Maternal Adiponectin Controls Milk Composition to Prevent Neonatal Inflammation

Zixue Jin¹, Yang Du¹, Adam G. Schwaid³, Ingrid W. Asterholm², Philipp E. Scherer², Alan Saghatelian³, and Yihong Wan¹

¹Department of Pharmacology, ²Touchstone Diabetes Center, Department of Internal Medicine, The University of Texas Southwestern Medical Center, Dallas, TX 75390, USA; ³Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA 02138, USA

Adiponectin is an important adipokine. Increasing evidence suggest that altered adiponectin levels are linked with metabolic and inflammatory disorders. Here we report an important yet previously unrecognized function of adiponectin in lactation by which maternal adiponectin determines the inflammatory status in the nursing neonates. Surprisingly, both maternal adiponectin over-expression in the transgenic mice and maternal adiponectin deletion in the knockout mice lead to systemic inflammation in the pups, manifested as transient hair loss. However, distinct mechanisms are involved. Adiponectin deficiency triggers leukocyte infiltration and production of inflammatory cytokines in the lactating mammary gland. In contrast, adiponectin overabundance increases lipid accumulation in the lactating mammary gland, resulting in excessive long chain saturated fatty acids (LcSFA) in milk. Interestingly, in both cases, the inflammation and alopecia in the pups can be rescued by TLR2/4 deletion because TLR2/4 double knockout pups are resistant. Mechanistically, LcSFA activation of inflammatory genes is TLR2/4-dependent and can be potentiated by pro-inflammatory cytokines, indicating that the inflammatory stimuli in both scenarios functionally converge by activating the TLR2/4 signaling. Therefore, our findings reveal adiponectin as a dosage-dependent regulator of lactation homeostasis and milk quality that critically control inflammation in the nursing neonates. Furthermore, these results suggest that inflammatory infantile disorders may result from maternal adiponectin dysregulation that can be treated by TLR2/4 inhibition.

Adiponectin is an adipocyte-derived hormone (1). It is a key modulator of lipid and glucose metabolism, and its dysfunction is associated with many metabolic disorders such as type 2 diabetes, insulin resistance and atherosclerosis (2, 3). It is also a modulator of the innate immunity, and its dysregulation is associated with chronic systemic inflammation, which can further exacerbate the metabolic syndrome (4, 5). However, the role of adiponectin in inflammation is unclear with seemingly contradictory findings reported (3). Adiponectin is suggested to have anti-inflammatory functions by stimulating a ceramidase activity because decreased adiponectin levels correlates with chronic inflammation associated with obesity, type 2 diabetes and cardiovascular diseases (3, 6). Nonetheless, adiponectin is also proposed to be proinflammatory because elevated adiponectin levels correlate with classic inflammatory and autoimmune diseases such as rheumatoid arthritis (RA) (7, 8), systemic lupus erythematosus (SLE) (9), inflammatory bowel disease (10) and type 1 diabetes (11). Therefore, further investigation is required to elucidate the mechanisms for how adiponectin controls inflammation (12).

Milk is the perfect food for all newborn mammals. However, our recent findings brought an unexpected twist that maternal genetic or dietary defects, such as PPARγ/VLDLR deficiency or high-fat-diet, can lead to the secretion of toxic milk that causes systemic inflammation in the neonates manifested as transient alopecia (13–15).
ceptually, our findings provide new insights to the etiology and the treatment of infantile metabolic and inflammatory disorders. Technically, by using alopecia as a visual readout that serves as the “eye of Drosophila”, our studies highlight the milk-neonate axis as an innovative in vivo experimental system to identify new regulators of metabolism and immunity.

Here we reveal that both maternal adiponectin deficiency and maternal adiponectin overabundance, via distinct mechanisms, cause lactation defects and milk disorders that lead to systemic inflammation and alopecia in the nursing neonates. These findings will enhance our understanding of how adiponectin modulates metabolism and immunity.

