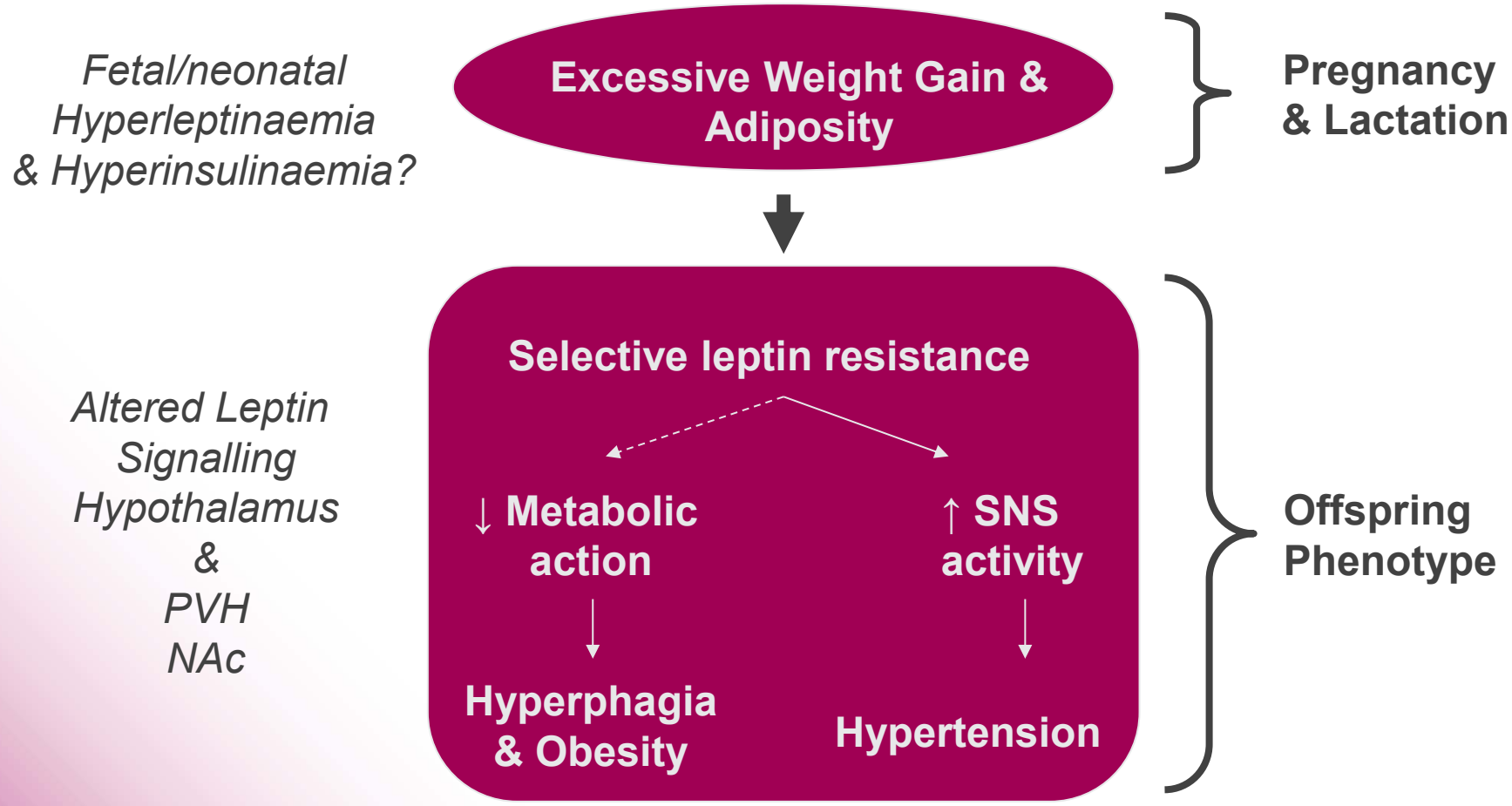
Overall Summary

- High leptin levels during a critical period for hypothalamic neurodevelopment can permanently influence the regulation of appetite and energy balance resulting in hyperphagia and obesity in the offspring of obese rodents.
- Experimental Hyperleptinaemia in the immediate postnatal period mimics the leptin resistant and obese phenotype we have previously described in offspring of obese rodents.
- Neonatal leptin exposure programmes a ‘selective leptin resistance’ in which the anorectic action of leptin is lost (the cardiovascular response to leptin is preserved).
- Hyperleptinaemia in the immediate postnatal period affects sucrose preference and reward-related receptor density in the mesolimbic dopamine pathway.
- **Increased perinatal leptin exposure secondary to maternal obesity may predispose to increased appetite, altered food preference and risk of obesity.**

Transgenerational 'Acceleration ' of Obesity?



