Cutting Edge: Intestinal IL-17A Receptor Signaling Specifically Regulates High-Fat Diet–Mediated, Microbiota-Driven Metabolic Disorders

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Abstract

Previous studies indicate that IL-17A plays an important role in mediating the intestinal microbiota and systemic metabolic functions. However, it is not known where IL-17RA signaling occurs to mediate these effects. To investigate this question, we used intestinal epithelial-specific (Il17raΔIEC) and liver-specific (Il17raΔLiver) IL-17RA knockout mice as well as littermate control mice. Our results indicate that intestinal IL-17RA signaling helps mediate systemic metabolic functions upon exposure to prolonged high-fat diet. Il17raΔIEC mice display impaired glucose metabolism, altered hormone and adipokine levels, increased visceral adiposity, and greater hepatic lipid deposition when compared with their littermate controls. We show that IL-17RA-driven changes in microbiota composition are responsible for regulating systemic glucose metabolism. Altogether, our data elucidate the importance of intestinal IL-17RA signaling in regulating high-fat diet–mediated systemic glucose and lipid metabolism.

Introduction

During recent years, the global prevalence of obesity has increased (1). Various factors, including diet, can influence obesity (1). Long-term consumption of a high-fat diet (HFD) can result in complications, including nonalcoholic fatty liver disease, by altering key metabolic tissues (2, 3). Additionally, pro-inflammatory Th17 cells and cytokines are induced under conditions that promote obesity and may mediate these disorders (4).

IL-17A is predominantly secreted from Th17, ILC3, and γδT cells and exerts protective and inflammatory functions (5). IL-17A signals via its receptor (IL-17RA/RC complex), which is expressed on a range of cells including enterocytes, hepatocytes, and adipocytes (5).

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Author Contributions
PK, SJG, and HH designed the experiments. Experiments were conducted by SG, HH, MB, MJP, PJ, CK, and HTW. Text was written by SG and edited by PK.

Conflicts of Interest Statement
The authors have no financial conflicts of interest.
Signaling by IL-17A to liver and adipose tissues is linked with steatosis, insulin resistance, and impaired glucose metabolism (2, 6).

Intestinal microbiota composition mediates host metabolic functions and weight gain (1, 3). An impaired microbiota can promote glucose intolerance and nonalcoholic fatty liver disease, and this phenotype was transmissible upon cohousing mice (7). Segmented filamentous bacteria (SFB) regulate Th17 polarization and IL-17A responses (8, 9). Furthermore, mice that specifically lack intestinal IL-17RA display impaired expression of genes that regulate the microbiota and increased colonization by SFB (10). SFB depletion is also associated with decreased IL-17A and protection from obesity-induced liver damage (11).

HFD-treated RORγt+/−, Il17−/−, and Il17ra−/− mice display greater hepatic steatosis and impaired (or similar) glucose tolerance when compared with wild-type controls (6, 11, 12). However, studies using anti–IL17A demonstrate that IL-17A may increase levels of inflammatory cytokines and aid the progression of hepatic steatosis (13). Because previous studies have solely used global cytokine or receptor knockout mice or generalized Ab–mediated cytokine neutralization, it is unclear where IL-17RA signaling occurs to mediate these effects. To address this, we used intestinal epithelium-specific and liver-specific IL-17RA knockout and littermate control mice. We demonstrate that intestinal IL-17RA signaling is specifically important for regulating systemic glucose and lipid metabolism.

**Materials and Methods**

**Mice:**

C57BL/6J, Villin-Cre (C57BL/6J background), and Albumin-Cre (C57BL/6J background) mice were purchased from The Jackson Laboratory. Generation and characterization of IL-17-floxed (Il17ra1fl/fl;Villin-cre) mice were performed as described (10). Il17ra1fl/fl;Albumin-cre mice were obtained from Dr. Jay Kolls. We placed 6-wk-old male mice on HFD (Bio Serve). All knockout and control mice were cohoused during this 6-wk period. All mice were separated according to genotype, and their weights were measured each week. Mice were housed in specific pathogen-free conditions at Stony Brook University. Studies were conducted with the approval of Stony Brook University Institutional Animal Care and Use Committee.

**Glucose tolerance test:**

Glucose tolerance tests (GTTs) were performed after 16 wk of HFD. Mice were placed in clean cages, weighed, and fasted for 5 h. An initial glucose reading (0 min) was obtained from a blood sample isolated from the tail. Blood glucose was measured using a Countour next (Bayer) monitor. Mice were intraperitonially injected with a body weight–specific volume (equivalent to 50% body weight) of glucose (200 ng/mL). Blood glucose was obtained 30, 60, 90, and 120 min post injection.
Cell line:
HepG2 cells were grown in 24-well plates and treated with pure media or media supplemented with 100 ng/mL IL-22 fusion protein (IL-22.Fc; Evive Biotech) or 100 ng/mL recombinant human leptin (rhLeptin; PeproTech) when growth reached 70% confluency. Cells were lysed in RLT buffer after 28 h, and RNA was isolated for RT-PCR analysis using a QIAGEN RNeasy Micro kit.