Materials and Methods

Mice

Adip-KO mice and Adip-TG (ap2-ΔGly-adiponectin transgenic) mice on a C57BL6 background have been described (16, 17). TLR2/4 DKO mice on C57BL6 background have been described (18, 19). Mice were fed ad libitum with irradiated standard chow (Harlan Laboratories). For mammary gland, milk and pup analyses, 8–10 week old mice were bred, and the litter sizes were normalized to six pups. Milk was collected as described (13–15). For cross-fostering, pups were switched on postnatal day 1. All protocols for mouse experiments were approved by the Institutional Animal Care and Use Committee of University of Texas Southwestern Medical Center.

Histology and staining

For immuno-fluorescence staining, skin and mammary gland were collected and immediately embedded in OCT compound in a dry ice/ethanol bath and stored at −80°C until cryo-sectioning. Frozen OCT sections were fixed in acetone for 10 minutes and stained with FITC-conjugated antibodies specific for CD11b/Mac-1 (BD Biosciences). The sections were washed twice and mounted with medium containing DAPI (Vector Laboratories). For Oil Red O staining, frozen OCT sections were fixed in formalin for 10 minutes, dipped in 60% isopropanol and then stained in Oil Red O working solution (60% 3 mg/ml Oil Red O in isopropanol) for 20 minutes. For H&E staining, tissues were immediately fixed in 4% paraformaldehyde (PFA), then processed, paraffin-embedded, sectioned, and H&E-stained according to standard protocols. For whole mount staining, mammary glands were fixed in 4% PFA overnight at 4°C, rinsed in PBS, stained in carmine alum solution overnight at room temperature, and then washed in 70%, 95% and 100% ethanol each for 1 hour. The tissues were cleared in xylene overnight before image acquisition.

Gene Expression Analyses

Tissue samples were snap frozen in liquid nitrogen and stored at −80°C. Anti-TNFα antibody was from Santa Cruz Biotechnology. Bone marrow cells were differentiated into macrophages with 20ng/ml M-CSF for 6 days, primed with 100ng/ml TNF-α and 100ng/ml IL-6 or PBS control for 5 hours, and then treated with 400 µM palmitic acid for 15 hours as described (20). RNA was extracted using Trizol (Life Technologies), treated with RNase-free DNase I, reverse transcribed into cDNA using an ABI High Capacity cDNA RT Kit, and analyzed using real-time quantitative PCR (SYBR Green) in triplicate. All RNA expression was normalized by two reference genes L19 and GAPDH, and similar results were observed; representative data for L19 normalization are shown as relative mRNA expression. Primer sequences and amplicon sizes are listed below.

Lipid Analysis by Mass Spectrometry

Organic soluble metabolites were extracted from milk with a 2:1:1 CHCl3/Methanol/H2O solution and homogenization as described (13–15). Samples were stored at −80°C and dissolved in CHCl3 prior to analysis. Ceramides were quantified by LC-MS/MS in positive ionization multiple reaction monitoring (MRM) mode as described (14). Shotgun lipidomic analyses were performed using an Agilent Triple Quadrupole mass spectrometer under neutral loss mode as described (14).

Statistical Analyses

All statistical analyses were performed with Student’s t-Test and represented as mean ± standard deviation (s.d.); *, P < .05; **, P < .01; ***, P < .005; ****, P < .001; n.s. nonsignificant (P > .05).

Results and Discussion

Pups nursed by Adiponectin-KO or Adiponectin-TG dam exhibit alopecia

We found that the pups born from Adiponectin-KO (Adip-KO) or Adiponectin-TG (Adip-TG) dams started to lose dorsal hair beginning postnatal day 20–25, which was typically recovered one week after weaning; whereas pups born from WT littermate control mice were normal (Figure 1A-B). These pups also exhibited lower body weight, indicating growth retardation (Figure 1C-D). To determine whether the alopecia and growth retardation was caused by maternal or offspring defect, we performed cross-fostering experiment. The results showed that the phenotype is dependent on maternal genotype because it is present in WT pups nursed by mutant dam but absent in mutant pups fostered by WT dam (Figure 1E-F). Although every litter from every Adip-KO or Adip-TG dam produced alopecia phenotype, on average, 78% of all pups nursed by Adip-KO dam and 62% of all pups nursed by AdipoQ-TG dam exhibited hair loss (Figure 1E-F). These results indicate that maternal adiponectin deficiency or overabundance causes postnatal abnormality in the neonates manifested as hair loss and growth retardation.