Developmental origin of leptin resistance



Schematic: mechanisms in fetal programming of obesity and hypertension.

Relevance to human obesity ?

- Rodents are altricial species: The relative maturity between the human brain and that of the rodent post-partum requires consideration.
- Non-human primates and sheep support translation to precocial species and provide evidence for postnatal development of neurite projections.
- Nothing is known about the sensitivity of the developing human hypothalamic circuitry to leptin in late pregnancy/early infancy.
- Susceptibility to the neurotrophic influences of leptin could also occur in both ante-natal and post-partum periods.
- Cord blood leptin concentration is elevated in neonates, falls rapidly post-partum, - developmental signal?.
- Maternal obesity associated with raised cord blood leptin (Catalano et al 2009).
- On-going RCT intervention studies in obese pregnant women offer a unique opportunity to study effects on childhood appetitive behaviour and obesity risk.

Thanks to:

Developmental Programming Research Group

Principle Investigators

Professor Lucilla Poston (Tommy's the Baby Charity)

Professor Clive Coen (Professor of Neuroscience)

Dr Paul Taylor (Senior Lecturer in Developmental Programming, HEFCE)

Postdoctoral Research Assistants

Dr Anne-Maj Samuelsson (Research Fellow, BHF)

Dr Olena Rudyk (Research Fellow, BHF)

Dr Shona Kirk (Research Fellow, BHF)

Dr Tim South (BBSRC).

Research Assistant

Mr Joaquim Plombo (British Heart Foundation)

PhD Students

Ms Phillippa Matthews (British Heart Foundation

Mr Marco Argenton (BHF)

Ms Xanthi Maragkoudaki (BBRSC)

Ms Angelina Mouralidarane

Ms Abigail Morris (BBSRC)

Collaborators

Dr Nina Balthasar (UK), Dr Mike Christie (KCL)

Dr Laura Cox (Texas) Dr Caroline Relton (UK)

Dr Susan Ozanne (UK) Dr Eugene Jensen (BE)

Dr Claude Remacle (BE), Dr Jude Oben (UCL).

