Leptin secretion after a high-fat meal in normal-weight rats: strong predictor of long-term body fat accrual on a high-fat diet

S. F. Leibowitz, G.-Q. Chang, J. T. Dourmashkin, R. Yun, C. Julien and P. P. Pamy
The Rockefeller University, New York, New York

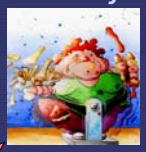
Am J Physiol Endocrinol Metab 290:258-267, 2006

Introduction

Obesity

- **Diet**
- **Activity**
- **Genetics**

etc.



- **Cardiovascular disease**
- **Diabetes mellitus**
- **Hypertension**
- **Atherosclerosis ,etc.**

susceptibility in individual subjects before the onset of disorder

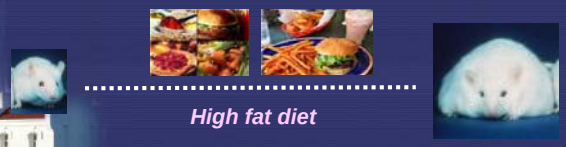
Obesity-resistance

Obesity-prone

Obesity-resistance (OR)

High fat diet

Obesity-prone (OP)



High fat diet

- Investigations in inbred mouse or rat strains with differential propensities toward obesity have been informative *(Bazin et al 1985, Smith et al 2000 & York 1998)*
- The detailed studies of these populations at young ages, before they become different in their body weight, are still lacking

Blood collection from chronic cardiac catheter & tail vein puncture

chronic cardiac catheter

tail vein puncture

This technique can reveal small endocrine changes in response to acute challenges

thus may allow one to detect subtle differences in animals at normal weight that are ultimately different in their propensity toward obesity

- Early studies using this approach have revealed an exaggerated cephalic phase insulin response to a glucose challenge in OP rats compared with OR rats *(Berthoud et al 1985, Powley et al 1985)*
- Also, in more recent investigations, OP rats are found to exhibit elevated levels of norepinephrine in response to glucose injection *(Levin et al 1999)*

- In clinical studies, normal-weight offspring of parents who are obese show greater insulin levels after infusions of β-endorphin *(Cozzolino et al 1996)*

- This evidence gives promise to the possibility that subtle endocrine responses that are markers of long-term body fat accrual can be detected in a preobese state in response to an experimental challenge

Basal or fasting levels of hormones and metabolites of already-obese animals compared with lean counterparts

obese animals

↑ **insulin**
leptin
glucose
triglycerides (TG)
nonesterified fatty acids (NEFA)

(Boivin et al 2000, Surwit et al 1995 & Wang et al 1998)

↓ **corticosterone (CORT)**

(Svec et al 1997 & Dourmashkin et al 2005)

In normal-weight animals *(Himaya et al 1997,Surina-Baumgartner et al 1996)*

HFM compared with premeal scores →

↑ **insulin, leptin, glucose & lipids**

little change in CORT

Clinical studies *(Guerci 2000, Imbeault 2001)*

lean

HFM-induced increase in insulin & lipids is significantly greater in the obese compared with lean subjects

obese

↑ **insulin**
lipids

- Thus the acute response after a meal may very likely contribute to the chronic endocrine and metabolic disturbances typically seen in obese subjects

- The focus of the present investigation, however, is on genetically heterogeneous animal populations

- In such populations, one must search for specific physiological or behavioral markers that can accurately identify distinct OP vs. OR subgroups while they are still at normal weight

Objective

- To compare meal-induced endocrine changes in rats that
  - had become obese vs. lean while on chronic high-fat diet
  - were still of normal weight on a low-fat diet → different weight gain (OP vs OR) while switched to a high-fat diet

- To determine whether tail vein blood collection could reveal endocrine changes similar to those seen with the chronic cardiac catheter

- To identify the distubances in hypothalamic peptides (galanin & NPY) that accompany elevated HFM-induced leptin in OP rats

Methods & Results

Animals and Diets

Adult, male Sprague-Dawley rats (220–240 g)

- individually housed (22°C, with lights off at 1:30 PM for 12 h)
- 7–10 days to acclimate to laboratory conditions

The constituents of high-fat diet (5.15 kcal/g)

50% fat → 80% lard + 20% vegetable oil

25% carbohydrate → 30% dextrin, 30% cornstarch + 40% sucrose

25% protein → casein with 0.03% L-cysteine hydrochloride

Supplemented with 4% minerals

3% vitamins

Weekly food intake measurements were taken to confirm a stable feeding pattern

Surgery & Blood sampling procedures

Experiments 1-2 → Chronic cardiac catheter (jugular vein)

