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

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

Introduction

- 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-prone (OP)
Obesity-resistance (OR)

• 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

- insulin
- leptin
- glucose
- triglycerides (TG)
- nonesterified fatty acids (NEFA)

(Boivin et al. 2000, Sunwit et al. 1995 & Wang et al. 1998)

- corticosterone (CORT)

(Svec et al. 1997 & Dourmashkin et al. 2005)

In normal-weight animals (Henaya et al. 1997, Sunina-Baumgartner et al. 1996)

- HFM compared with premeal scores
  - insulin, leptin, glucose & lipids
  - little change in CORT

Clinical studies (Guerci 2000, Imbeault 2001)

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

- 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 disturbances in hypothalamic peptides (galanin & NPY) that accompany elevated HFM-induced leptin in OP rats
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
- 4% minerals
- 3% vitamins

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

Surgery & Blood sampling procedures

Chronic cardiac catheter (jugular vein)

Experiments 1-2

Tail vein puncture

Experiments 3-5

rotating cantilever beam

HFM challenge test (40 kcal)

At dark onset

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

Æ close to a natural intermeal interval
Æ consistent postmeal changes in hormones and metabolites

2 h before the nocturnal feeding cycle

remove lab chow diet

To control for the size of the test meal

15–20% of the total group

40 kcal in 2h interval

< 38 kcal in 2h interval

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.

<table>
<thead>
<tr>
<th></th>
<th>Intra-assay coefficient of variation</th>
<th>Inter-assay coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin</td>
<td>4.1</td>
<td>3.0</td>
</tr>
<tr>
<td>Insulin</td>
<td>4.3</td>
<td>8.5</td>
</tr>
<tr>
<td>CORT</td>
<td>7.1</td>
<td>7.2</td>
</tr>
<tr>
<td>TG</td>
<td>1.25</td>
<td>1.6</td>
</tr>
<tr>
<td>NEFA</td>
<td>1.1</td>
<td>2.1</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.6</td>
<td>8.4</td>
</tr>
</tbody>
</table>

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

Hormone and metabolite assays were performed at different times for the different experiments.

<table>
<thead>
<tr>
<th>Clinical parameter</th>
<th>Pre-HFM</th>
<th>Post-HFM</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>44±18</td>
<td>50±10</td>
<td></td>
</tr>
<tr>
<td>Fat weight (g)</td>
<td>15-19</td>
<td>26-32</td>
<td></td>
</tr>
<tr>
<td>24h food intake (kcal)</td>
<td>105±15</td>
<td>115±27</td>
<td>*</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Clinical parameter</th>
<th>Pre-HFM</th>
<th>Post-HFM</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/ml)</td>
<td>-6.4±0.3</td>
<td>-2.0±0.5</td>
<td></td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>-19±0.2</td>
<td>-2.1±0.2</td>
<td></td>
</tr>
<tr>
<td>CORT (ng/ml)</td>
<td>22±11</td>
<td>16±11</td>
<td></td>
</tr>
</tbody>
</table>

CORT = corticosterone

*p < 0.05

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

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Lean (n=7)</th>
<th>Obese (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEFA (ng/ml)</td>
<td>0.9±0.1 †</td>
<td>1.6±0.1 †*</td>
</tr>
<tr>
<td>Glucose (ng/ml)</td>
<td>143±22</td>
<td>151±27*</td>
</tr>
<tr>
<td>TG (ng/ml)</td>
<td>110±7</td>
<td>134±8†</td>
</tr>
</tbody>
</table>

TG = triglyceride, NEFA = nonesterified fatty acids

* p < 0.05 obese vs lean subgroups
† p < 0.05 postmeal vs premeal values
‡ 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**

<table>
<thead>
<tr>
<th></th>
<th>Obesity-resistant (n=7)</th>
<th>Obesity-prone (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat weight (g)</td>
<td>16±20</td>
<td>27±31</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>45±10</td>
<td>51±17</td>
</tr>
<tr>
<td>24h food intake (kcal)</td>
<td>89±12</td>
<td>95±15</td>
</tr>
</tbody>
</table>