British Heart Foundation

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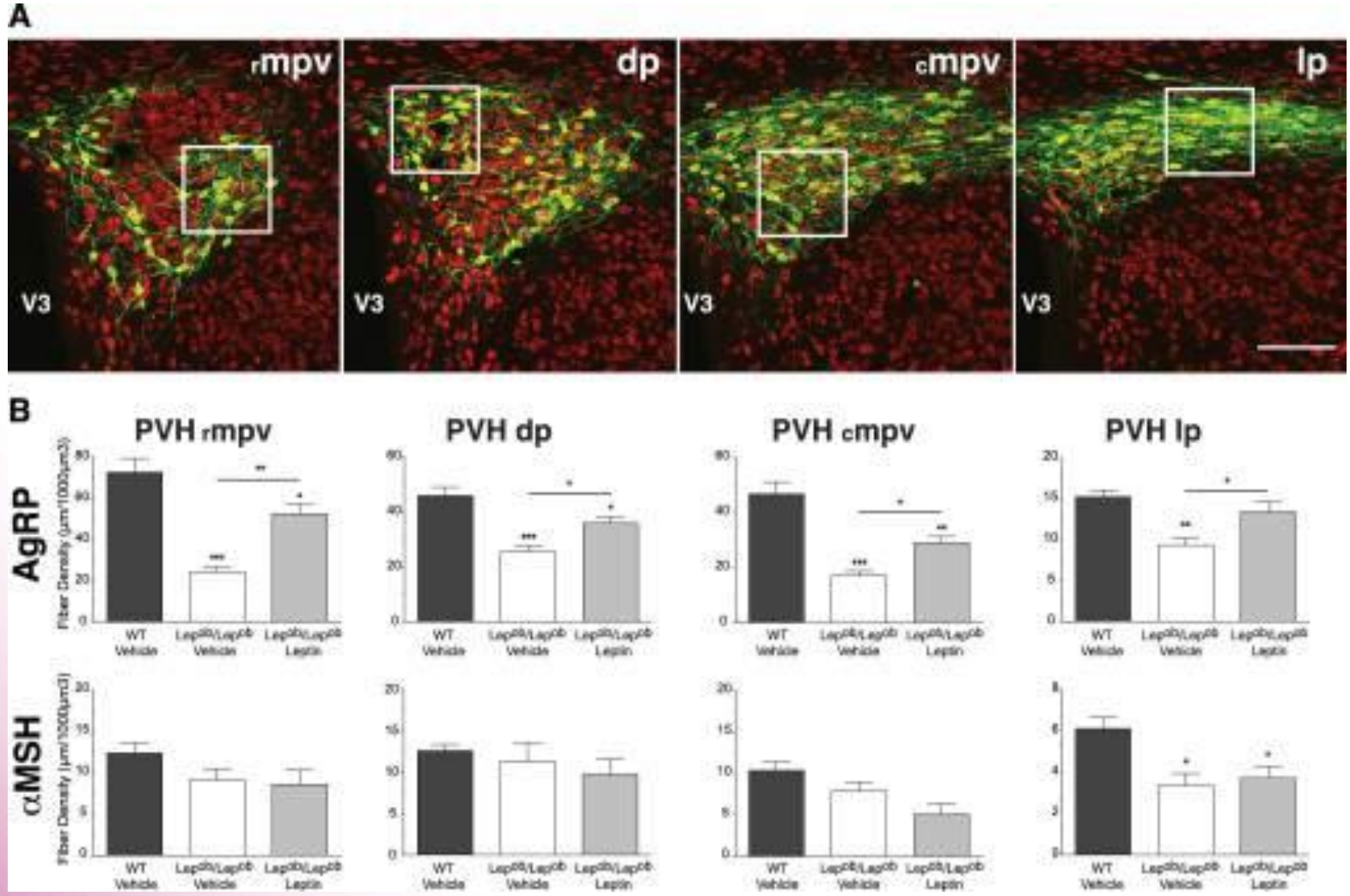
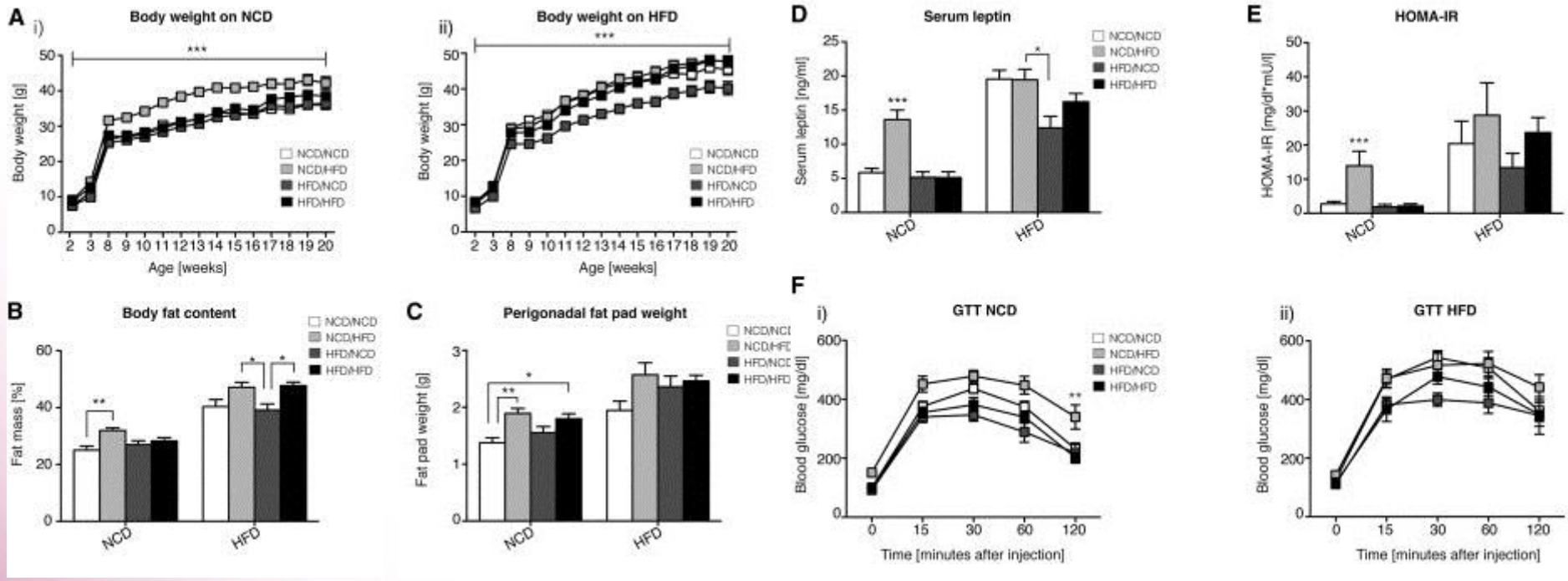
Leptin impacts the peptidergic innervation of preautonomic divisions in adult Ob/Ob mouse PVH