Experiments 3-5 → Tail vein puncture

rotating cantilever beam

HFM challenge test (40 kcal)

remove lab chow diet

lab chow

2 h before the nocturnal feeding cycle

At dark onset

high-fat diet (50% fat) for a 2-h period

→ close to a natural intermeal interval

→ consistent postmeal changes in hormones and metabolites

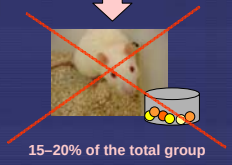
To control for the size of the test meal

40 kcal

39-40 kcal *in 2h interval*

40 kcal

< 38 kcal *in 2h interval*



15–20% of the total group

Hormone and Metabolic determinations

Hormone

- insulin
- leptin
- CORT

→ RIA kits

CORT = corticosterone

Metabolite

Glucose → glucose Trinder Reagent Kit

TG → TG Assay Kit

NEFA → NEFA C Kit

TG = triglyceride

NEFA = nonesterified fatty acids

Intra- & Inter-assay coefficient of variation

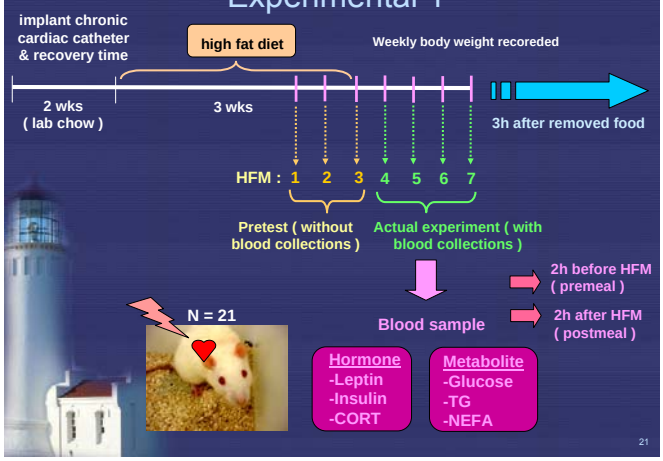
The hormone and metabolite assays were performed at different times for the different experiments

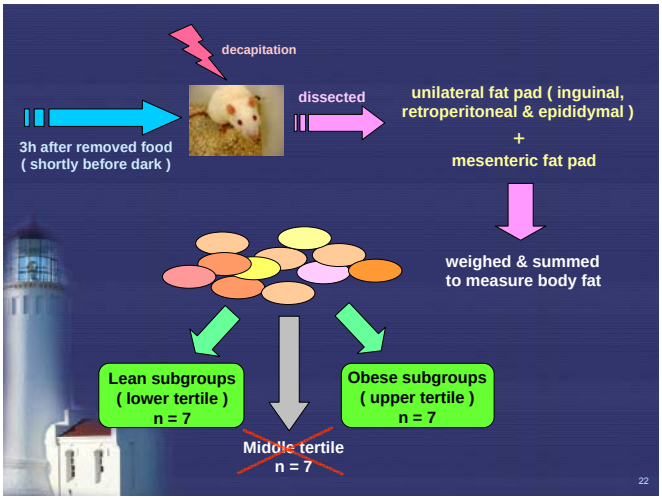
	Intra-assay coefficient of variation	Inter-assay coefficient of variation
Leptin	4.1	3.0
Insulin	4.3	8.5
CORT	7.1	7.2
TG	1.25	1.6
NEFA	1.1	2.1
Glucose	5.6	8.4

Data analysis

- All values are expressed as means ± SE
- Comparison between groups were tested using either a two-way ANOVA followed by a Bonferroni post hoc test for multiple comparison between groups or an unpaired Student's *t*-test when appropriate
- Correlations between within-group measures were performed using a Pearson's product moment correlation
- Significant : $p < 0.05$

Experimental 1





HFM-induced endocrine changes in already obese rats with chronic cardiac catheters

	Lean subgroup (n = 7)	Obese subgroup (n = 7)	
Body weight (g)	441±8	506±10	*
Fat weight (g)	15-19	26-32	*
24h food intake (kcal)	105±5	115±7	

*$p < 0.001$

Concentrations of hormone before and after HFM in lean vs obese rats with a chronic cardiac catheter