*p < 0.001

Leptin 2h before and after HFM

* p < 0.05

Insulin 2h before and after HFM

* p < 0.05
Pre-meal Post-meal

Triglyceride 2h before and after HFM

• 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

Blood sample

Hormone
- Leptin
- Insulin
- CORT

Metabolite
- Glucose
- TG
- NEFA

2h before HFM 2h after HFM

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

<table>
<thead>
<tr>
<th></th>
<th>Obesity-resistant (n = 12)</th>
<th>Obesity-prone (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat weight (g)</td>
<td>18-21</td>
<td>30-33</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>465±30</td>
<td>510±41</td>
</tr>
</tbody>
</table>

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)
High fat pad weight (n = 12)

Middle group (n = 12)
Obesity-resistant (OR)
Obesity-prone (OP)

compared with initial endocrine measurements

Prospective analysis

Post-HFM leptin

Low (2.5-3.9 ng/ml)
18±2
60% heavier (p < 0.001)

High (4.2-8.1 ng/ml)
29±3

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 recorded
lab chow
chronic high fat diet 5 wks

Pretest (without blood collections)
Actual experiment (with blood collections)
Blood sample

Hormone
-Leptin
-Insulin
-CORT

Metabolite
-Glucose
-TG
-NEFA

2h before HFM
2h after HFM

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

After 5 wk high-fat diet

<table>
<thead>
<tr>
<th>OR subgroups</th>
<th>OP subgroups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat weight (g)</td>
<td>40-55</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>57±8</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>6.3 ±0.6</td>
</tr>
</tbody>
</table>

p < 0.001

OP > OR: 75%
Positive correlation between fat pad weight and blood leptin levels

\[ r = 0.62 \quad (p < 0.001) \]

\( N = 70 \)

Prospective analysis

- Low (3.4±0.4 ng/ml)
- High (7.0±0.7 ng/ml)

Fat weight (g) 26±1.7
- 70% heavier (\( p < 0.001 \))

Experimental 5

- Pretest (without blood collections)
- Actual experiment (with blood collections)

Real-time quantitative PCR

- PVN = paraventricular nuclei
- ARC = arcuate nuclei

- Hormone
  - Leptin
  - Insulin
  - CORT

- Metabolite
  - Glucose
  - TG
  - NEFA

- Trunk blood
- Brain

Measurement of hypothalamic peptide by real-time quantitative PCR
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)

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

- 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.

Premeal, basal hormone 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
Low-fat diet vs normal endocrine profiles in OP & OR (Levin et al 1999)

High-fat diet (1 wk) vs OP rats disturbances in basal levels of endocrine profiles (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 1994) and humans (Murphy et al 1996, Romon et al 1999 & Surina et al 1993)

Cardiac catheter vs 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

No group different in NEFA, glucose, and CORT

Consistent with the present results (Raben et al 2000)

- 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 levelsÆ 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 subjects vs lean subjects

- Leptin, insulin & TG
- HFM
- 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
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 identify 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)
  - Insulin (depend on dose & nutritional status)

  - HFM-induced suppression of fat oxidation in OP subjects
  - 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 rats 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

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

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

- OP rats on HFM
  - leptin 
  - leptin resistance 
  - NPY expression 
  - OP rats 
  - NPY expression 
  - high-fat diet intake 
  - Obesity 
  - OP rats on HFM

- OP rats 
  - NPY expression 
  - high-fat diet intake 
  - Obesity 

- OP rats 
  - NPY expression 
  - high-fat diet intake 
  - Obesity 

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

- GAL less sensitive to leptin
  - GAL

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
Conversion of mRNA to cDNA by Reverse Transcription

1. Oligo-dT primer is bound to mRNA
2. Reverse transcriptase (RT) copies first cDNA strand
3. Reverse transcriptase digests and displaces mRNA and copies second strand of cDNA
4. Double strand cDNA

First round of cDNA synthesis (4 strands)

A. Double strand DNA
B. Denature
C. Anneal primers
D. Polymerase binds
E. Copy strands
F. Denature