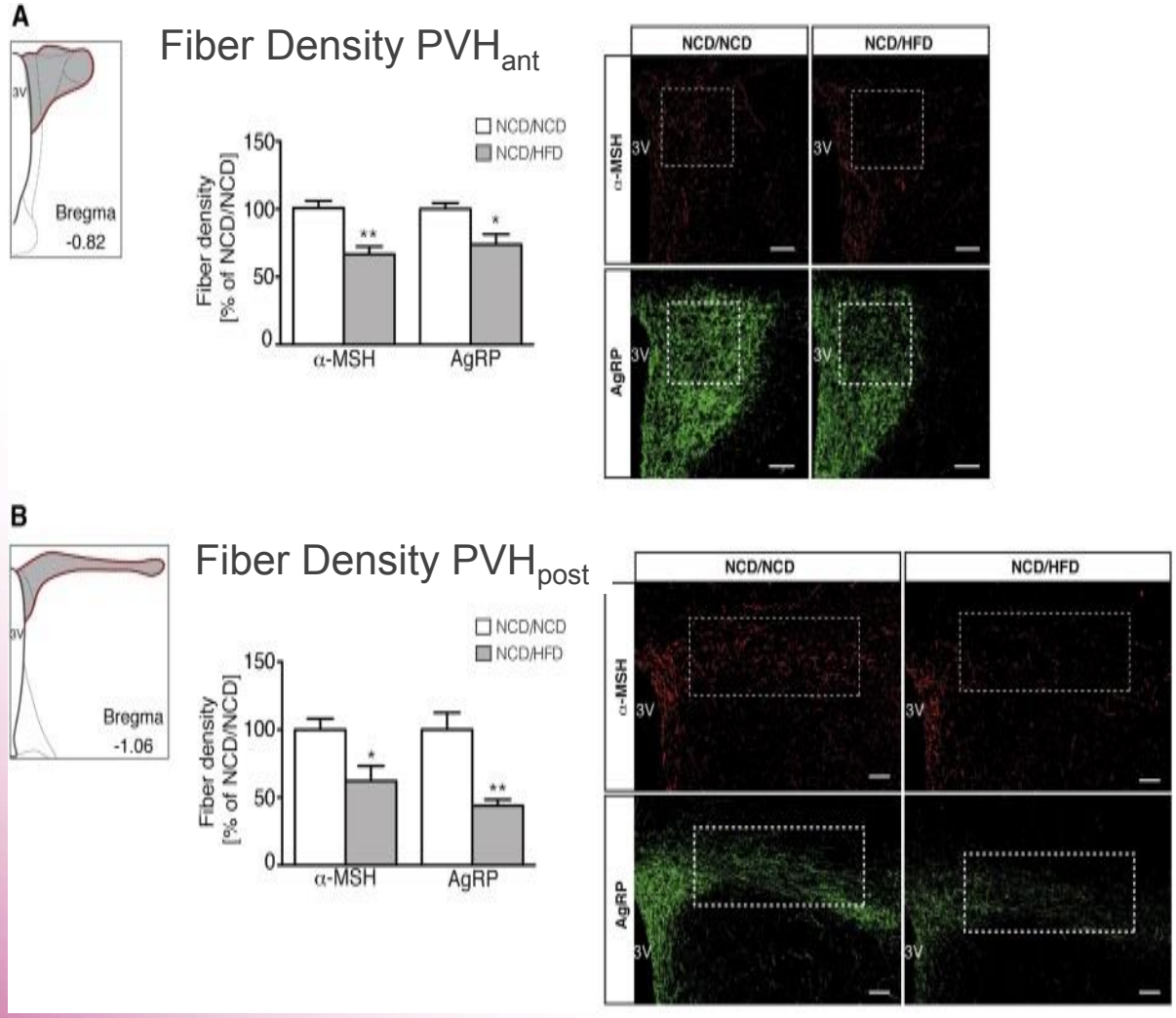
Bouyer & Simerly J Neurosci. 2013 January 9; 33(2): 840–851.