Pups nursed by Adip-KO or Adip-TG dam exhibit skin inflammation

Histological analysis by H&E staining revealed that the skin of the pups with alopecia exhibited hyperplasia and
follicular cysts formation (Figure 2A). Based on our previous studies (13–15, 20), we examined whether the alopecia in the suckling pups was caused by inflammation. Immunofluorescence staining using a macrophage marker CD11b reveals that there was an increased leukocyte infiltration in the skin of the pups nursed by either Adip-KO dam or Adip-TG dam compared to control pups nursed by WT dam (Figure 2B). Moreover, in both cases, the expression of inflammatory markers was significantly elevated in both skin and intestine of the defective pups (Figure 2C-D). These results indicate that maternal adiponectin deficiency or overabundance can both cause systemic inflammation in the nursing neonates.

**Adip-KO and Adip-TG dams exhibit distinct defects in the lactating mammary gland**

To determine the lactation defects, we performed H&E staining, CD11b immunofluorescence staining, and Oil
Red O staining of the mammary gland collected on lactation day 10. The results showed that there was an increased leukocyte infiltration in the Adip-KO gland but not in the Adip-TG gland; in contrast, there was more adipocytes, more and larger lipid droplets in the mammary epithelial cells, as well as excessive lipid accumulation in the Adip-TG gland but not in the Adip-KO gland (Figure 3A). These observations suggest that the inflammation in the pups nursed by Adip-KO and Adip-TG dams may be triggered by distinct stimuli from the milk.

We also observed different developmental defects in the lactating mammary gland from Adip-KO and Adip-TG

![Image](image_url)

**Figure 2.** Pup hair loss is caused by follicular cysts and inflammation in the skin. (A) H&E staining showed skin hyperplasia and follicular cysts (FC, indicated by arrows) in P20 pups nursed by adip-KO or adip-Tg dams. Scale bars, 100 μm. B–E, Hair loss in pups nursed by adip-KO or adip-Tg dams was associated with a systemic inflammatory response. B, Immunofluorescence staining showed an increased infiltration of CD11b+ leukocytes in the skin. Scale bars, 25 μm. C–D, RT-QPCR analysis showed an increased expression of proinflammatory cytokines in the skin (C) and intestine (D) (n = 6). Pups of the same genotype were compared.
Figure 3. Adiponectin deletion or overabundance causes distinct changes in the lactating mammary gland. (A) Lactating mammary glands from Adip-KO dams exhibited increased leukocyte infiltration, whereas lactating mammary glands from Adip-Tg dams exhibited increased lipid accumulation. Mammary glands were collected on lactation day 10 and subjected to H&E, CD11b immunofluorescence, or Oil Red O staining. Green arrows indicate leukocytes. Red arrows indicate adipocytes. Blue arrows indicate larger lipid droplets in the lactating mammary epithelium of Adip-Tg dams. Scale bars, 25 μm. B, Whole mount staining showed a reduced alveolar expansion in P15 (pregnancy day 15) mammary gland from Adip-Tg dams but not Adip-KO dams. Scale bars, 60 μm. Histomorphometric quantification of lobuloalveolar area (%) and alveoli counts per gland is shown (n = 6). C, RT-QPCR analysis showed that involution-associated proapoptotic genes were increased whereas antiapoptotic genes were decreased in L10 Adip-KO glands but not in L10 Adip-Tg glands (n = 6). Gene expression relative to L19 (top) or GAPDH (bottom) showed...
dams. Adip-TG glands exhibited a reduced alveolar expansion during pregnancy and lactation, resulting in more adipocytes (Figure 3A-B). Adip-KO glands exhibited an early involution, leading to more apoptosis and less ductal/alveolar structures in the L10 mammary glands (Figure 3A,C). These developmental defects can compromise efficient lactation and reduce milk production, which is consistent with the lower body weight in the pups nursed by Adip-KO and Adip-TG dams (Figure 1C-D).