	Leptin (ng/ml)	Insulin (ng/ml)	CORT (ng/ml)
Lean (n=7)			
Pre-HFM	6.9±0.3	1.5±0.2	224±17
Post-HFM	8.1±0.8†	2.1±0.2†	176±16† ↓
Obese (n=7)			
Pre-HFM	8.7±0.9*	2.4±0.2*	221±11
Post-HFM	12.0±1.5*†‡	3.8±0.1*†‡	180±14† ↓

CORT = corticosterone

$p < 0.05$

* $p < 0.05$ obese vs lean subgroups
† $p < 0.05$ postmeal vs premeal valuese
‡ $p < 0.05$ magnitude of different between postmeal & premeal scores comparison between obese vs lean

Concentrations of metabolite before and after HFM in lean vs obese rats with a chronic cardiac catheter

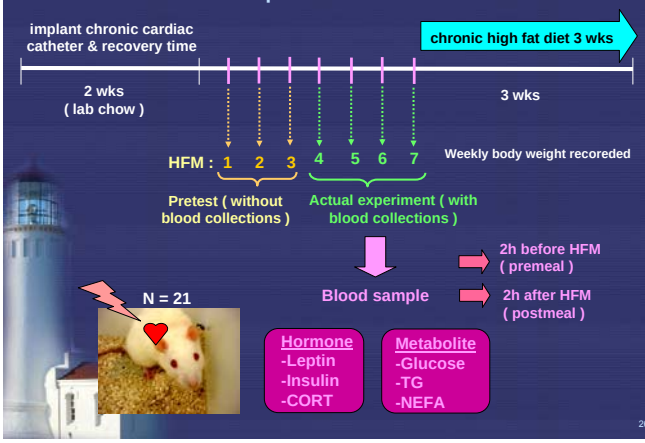
	NEFA (ng/ml)	Glucose (ng/ml)	TG (ng/ml)
Lean (n=7)			
Pre-HFM	0.9±0.1	143±2.2	110±7
Post-HFM	1.3±0.2†	139±2.6	134±9†
Obese (n=7)			
Pre-HFM	1.0±0.1	151±2.7*	134±12*
Post-HFM	1.9±0.3*†	144±1.7*	198±11*†‡

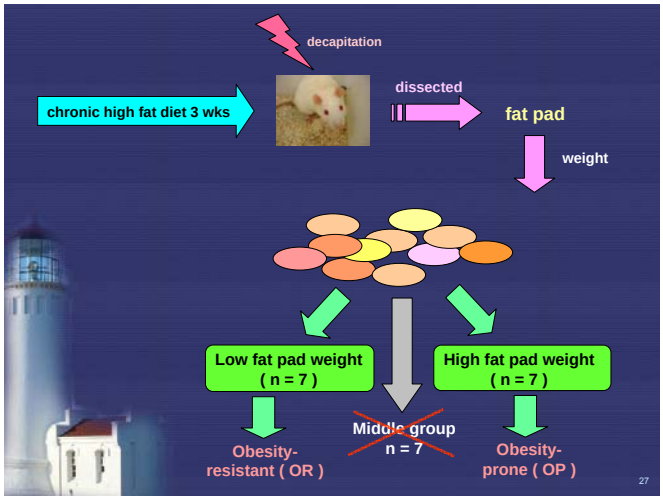
TG = triglyceride ,NEFA = nonesterified fatty acids

p < 0.05

* p < 0.05 obese vs lean subgroups
† p < 0.05 postmeal vs premeal valuese
‡ p < 0.05 magnitude of different between postmeal & premeal scores comparison between obese vs lean

Experimental 2



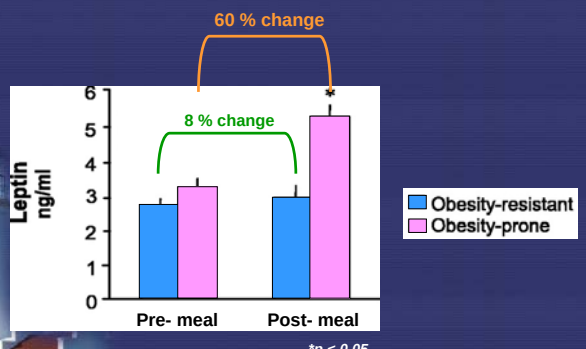


HFM-induced endocrine changes in normal-weight OP rats with chronic cardiac catheter

	Obesity-resistant (n = 7)	Obesity-prone (n = 7)	
Fat weight (g)	16-20	27-31	*
Body weight (g)	455±10	510±17	*
24h food intake (kcal)	89±12	95±5	