Maternal HFD Feeding Exclusively during Lactation Predisposes the Offspring for Metabolic Disorders



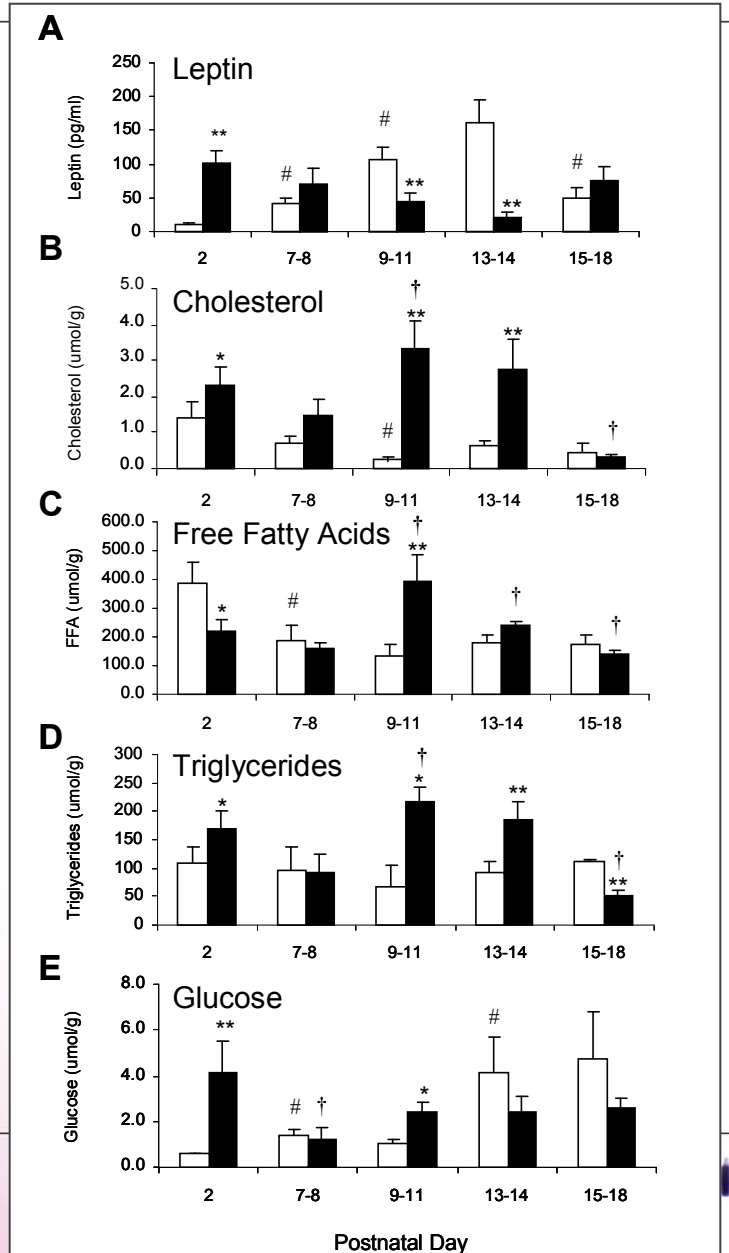
Vogt et al. Cell, Volume 156, Issue 3, 2014, 495 - 509

Maternal HFD Feeding Exclusively during Lactation Predisposes the Offspring for Metabolic Disorders and Impairs Axonal Projections of ARH Neurons to Intrahypothalamic Target Sites



POMC-specific IR Deficiency in NCD/HFD offspring Rescues POMC Axonal Projections to Preautonomic Regions in the PVH

