Analysis of Inflammatory Signaling in Repro-Metabolic Syndrome

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INTRODUCTION

- We have previously demonstrated that the combination of infused free fatty acids and lipids, administered via a hyperinsulemic, euglycemic clamp, acutely reduces LH and FSH in healthy, non-obese men and women [1].
- We have adopted the term 'reprometabolic syndrome' to describe the metabolic dysfunction and relative hypogonadotrophic hypogonadism characteristic of women with obesity.
- We sought to determine the underlying cause for the rapid (within 6 hours) induction of reprometabolic syndrome in normal weight women.
- Obesity is a state of chronic low-grade inflammation, characterized by a milieu of excess pro-inflammatory cytokines and chemokines, and a decrease in anti-inflammatory cytokines [2,3].
- We have previously shown that low dose transdermal estrogen pre-treatment selectively reduced inflammatory cytokines in women with obesity accompanied by an increase in LH pulse amplitude and GnRH stimulated FSH [4].
- Thus, we hypothesize that inflammatory signaling to the pituitary may impair gonadotropin synthesis and secretion, explaining the previously demonstrated link between infertility and obesity.
- In order to identify potential mediators of insulin and lipid-related reproductive endocrine dysfunction, we examined serum levels of inflammatory markers.

METHODS

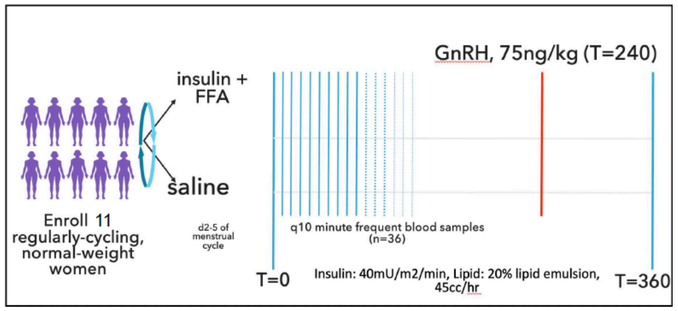
- This study was performed as a a secondary analysis of an ongoing study. 11 reproductive aged women of normal BMI (<25 kg/m2), with regular menstrual cycles, were recruited with IRB approval. All subjects were studied in the early follicular phase of the menstrual cycle (Day 2-5).
- Each participant underwent infusion of either saline or insulin (40mg/kg/min) plus free fatty acid (Intralipid), for 6 hours, in sequential cycles in random order. Euglycemia was maintained by glucose infusion. Frequent blood sampling (q10 min) was performed.
- To assess the inflammatory milieu, blood samples from 180-230 min (at which time steady state lipid and insulin levels were achieved) were pooled from each subject and analyzed for cytokines, interleukins, chemokines, adipokines, Fibroblast Growth Factor-21 (FGF-21) and markers of endoplasmic reticulum (ER) stress (CHOP and GRP78) (Table 1).
- Wilcoxon signed-rank tests were used to compare results across experimental conditions. The level of significance was set at $p < 0.05$.

DEMOGRAPHICS

Parameter	Mean ± SD
Total Enrollment	8
Age (y)	26.33 ± 4.87
BMI (kg/m^2)	22.22 ± 1.32
Weight (kg)	60.61 ± 3.75
Height (cm)	165.43 ± 4.85
Waist Circumference (cm)	69.43 ± 15.29
Waist-Hip Ratio	0.83 ± 0.09
TSH (mIU/ml)	1.43 ± 0.46
Prolactin (ng/ml)	9.68 ± 3.99
Cycle Length (days)	27.50 ±1.41

FIGURE 1: STUDY DESIGN

Depiction of each study arm and timeline. Crossover study of 11 NWW. Bolus of GnRH administered at 240 min in both visits, done to measure pulsatility as part of a related but separate study.



Inclusion Criteria

- Regular menstrual cycles
- Normal Prolactin, TSH, & HbA1c
- < 4 hours vigorous exercise/week

Exclusion Criteria

- Pregnancy
- Diabetes
- Triglycerides > 250 mg/dL
- Chronic inflammatory conditions
- Liver, kidney, or lung disease
- Abnormal cardiac function
- Anemia
- Hormonal medications
- Soy or egg allergy

RESULTS

The Effect of Insulin + Fatty Acid Infusion on Inflammatory Molecules and ER Stress