**Adip-KO dams produce more proinflammatory cytokines in the lactating mammary gland**

Consistent with the increased leukocyte infiltration in the Adip-KO lactating mammary gland (Figure 3A), the expression of many proinflammatory cytokines was significantly elevated, including IL-6, TNF-α, IL-1β, MMP9, IL-12P35, IL-12P40 (Figure 4A). Moreover, the expression of several enzymes that produce proinflammatory lipids such as prostaglandin and leukotriene was also elevated in Adip-TG mice, whereas the expression of adiponectin in the lactating mammary gland was ablated in Adip-KO mice but elevated by 3.4 fold in Adip-TG mice (Figure 4A-B). The expression of adiponectin in the lactating mammary gland was ablated in Adip-KO mice but elevated by 3.4 fold in Adip-TG mice, whereas the expression of adiponectin receptors was unaltered (Figure 4C). In accordance with the elevated mRNA expression of proinflammatory cytokines (Figure 4A) and lipid oxidation enzymes in the mammary gland of Adip-KO mice (Figure 4B), the protein levels of TNFα was higher in the milk from Adip-KO dams (Figure 4D) and the levels of oxidized fatty acids were increased in the skin of the pups nursed by Adip-KO dams (Figure 4E). These data suggest that the alopecia and inflammation in the pups nursed by Adip-KO dams may be triggered by the excessive inflammatory cytokines in the milk.

**Adip-TG dams produce more long chain saturated fatty acids in the milk**

Previous studies report that adiponectin overabundance in the Adip-TG mice promotes the clearance of triglycerides and fatty acids (FAs) from circulation (16, 21). Thus, we hypothesize that more fat may be transferred to the lactating mammary gland and secreted into milk in the Adip-TG dams. Consistent with the increased lipid accumulation and larger milk lipid droplets in the Adip-TG lactating mammary gland (Figure 3A), the lipid content in the milk was also significantly higher in Adip-TG mice compared to WT controls or Adip-KO mice (Figure 4F).

Triglycerides make up 98% of milk lipids, and their FAs derive from both de novo synthesis in the lactating mammary gland which generates predominantly short and medium chain (C8-C12) FAs, as well as adipose tissue and circulation that contains mainly long chain (C16-C20) FAs (22). Previous studies show that inflammation is specifically activated by saturated FAs but not unsaturated FAs (23–27); and by long chain FAs (C16:0, C18:0) but not medium/short chain (C8:0-C12:0) FAs (28). Therefore, we investigated the FA composition of milk triglycerides by shotgun lipidomic analysis using neutral loss mass spectrometry (29). The results showed that the FA elongation ratio (C18:0/C10:0) (Figure 4G) was 3.6-fold higher in the Adip-TG milk, indicating a higher percentage of long chain FAs. Moreover, the FA saturation ratio (C18:0/C18:2) was also 1.9-fold higher in the Adip-TG milk (Figure 4H), indicating a higher percentage of saturated FAs. Particularly, the level of palmitic acid (C16:0), a representative long-chain saturated fatty acid (LcSFA) that induces inflammation via TLR4 activation (27, 30–34), was 2.3-fold higher in the Adip-TG milk compared to WT milk (Figure 4I). In contrast, these changes were not observed for Adip-KO milks (Figure 4G-I).

Saturated FAs induce the biosynthesis of ceramides (Cer) and glucosylceramides (GlcCer) to promote inflammation and metabolic diseases (35, 36). We found that there were also higher levels of Cer and GlcCer in the Adip-TG milk (Figure 4J). This is a local effect in the lactating mammary gland as systemic ceramide levels were lower in the Adip-TG mice (6). Together, these results suggest that the alopecia and inflammation in the pups nursed by Adip-TG dams may be triggered by the excessive LcSFAs and ceramides in the milk.

