Adipocyte Dysfunction in a Mouse Model of Polycystic Ovary Syndrome (PCOS): Evidence of Adipocyte Hypertrophy and Tissue-Specific Inflammation

Joseph S. Marino, Jeffrey Iler, Abigail R. Dowling, Streamson Chua, Jens C. Bruning, Roberto Coppari, Jennifer W. Hill

Abstract

Clinical research shows an association between polycystic ovary syndrome (PCOS) and chronic inflammation, a pathological state thought to contribute to insulin resistance. The underlying pathways, however, have not been defined. The purpose of this study was to characterize the inflammatory state of a novel mouse model of PCOS. Female mice lacking leptin and insulin receptors in pro-opiomelanocortin neurons (IR/LepR mice) and littermate controls were evaluated for estrous cyclicity, ovarian and adipose tissue morphology, and body composition by QMR and CT scan. Tissue-specific macrophage infiltration and cytokine mRNA expression were measured, as well as circulating cytokine levels. Finally, glucose redrome (PCOS): Evidence of

Adipocyte Hypertrophy and Tissue-Specific Inflammation. PLoS ONE 7(10): e48643. doi:10.1371/journal.pone.0048643

Editor: Stephen Franks, Imperial College London, United Kingdom

Received: May 29, 2012; **Accepted:**

Examination of Inflammatory Markers

Immunohistochemistry was performed on perigonadal adipose tissue collected from 4 month old IR/LepR and IR/LepR (control) mice. Briefly, tissue was fixed overnight in 10% formalin and paraffin embedded before sectioning. Following 30 minutes of blocking, sections were incubated overnight at 4°C with rat anti-mouse F4/80 (abCam) at a dilution of 1:50. Alexa Fluor 488 (Invitrogen) secondary was used to visualize F4/80 positive cells, and nuclei were labeled with DAPI (Vector Labs). For each set of slides immunostained for F4/80, one cross section from an IR/LepR mouse was used as an antibody control to test specificity of the primary antibody.

For QPCR analysis, RNA was isolated from perigonadal fat, liver, and ovary from 4 month old IR/LepR and IR/LepR (control) mice using Qiagen Rneasy kit (Qiagen). cDNA was reverse transcribed using Applied Biosystems high capacity RT kit (Applied Biosystems). F4/80, and cytokine gene expression was measured using a Step One Plus (Applied Biosystems) system and expression levels were normalized to 18S (adipose) or GAPDH (ovary and liver). Changes in mRNA expression were calculated using 2 and expressed as a fold change compared with controls (IR/LepR). Sequences for primers used from 5′ to 3′ are: F4/80 forward CTTTGGCTATGGGCTTCCAGTC and reverse GCAAGGAGGACAGAGTTTATCGTG, CD11c forward CTGGATAGCCTTTCTTCTGCTG and reverse GCACACTGTGCCGAACTCA, IL-1β forward CAACCAACAAGTGATATTCTCCATG and reverse GATCCACACTCTCCAGCTGCA, IL-6 forward CTGCAAGAGCTTCCATCCAGTT and reverse GAAGTAGGGAAGGCCGTGG,18s forward TTGACGGAAGGGCACCACCAG and reverse GCACCACCACCCACGGAATCG and GAPDH forward ATGTTTGTGATGGGTGTGAA and reverse ATGCCAAAGTTGTCATGGAT.

Serum analysis

Blood from randomly cycling adult female mice (4 months old) was collected transcardially at 3 hours after lights on to avoid any potential influence of increased gonadal steroids in the afternoon of certain phases of the estrous cycle. Serum was analyzed as follows. Triglycerides were analyzed with commercially available reagents (Pointe Scientific, Inc.). Total cholesterol, HDL, and LDL/VLDL were analyzed with a commercially available kit (BioAssay Systems), with a lower limit of detection of 5 mg/dl. C-reactive protein (Alpco) analysis was performed according to the manufacturer's protocol with a lower limit of detection of 0.39 ng/ml. Cytokine concentrations were analyzed using a Bio-Plex cytokine array (Bio-Rad) and measured using the Bio-Plex Plate Reader (Bio-Rad) with a lower limit of detection of 0.2

We then tested whether impaired ovulation in IR/LepR mice results from POMC neurons failing to communicate key permissive signa

Glucose tolerance was not impaired when normalized to initial glucose levels (Fig 5c), indicating that pregnancy induced a similar increase in blood glucose levels in each group of mice. In addition, HOMA-IR values did not differ between the two groups (not shown). Therefore, IR/LepR mice are hyperglycemic before and during pregnancy, without evidence of gestational glucose intolerance.