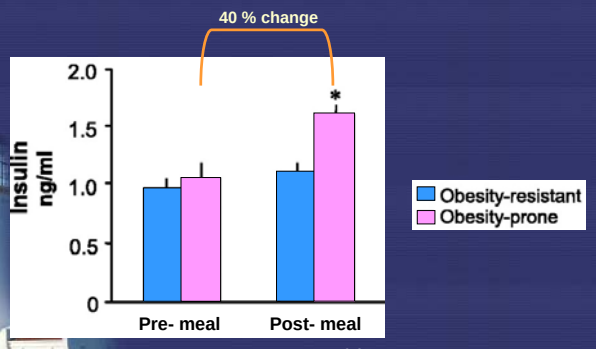
**p < 0.001*

Leptin 2h before and after HFM



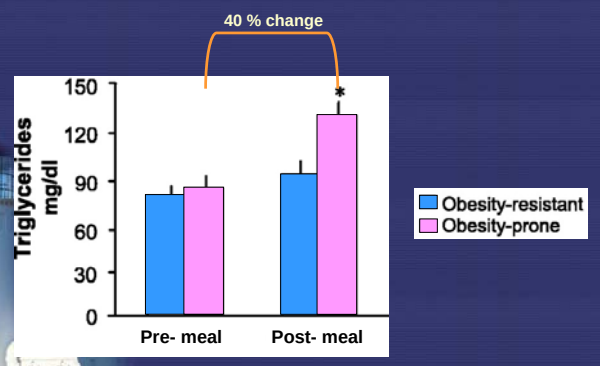
**p < 0.05*

Insulin 2h before and after HFM



**p < 0.05*

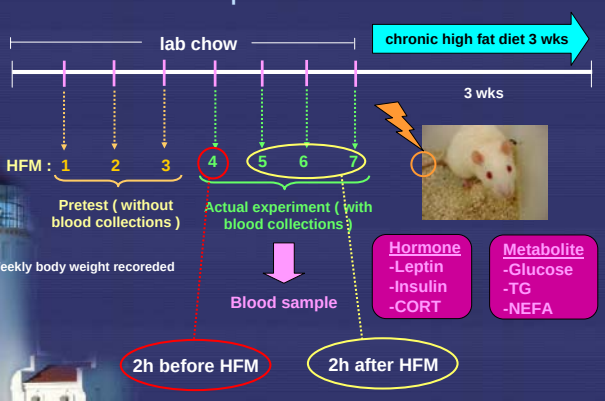
Triglyceride 2h before and after HFM



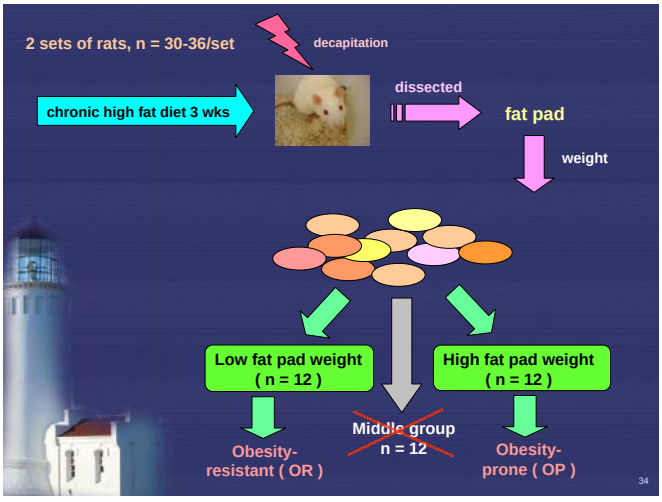
*p < 0.05

- OP rats at normal weight showed early signs of obesity, responding similarity to the obese rats of experimental 1 in their endocrine changes induced by fat-rich meal
- Leptin showed the strongest response to high fat meal

Experimental 3



2 sets of rats, n = 30-36/set



HFM-induced similar endocrine changes in normal –weight OP rats when using a simpler tail vein blood collection

	Obesity-resistant (n = 12)	Obesity-prone (n = 12)	
Fat weight (g)	18-21	30-33	*
Body weight (g)	465±30	510±41	*

p < 0.001

Similar body weight in OR & OP rats (~340 g) before high-fat diet

- Similar result between 2 groups → HFM-induced endocrine changes are reproducible
- Tail vein blood gave similar interaction as cardiac catheter blood collection → simpler tail vein puncture may be a valid method for identifying OR & OP rats
- The greatest change was still detected in post-HFM leptin
- Positive correlation between fat pad vs leptin level across the entire group (N = 30-36)
  - → group 1 : r = +0.58, p < 0.05
  - → group 2 : r = +0.64, p < 0.001