**TLR2/4 DKO pups are resistant to alopecia when nursed by Adip-KO or Adip-TG dams**

TLR2/4 signaling is an important nexus of inflammatory response pathways. TLR2/4 can be activated by LcSFAs such as palmitic acid (PA) (27, 30–34), which are excessive in Adip-TG milk (Figure 4G-I). TLR2/4 function can also be primed and synergized by proinflammatory cytokines (37–39), which are excessive in Adip-KO milk. We found that macrophage activation by palmitic acid, a highly abundant FA in milk, was significantly augmented by TNFα and IL-6, two cytokines that were elevated in the Adip-KO lactating mammary gland (Figure 4K).

We next investigated whether the neonatal inflammation triggered by the toxic milk produced by Adip-KO and

---

**Legend to Figure 3 Continued. . .**

similar results.
Figure 4. Increased inflammatory cytokines in Adip-KO mammary glands and excessive long-chain saturated fatty acids in Adip-Tg milk. (A) Expression of proinflammatory cytokines was increased in the lactating mammary glands from Adip-KO dam but not from Adip-Tg dams (n = 6). B, Expression of enzymes that synthesize proinflammatory lipids was increased in the lactating mammary glands from Adip-KO dam but not from Adip-Tg dams (n = 6). C, Expression of adiponectin, adiponectin receptor 1 (Adip-R1) and adiponectin receptor 2 (Adip-R2) in the lactating mammary glands (n = 6). D, Western blot showed that milk from Adip-KO dams, but not from Adip-Tg dams, had increased TNFα protein levels.

Equal volume (15 μl) of milk samples were loaded. E, LC-MS analysis showed that levels of hydroxyl fatty acids were increased in the skin of the pups nursed by Adip-KO dams but not Adip-Tg dams (n = 6). F, Milk from Adip-Tg dams, but not from Adip-KO dams, had increased lipid content (n = 3). G–J, LC-MS analyses of milk lipids. G, Milk from Adip-Tg dams, but not from Adip-KO dams, contained increased fatty acid elongation ratio, indicating a higher percentage of long chain fatty acids (n = 3). H, Milk from Adip-Tg dams, but not from Adip-KO dams, contained increased fatty acid saturation ratio, indicating a higher percentage of saturated fatty acids (n = 3). I, Milk from Adip-Tg dams, but not from Adip-KO dams, contained...
Adip-TG dams can be rescued by TLR2/4 deletion. When fostered by Adip-KO or Adip-TG dams, while WT control pups still developed hair loss, TLR2/4 double knockout (DKO) pups were completely resistant (Figure 5A-B); while the expression of inflammatory genes was still elevated in WT control pups, this increase was largely abolished in TLR2/4 DKO pups (Figure 5C-D). Interestingly, the lower body weight in the pups nursed by Adip-KO or Adip-Tg dams was not significantly rescued by TLR2/4 deletion (Figure 5A-B), suggesting that TLR2/4 deletion specifically abolished the systemic inflammation caused by altered milk composition but not the growth retardation caused by mammary gland developmental defects. Consistent with these observations, palmitic acid induction and cytokine potentiation of inflammatory genes were attenuated in TLR2/4 DKO macrophages (Figure 5E). These results indicate that, despite the distinct inflammatory stimuli from Adip-KO and Adip-TG milk, the inflammatory signaling converges at TLR2/4 activation in

Legend to Figure 4 Continued. . .
higher levels of palmitic acid (PA) (n = 3). J, Milk from Adip-Tg dams, but not Adip-KO dams, contained higher levels of ceramides (Cer) and glucosylceramide (GlcCer) (n = 3). K, Palmitic acid (PA) induction of inflammatory genes was potentiated by cytokines. Bone marrow cells were differentiated into macrophages with 20ng/ml M-CSF for 6 days, primed with 100ng/ml TNF-α and 100ng/ml IL-6 or PBS control for 5 hours, and then treated with 400 μM palmitic acid (PA) for 15 hours before gene expression analysis (n = 3).