Milk Contents (Pre-weaning Stomach Contents)



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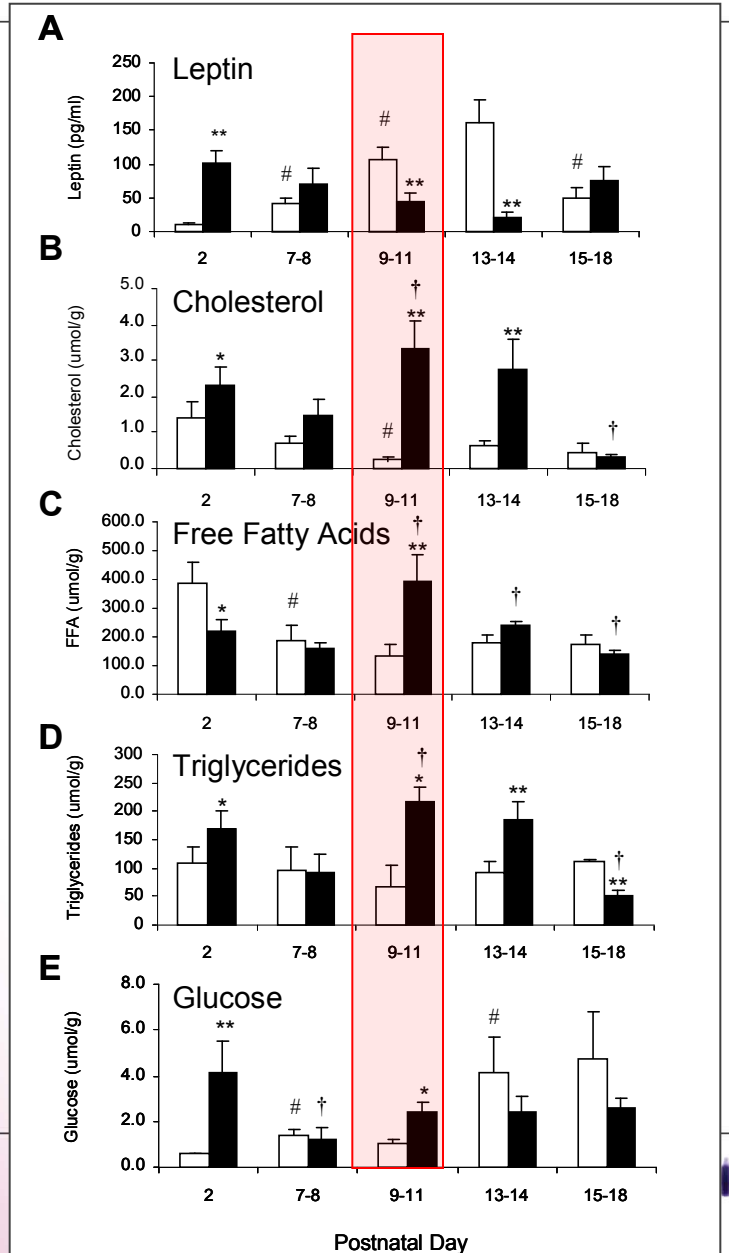
Milk Contents (Pre-weaning Stomach Contents)



KING'S HEALTH PARTNERS

30 day Body weights and Organ weights in L-Tx rats

	male		female	
	S-Tx	**L-Tx**	**S-Tx**	**L-Tx**
Body weight (g)	63.5±2.6	65.2±1.2	62.0±1.1	63.5±1.9
Inguinal WAT (mg)	0.18±0.03	0.20±0.03	0.15±0.03	0.14±0.01
Heart weight (mg)	0.35±0.02	0.41±0.01*	0.35±0.01	0.42±0.01*
Liver weight (mg)	2.53±0.13	2.58±0.10	2.33±0.10	2.60±0.32
Heart weight (mg/g BW)	5.53±0.17	6.34±0.28*	5.64±0.18	6.52±0.09*
Liver weight (mg/g BW)	40.2±2.9	39.1±1.7	38.7±2.0	42.3±2.6

Table 1. *Body weight, inguinal WAT, and heart -weight in 30 day- old male and female rats treated with saline (S-Tx) or leptin (L-Tx) in neonatal period.* Values given as mean± SEM **P<0.05 versus saline using t-test, n=4-8.*

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