Retrospective analysis

Low fat pad weight (n = 12) → Obesity-resistant (OR)

~~Middle group n = 12~~

High fat pad weight (n = 12) → Obesity-prone (OP)

compared with initial endocrine measurements

Retrospective analysis

After 3 wk high-fat diet

	OR-low body fat	OP-high body fat
Post-HFM leptin (ng/ml)	3.3±0.3	5.8±0.5
Post-HFM insulin (ng/ml)	1.3±0.2	1.9±0.3
Post-HFM TG (ng/ml)	91±15	128±13

group 1 : 74%
group 2 : 80%
} OP > OR

p < 0.001

Prospective analysis

Post-HFM leptin

- Low (2.5-3.9 ng/ml) → Fat weight (g) 18±2
- High (4.2-8.1 ng/ml) → 29±3

60 % heavier

(p < 0.001)

2h after HFM tail vein leptin was the strongest early marker of body fat accrual on a high-fat diet

Experimental 4

Daily body weight recoreded

lab chow → chronic high fat diet 5 wks

HFM : 1 2 3 4 5 6 7

Pretest (without blood collections)

Actual experiment (with blood collections)

5 wks

N = 70

Blood sample

Hormone
-Leptin
-Insulin
-CORT

Metabolite
-Glucose
-TG
-NEFA

2h before HFM

2h after HFM

chronic high fat diet 5 wks

decapitation

dissected → fat pad → weight

Low fat pad weight (n = 23) → Obesity-resistant (OR)

~~Middle group n = 23~~

High fat pad weight (n = 23) → Obesity-prone (OP)

Validation of HFM-induced leptin in differentiating distinct OR and OP subgroups

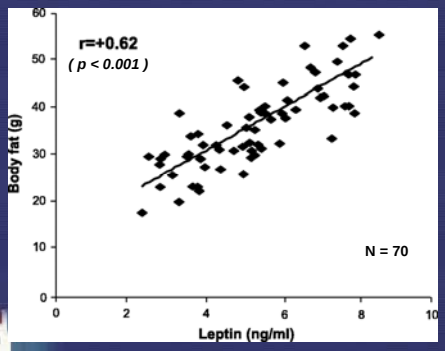
After 5 wk high-fat diet

	OP subgroups	OR subgroups
Fat weight (g)	40-55	20-32
Body weight (g)	571±8	510 ±5
Leptin (ng/ml)	6.3 ±0.6	3.6 ±0.4