Figure 5. IR/LepR females are hyperglycemic and show hyperglycemia during pregnancy.
A. IR/LepR female glucose levels under basal conditions (n = 6–7) ** p<0.01. B. Fasted glucose levels in a second cohort of females, before pregnancy and on gestational day 12 and 15 (Black circles are control dams, open triangles are IR/LepR dams; n = 11–12) * p<0.05, compared with controls at same timepoint; p<0.05, compared with pre-pregnancy values of same group. C. Glucose tolerance testing (2 g/kg) performed on day 15–18 of gestation. (Black circles are control dams, open triangles are IR/LepR dams; n = 6). Mean ± SEM.
doi:10.1371/journal.pone.0048643.g005

Discussion

Mice lacking leptin receptors and insulin receptors in POMC cells (IR/LepR mice) were originally developed to study the control of food consumption by POMC neurons in the hypothalamus [37]. Both insulin and the adipokine leptin communicate the status of adiposity stores to the brain [47], [48]. POMC neurons modulate food intake, energy use, and hepatic glucose production, and may change these parameters independently [34], [35], [36]. High parasympathetic output (or low sympathetic output) transmitted through the vagus nerve to the liver reduces hepatic glucose production by inhibiting hepatic enzymes involved in gluconeogenesis and by activating enzymes promoting glycogen synthesis. This output is modulated by neurons of the hypothalamus, including POMC neurons [49], [50] and seems to result in excess HGP in IR/LepR mice. Likewise, POMC neurons project multisynaptically to brown adipose tissue [51] and can thereby change oxygen consumption and basal metabolic rate. Given the suppressed oxygen consumption in IR/LepR mice without changed physical activity levels [37], this pathway likely underlies the increased adiposity in older IR/LepR mice.

Both leptin and insulin signaling in POMC neurons can change neuronal activity as well as the production and processing of the POMC gene product [37], [52]. Importantly, a single nucleotide polymorphism of the POMC gene is associated with PCOS risk in women [53]. Hence, further characterization of POMC neuronal function and regulation is important to understand the potential contribution of this pathway to PCOS.

We have recently shown that the IR/LepR mouse displays hyperandrogenemia, lengthened estrous cycles, and reduced fertility, arguably qualifying it for a PCOS “diagnosis.” However, the ovaries of IR/LepR mice do not show excess numbers of small antral follicles, i.e. polycystic ovaries. Most women with polycystic ovaries are asymptomatic [54], and diagnosis of PCOS does not require the presence of polycystic ovaries [55]. Thus, the IR/LepR mouse might best serve a model of the subset of PCOS patients diagnosed because of impaired ovulation and hyperandrogenemia. Interestingly, not all IR/LepR mice are affected by anovulation or infertility. The cause of the incomplete penetrance of this phenotype is the subject of ongoing studies.

(remaining to be tested) is that inadequate adipogenesis in the face of a positive energy balance drives adipocyte dysfunction, chronic inflammation, and insulin resistance.

Although circulating markers of chronic inflammation are elevated in women with PCOS, the source of their production is unclear. Manneras-Holm and colleagues found no increase in macrophage density in subcutaneous adipose tissue from women with PCOS [5]. The antibody used in that study, how

1. CrossRef • PubMed/NCBI • Google Scholar
2. CrossRef • PubMed/NCBI • Google Scholar
3. CrossRef • PubMed/NCBI • Google Scholar
4. CrossRef • PubMed/NCBI • Google Scholar
5. CrossRef • PubMed/NCBI • Google Scholar
6. CrossRef • PubMed/NCBI • Google Scholar

7. CrossRef • PubMed/NCBI • Google Scholar
8. CrossRef • PubMed/NCBI • Google Scholar
9. CrossRef • PubMed/NCBI • Google Scholar
10. CrossRef • PubMed/NCBI • Google Scholar
11. CrossRef • PubMed/NCBI • Google Scholar
12. CrossRef • PubMed/NCBI • Google Scholar
13. CrossRef • PubMed/NCBI • Google Scholar
14. CrossRef • PubMed/NCBI • Google Scholar
15. CrossRef • PubMed/NCBI • Google Scholar
16. CrossRef • PubMed/NCBI • Google Scholar
17. CrossRef • PubMed/NCBI • Google Scholar
18. CrossRef • PubMed/NCBI • Google Scholar
19. CrossRef • PubMed/NCBI • Google Scholar
20. CrossRef • PubMed/NCBI • Google Scholar
21. CrossRef • PubMed/NCBI • Google Scholar
22. CrossRef • PubMed/NCBI • Google Scholar
23. CrossRef • PubMed/NCBI • Google Scholar
24. CrossRef • PubMed/NCBI • Google Scholar
25. CrossRef • PubMed/NCBI • Google Scholar
26. CrossRef • PubMed/NCBI • Google Scholar

2D.001 Tc[(Cro)4.8(s)-3.8(s)5.8(R)-.6(e)]TJETq1 i 101.16 403.86 26.22 7.08 reW nBT6.3 0 pV7 6.3 232.44 176.8201 Tm0 3.8(-8.7(Sc)-7.7(hol)-9.3(a)]TJETq1 ie)]TJETq1 i 101.16 463.1

31.

CrossRef • PubMed/NCBI • Google Scholar

32.

CrossRef • PubMed/NCBI Google Schola

55.