Figure 2: Cytokines

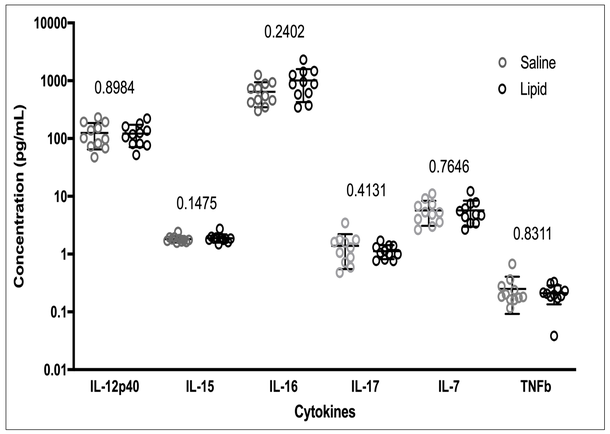


Figure 3: Proinflammatory

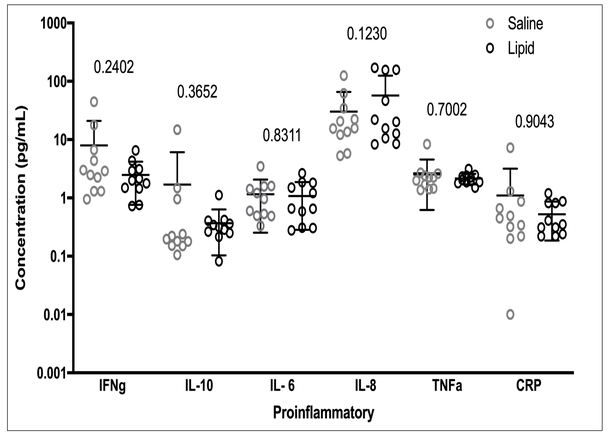


Figure 4: Chemokines

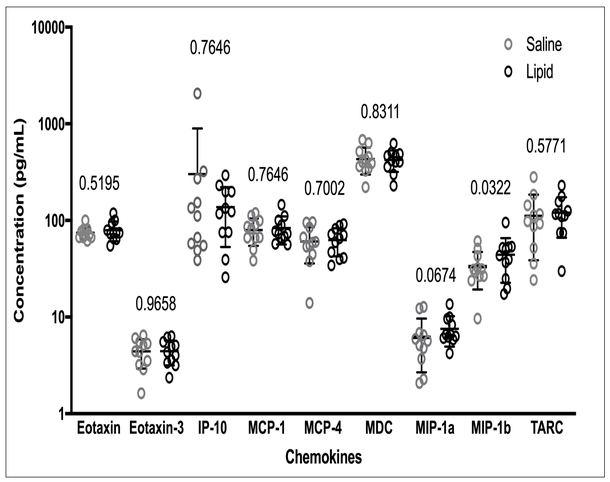
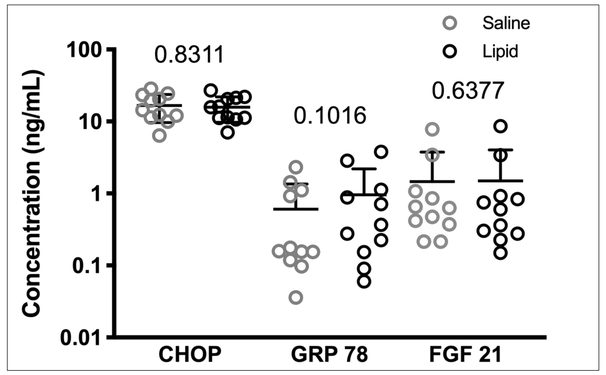


Figure 5: ER Stress Markers and FGF-21



ER stress has been implicated in the pathogenesis of obesity and may modulate pituitary gonadotropin secretion and ovarian function. FGF-21 is a regulator of glucose and lipid metabolism with increased level in obesity, which has been implicated in neuroendocrine control of female reproduction

SIGNALING MOLECULES MEASURED

hsCRP	Eotaxin	Eotaxin-3	IFN-γ
IL-6	IL-7	IL-8	IL-10
IL-12p40	IL-17	IL-15	IL-16
IP-10	MCP-1	MCP-4	MDC
MIP-1β	TARC	TNF-α	TNF-β
MIP-1α	CHOP	GRP78	FGF-21

Table 1: Signaling Molecules

RESULTS

- Induction of Repro-Metabolic syndrome was confirmed by a decrease in LH and FSH pulse amplitude and the development of insulin resistance (not shown).
- With the exception of MIP-1b, no significant differences were observed in any of the cytokines, proinflammatory molecules, chemokines, hormones, or ER stress markers tested.

CONCLUSIONS

- Infusion of lipid and insulin to mimic the metabolic syndrome of obesity was not associated with an increase in inflammatory markers.
- Our results imply that the endocrine disruption and adverse reproductive outcomes of obesity are not a consequence of the inflammatory environment but may be mediated by direct lipotoxic effects on the hypothalamic-pituitary-gonadal axis.

REFERENCES

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