Figure 5. TLR2/4 deletion in the pups rescues the inflammation and hair loss. (A) TLR2/4 DKO pups, when fostered by Adip-KO dams, were resistant to alopecia. B, TLR2/4 DKO pups, when fostered by Adip-Tg dams, were resistant to alopecia. C, TLR2/4 DKO pups, when fostered by Adip-KO dams, were resistant to the increased inflammatory gene expression in the skin. D, TLR2/4 DKO pups, when fostered by Adip-Tg dams, were resistant to the increased inflammatory gene expression in the skin. E, Palmitic acid (PA) induction and cytokine potentiation of inflammatory genes were attenuated in TLR2/4 DKO macrophages. Bone marrow cells were differentiated into macrophages with 20ng/ml M-CSF for 6 days, primed with 100ng/ml TNF-α and 100ng/ml IL-6 or PBS control for 5 hours, and then treated with 400 μM palmitic acid (PA) for 15 hours before gene expression analysis (n = 3).
the nursing neonates and can be prevented by TLR2/4 inhibition.

Our study reveals maternal adiponectin as a key genetic program that controls metabolic and immune homeostasis during lactation to ensure normal milk composition and prevent neonatal inflammation. Interestingly, this regulation is dosage-dependent because adverse effects can result from either adiponectin deficiency or adiponectin overabundance. On one hand, lack of maternal adiponectin causes increased production of inflammatory cytokines in the lactating mammary gland, which in turn primes and synergizes fatty acid activation of TLR2/4 signaling pathway in the neonates, leading to an overt inflammatory response. On the other hand, excessive maternal adiponectin promotes triglycerides accumulation in the lactating mammary gland, causing higher levels of long-chain saturated fatty acids in milk, which in turn activate TLR2/4 to mount an inflammatory response in the newborns (Figure 6). Thus, adiponectin control of inflammation involves the regulation of both metabolism and immunity. These findings demonstrate that physiological level of adiponectin must be within an optimal window to prevent inflammation, providing insights to the perplexing and seemingly contradictory observations of its both antiand proinflammatory effects in experimental and clinical investigations.

Changes of the lipid or cytokine content in the milk may also alter the microbiome of the neonatal gut, which in turn may modulate local immune cell function and/or nutrient uptake, and consequently contribute to the systemic inflammation phenotype in the offspring. In future studies, it would be interesting to compare the major gut phyla in the pups nursed by Adip-KO or Adip-Tg dams vs. pups nursed by WT dams.

The etiology of several inflammatory infantile disorders is still unclear, such as asthma, eczema, inflammatory bowel disease, necrotizing enterocolitis (NEC) and neonatal onset multisystem inflammatory disease. Our findings suggest that these diseases may originate from milk disorders caused by maternal dysfunction of adiponectin, in addition to our previously identified genetic or dietary factors (13–15). Importantly, we found that the inflammatory insults in both maternal adiponectin deficiency and maternal adiponectin overabundance converge to TLR2/4 activation, and TLR2/4 deletion in the offspring confers resistance. Therefore, our findings reveal that TLR2/4 inhibition may represent a novel strategy for the prevention and treatment of infantile inflammatory disorders.

Acknowledgments

We thank UTSW Molecular Pathology Core for histology. Y.W. is a Virginia Murchison Linthicum Scholar in Medical Research. This work was in part supported by March of Dimes (#6-FY13–137, YW), The Welch Foundation (I-1751, YW), NIH (R01 DK089113, YW), CPRIT (RP130145, YW), DOD BCRP Idea Award (W81XWH-13-1-0318, YW) and UTSW Endowed Scholar Startup Fund (YW). The authors declare that they have no financial conflict of interest.

Address all correspondence and requests for reprints to: Assistant Professor Yihong Wan, Ph.D., Email: yihong.wan@utsouthwestern.edu, Univ of Texas Southwestern Medical Center - Department of Pharmacology, 6001 Forest Park Road, Rm ND8.502B, Dallas, TX, UNITED STATES, 75390–9041, Phone: 214–645-6062

This work was supported by .