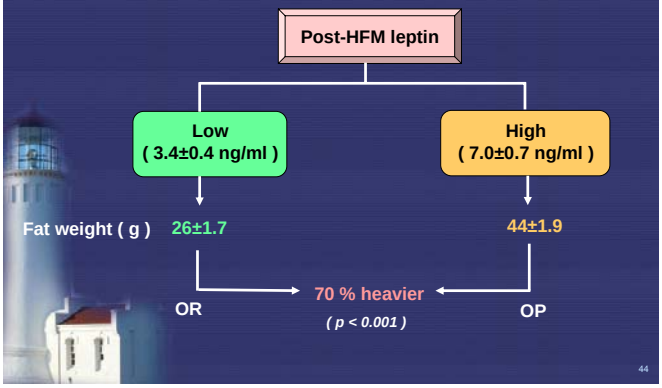
→ OP > OR : 75 %

p < 0.001

Positive correlation between fat pad weight and blood leptin levels

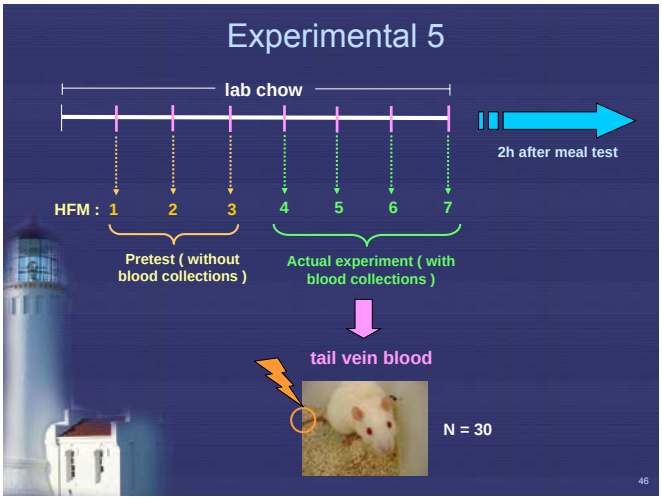


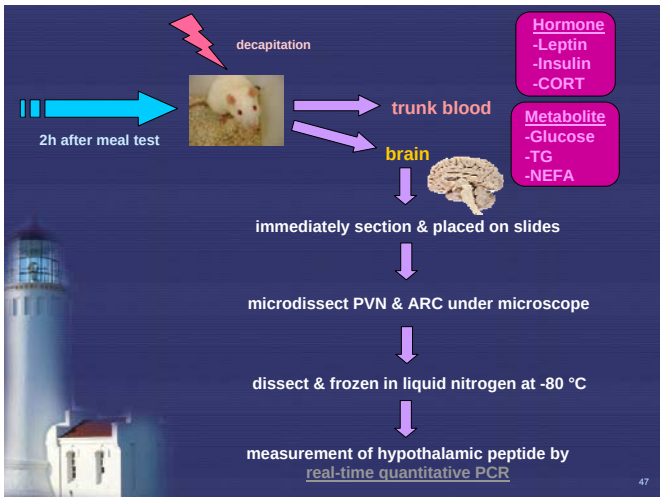
Prospective analysis



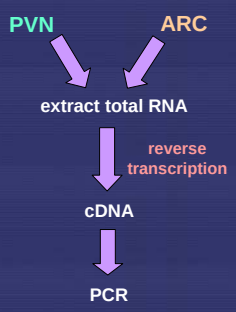
- Experimental 3 & 4 indicate that measurement of post-HFM leptin permits one to identify rats at normal weight (330-350 g) that are prone to obesity

Experimental 5





Real-time quantitative PCR



PVN = paraventricular nuclei
ARC = arcuate nuclei

lowest ····· *leptin level* ·····→ *highest*

1 2 3 4 5 6

N = 30

Subgrouped (n = 5/group) according to post-HFM leptin :
- OP → highest post-HFM leptin (6.5±0.5 ng/ml)
- OR → lowest post-HFM leptin (3.2±0.3 ng/ml)

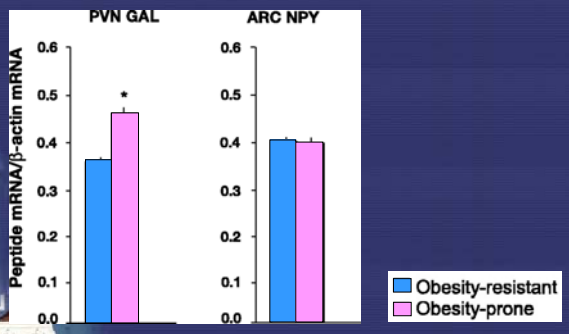
Galanin (GAL) & Neuropeptide Y (NPY)

GAL → positively related to dietary fat and body fat accrual on a high-fat diet

NPY → stimulated food intake and is more specificity controlled by leptin

leptin ⊖ ·····→ **NPY**

Phenotype of normal-weight OP rats identified post-HFM leptin level



PVN GAL = expression of galanin in paraventricular nuclei
ARC NPY = expression of neuropeptide Y in arcuate nuclei

$*p < 0.05$

Discussion

- The results of these experiments demonstrated that OP rats at normal body weight show very early signs of obesity in their HFM-induced changes in leptin, insulin, and TG
- The rise in leptin after an HFM challenge was found to be the strongest correlate of long-term body fat accrual
- This measurement
  - identified distinct OR and OP subgroups at normal weight
  - revealed in OP rats a greater sensitivity to dietary fat, as reflected by an exaggerated HFM-induced rise in PVN GAL expression

Premeal, basal hormome levels in obese and OP rats

- Trunk blood : *(Boivin et al 2000 & Wang et al 1998)*
  basal levels of leptin, insulin, TG & glucose obese > lean
- Blood from chronic cardiac catheter (present study)
  basal premeal :
  - leptin
  - insulin
  - TG
  - glucose

  } obese > lean

  no different in NEFA & CORT

(Levin et al 1999)

rats

humans

low-fat diet → normal endocrine profiles in OP & OR

(Giacco et al 2003 & Raben et al 2000)

OP rats

high-fat diet (1 wk) → disturbances in basal levels of endocrine profiles

body fat ↑

(Leibowitz et al 2004)

HFM-induced rise in leptin, insulin and lipid levels

In normal weight rats

2h after HFM vs premeal at dark onset

↑ leptin, insulin, TG & NEFA

no change in glucose

In the few studies that have made such comparisons between pre- and postmeal levels, similar meal-induced changes in normal weight subjects have been described in rats (West et al 1989) and humans (Murphy et al 1995, Romon et al 1999 & Surina et al 1993)

↓ CORT levels during the first 2 h of the dark cycle → circadian-related shift

(Tempel et al 1994)

Cardiac catheter

post-HFM : leptin ↑ insulin lipids

Tail vein puncture

This validates the utility of this simpler method of blood collection for revealing subtle, meal-induced endocrine responses in subjects with normal basal levels of hormones and metabolites

Greater HFM-induced rise in leptin, insulin and TG in obese and OP rats

blood collected via cardiac catheter both before and after a meal

lean subjects

obese subjects

HFM

Leptin, insulin & TG

Leptin, insulin & TG ↑ X 2

premeal levels of the hormones and metabolites exhibited a twofold greater rise after the HFM in leptin, insulin, and TG

No group different in NEFA, glucose, and CORT

Consistent with the present results (Raben et al 2000)

post-HFM leptin levels in normal-weight ↑

normal premeal leptin levels

leptin

OP rats are less sensitive to the anorectic effects of centrally administered leptin (Levin et al 2002)

Thus meal-related changes in leptin in OP subjects with normal basal levels → early indications of a resistance & disturbance in fat metabolism

Prediction of obesity by high post-HFM leptin levels

Need : predictors or early endocrine markers that can reliably and accurately predict future weight gain on a chronic high-fat diet

obese → positive relationships to fat mass

measurements of → basal insulin levels (Ravussin et al 1999 & Pagliassotti et al 1994)

→ basal leptin levels (Byrnes et al 1999 & Raben et al 2000)

In normal-weight rats

measurements of → fasting levels of TG levels (Ji et al 2003 & Levin et al 1992)

strong correlates of future weight gain on a high-calorie diet

- This study
  → measurement of leptin after an HFM was the strongest & most consistent correlate of long-term body fat accrual
  → also the most effective in accurately identity OR & OP rats
- Both insulin & TG levels after HFM were positively related to body fat

Mechanisms underlying increased HFM-induced leptin in normal-weight OP rats

- **Basal levels of leptin are known to be strongly positively correlated with body fat on a high-fat diet** *(Fried et al 2000)*

Dietary fat (critical factor)

(Coleman et al 1999 & Stefan et al 2001)

Insulin (depend on dose & nutritional status)

→ **leptin** ↑

insulin → accelerated fat synthesis

HFM-induced suppression of fat oxidation in OP subjects  *reflect* → **early resistance to the effects of leptin**

(Giacco et al 2003 & Raben et al 1999)

Disturbances in hypothalamic peptides that accompany elevated HFM-induced leptin in OP rats

OP rarts → expression of GAL gene in PVN ↑

High-fat diet has significantly greater impact in the OP subjects

↓

they are more sensitive to the stimulatory effect of dietary fat and lipids on PVN GAL

(Chang et al 2004 & Leiboweitz et al 2004)

- **Increase in GAL expression and peptide in OP rats is likely to contribute to their early development of obesity on a high-fat diet**

supported evidences

Acute GAL injection → **stronger feeding-stimulatory effect on high-fat diet compared to low-fat diet**

metabolic effect

↓ **energy expenditure & sympathetic nervous system activity**

↓ **stimulation of carbohydrate over fat metabolism**

(Leibowitz et al 2004, Menendez et al 1992 & Nagase et al 2002)

Chronic GAL injection → **body weight & body fat accrual most strongly on a high-fat diet** ↑

(Yun et al 2005 & Hohmann et al 2003)

No difference in NPY mRNA in the ARC of OP compared with OR rats

GAL → less sensitive to leptin

↑ **leptin** → ↑ **GAL** *(Leibowitz et al 2004)*

Thus, in response to an HFM, measurements of PVN GAL expression are more revealing of significant differences between OR and OP subjects, with the latter showing greater responsiveness to the fat content of a meal