Table 1.

<table>
<thead>
<tr>
<th>Mouse Gene</th>
<th>Primer-F</th>
<th>Primer-R</th>
<th>Amplicon (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>TGGGCTCTCAAAGGAAAGAATC</td>
<td>GGTATTGCTTGGGATCCACT</td>
<td>93</td>
</tr>
<tr>
<td>MMP9</td>
<td>CCAAGGGTGACCGCTGCTTCT</td>
<td>GCACGCTTGAATGTCAGACTGAC</td>
<td>74</td>
</tr>
<tr>
<td>IL-6</td>
<td>TCCCCATCTCTCATGAGCTG</td>
<td>CCTCTCTCCCTCTGAGCACTG</td>
<td>90</td>
</tr>
<tr>
<td>TNF-α</td>
<td>CAGCCCGATGAGGGTGATCCTT</td>
<td>GTGTTGGGAGGAGACGCAGTA</td>
<td>82</td>
</tr>
<tr>
<td>IL-12P35</td>
<td>AAATGAAGCTGGTGACGCTTCT</td>
<td>AGATAGCCACATCCTCCTGGA</td>
<td>73</td>
</tr>
<tr>
<td>IL-12P40</td>
<td>CATCACGAAACGGTCAAAGC</td>
<td>GGGGAACCATGCCCACCTT</td>
<td>96</td>
</tr>
<tr>
<td>COX-2</td>
<td>ATCAGAAACCAGGATGCTTCTT</td>
<td>CCAAGGAGGATGTTGATGATTAG</td>
<td>129</td>
</tr>
<tr>
<td>Epha1</td>
<td>GTGCCCACACTGGCTATTCAG</td>
<td>GGCTTACCTGCCCTCACCACTT</td>
<td>75</td>
</tr>
<tr>
<td>Alox5</td>
<td>GTGCCTCATGAGAGAGCTTACT</td>
<td>CCCATCCTCAACAGCCTCTA</td>
<td>76</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>CAGTTGAGTCTGGACGACAAACA</td>
<td>GAACAGGAGAGCTGCAACAG</td>
<td>59</td>
</tr>
<tr>
<td>Adip-R1</td>
<td>TCCCATGTCGCCGTCCTTG</td>
<td>AGTGCATGGTGGTACAACA</td>
<td>138</td>
</tr>
<tr>
<td>Adip-R2</td>
<td>GTGGAGCTACGAGACATAC</td>
<td>TGGGGCTTACCTGAGGA</td>
<td>102</td>
</tr>
<tr>
<td>L19</td>
<td>GGTCTCTGCTGGGATGCTTCCAA</td>
<td>CCCATCCTTGATGCTTCCAC</td>
<td>82</td>
</tr>
<tr>
<td>GAPDH</td>
<td>ATCCGACTGATGATGACATCA</td>
<td>CCCCAGATGCTGCTTCA</td>
<td>51</td>
</tr>
</tbody>
</table>
Dual Roles of Adiponectin in Lactation Endocrinology

Figure 6. A schematic diagram for the dosage-dependent regulation of neonatal inflammation by maternal adiponectin via distinct mechanisms. Maternal adiponectin deficiency increases inflammatory cytokines in the mammary gland and milk, which potentiates the ability of milk fatty acids to activate inflammatory genes in a TLR2/4-dependent manner. Maternal adiponectin overabundance increases the levels of long-chain saturated fatty acids in the milk, which activate inflammatory genes in a TLR2/4-dependent manner. Both cause a systemic inflammatory response in the nursing neonates manifested as alopecia. Our findings indicate that fatty acids activation of TLR2/4 is an important proinflammatory component in both Adip-Tg milk (which has increased abundance of saturated fatty acids) and Adip-KO milk (which has increased proinflammatory activity of saturated fatty acids due to the excessive cytokines).

References