OP rats on HFM

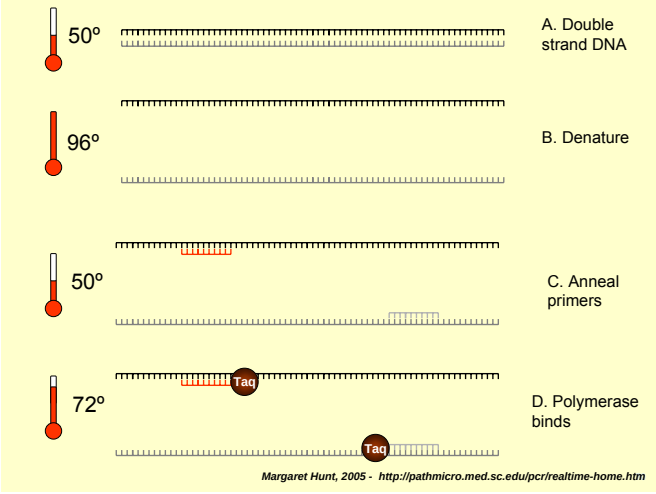
leptin ↑ → **leptin resistance** → **NPY expression** ✗ → ✗ **food intake** → **Obesity**

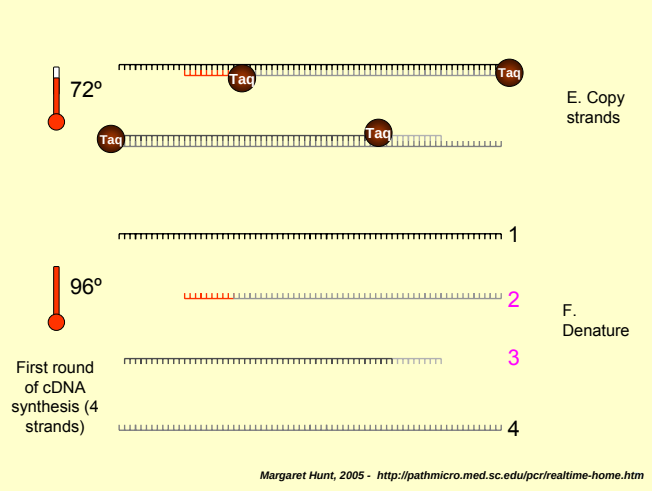
PVN GAL ↑ → **high-fat diet intake** ↑ → **Obesity**

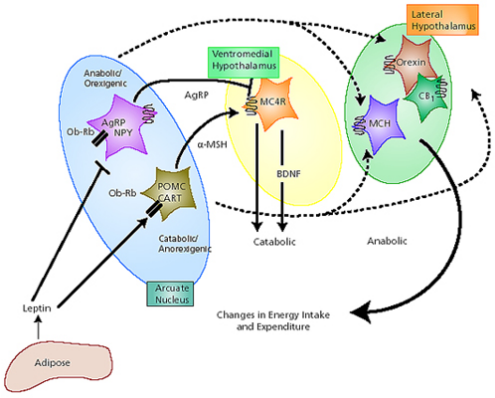
fat tissue ↑ → **leptin**


Thank You
67


AAAAA
RT
TTTTT
Oligo dT primer is bound to mRNA
RT
AAAAA
TTTTT
Reverse transcriptase (RT) copies first cDNA strand
AAAAA
RT
TTTTT
Reverse transcriptase digests and displaces mRNA and copies second strand of cDNA
Double strand cDNA
Conversion of mRNA to cDNA by Reverse Transcription
Margaret Hunt, 2005 - http://pathmicro.med.sc.edu/pcr/realtime-home.htm


50º
A. Double strand DNA
96º
B. Denature
50º
C. Anneal primers
72º
Taq
Taq
D. Polymerase binds
Margaret Hunt, 2005 - http://pathmicro.med.sc.edu/pcr/realtime-home.htm


72º
Taq
Taq
Taq
Taq
E. Copy strands
1
96º
2
3
F. Denature
First round of cDNA synthesis (4 strands)
4
Margaret Hunt, 2005 - http://pathmicro.med.sc.edu/pcr/realtime-home.htm


Lateral Hypothalamus
Orexin
CB1
MCH
Ventromedial Hypothalamus
MC4R
AgRP
α-MSH
BDNF
Catabolic
Anabolic
Anabolic/ Orexigenic
AgRP NPY
Ob-Rb
POMC CART
Ob-Rb
Catabolic/ Anorexigenic
Arcuate Nucleus
Leptin
Adipose
Changes in Energy Intake and Expenditure