IL-22.Fc treatment:
C57BL/6J mice were i.p. injected with pure PBS or 80 μg IL-22.Fc (in PBS). Mice were euthanized after 24 h. Liver tissues were collected for RT-PCR analysis.

Antibiotics treatment:
An antibiotic mixture containing vancomycin (0.5 g/L), ampicillin (0.5 g/L), neomycin (1 g/L), and metronidazole (1 g/L) was used (14). Normal drinking water was replaced with the antibiotics starting on week 12 of HFD. Fresh antibiotics were provided every 2 d during the remaining 4 wk.

RT-PCR:
Trizol-based RNA isolation was performed for tissues. Transcription of RNA to cDNA was performed using Bio-Rad iScript. RT-PCR was performed using Bio-Rad SsoAdvanced Supermix or SYBR Green. Primer probes were used from Applied Biosystem and Integrated DNA Technologies (Supplemental Table 1).

Bacterial DNA was isolated from feces for RT-PCR and 16S rRNA sequencing. DNA was isolated using QiAMP stool DNA extraction kit. Bacterial abundance was examined using primer probes from Integrated DNA Technologies (Supplemental Table 1). The 16S rRNA sequencing was performed as described (15). The 16S rRNA data has been uploaded to the SRA database (PRJNA760519).

H&E staining:
Tissues were fixed in formalin. Paraffin-embedded tissue sections (5 μm) were deparaffinized in xylene and rehydrated with an ethanol wash. Tissues were stained with Mayer’s hematoxylin solution (VWR International) for 2 min and washed under running tap water. A 30 s differentiation with 95% ethanol was performed. Samples were stained with eosin (VWR International) for 2 min. Slides were dehydrated in ethanol, cleared with xylene, and mounted.

Oil Red O staining:
Liver tissue was frozen in OCT compound medium. Blocks were sectioned (5 μm) and sections were dried at room temperature and stained with Oil Red O (ORO) Isopropanol Solution (Electron Microscopy Sciences) solution (3:2 ratio of ORO to H₂O). Slides were washed with running tap water for 30 min, dried, and mounted. Images were acquired at 40X magnification. ImageJ was used to evaluate percent area coverage of tissue by staining.
Luminex multiplex assay:

Serum samples were collected from mice and analyzed via multiplex analysis (Luminex). The Mouse Metabolic Hormone Panel (MilliporeSigma) and Mouse Th17 Panel (MilliporeSigma) were used. Samples were run at the Stony Brook School of Medicine Genomics Core (Bio-Plex 200) and Vanderbilt University Hormone Assay and Analytical Services Core (MagPix).

Results and Discussion

Intestinal IL-17RA signaling regulates systemic glucose metabolism and tissue physiology

Because the intestine is a major site for upregulating the uptake of nutrients and mediating metabolic processes, we analyzed if intestinal IL-17RA signaling specifically influences HFD-induced disorders (16). To do so, we used Il17raΔIEC and Il17rafl/fl (littermate control) mice. Knockdown of colonic Il17ra was confirmed (Fig. 1A). Il17raΔIEC and Il17rafl/fl mice display no differences in weight gain upon HFD treatment (Fig. 1B). However, Il17raΔIEC mice display impaired glucose metabolism (Fig. 1C). Because glucose metabolism was impaired, we investigated if systemic changes could be observed in the white adipose tissue and liver. Interestingly, an increase in relative epididymal white adipose tissue (eWAT) weight was observed in Il17raΔIEC mice (Fig. 1D). We also examined transcript levels of lipid metabolism and inflammation genes of the eWAT but did not observe any differences (Supplemental Fig. 1A). H&E staining of the liver revealed greater lipid accumulation in Il17raΔIEC mice, and this was confirmed by ORO staining (Fig. 1E, 1F). Upon analyzing the expression of key inflammatory and metabolic genes in the liver, Il17raΔIEC mice displayed increased expression of Fasn, which aids in the synthesis of lipids (Supplemental Fig. 1B). Increased expression of Fasn has been observed in livers that develop steatosis, and inhibition of hepatic FASN decreases hepatic triglyceride levels and improves glucose metabolism (17, 18). Collectively, our data suggest that intestinal IL-17RA signaling may regulate systemic glucose levels and adiposity.

Hepatic IL-17RA signaling is not essential in regulating glucose metabolism

HFD and IL-17A promote lipid accumulation and inhibit insulin signaling in the liver, and hepatic glucose use is closely related to intestinal function (2, 11). Therefore, we examined the effects of HFD treatment on Il17raΔLiver and Il17rafl/fl mice. Knockdown of hepatic Il17ra was confirmed (Fig. 2A). HFD did not affect weight gain between Il17raΔLiver and Il17rafl/fl mice (Fig. 2B). Likewise, no differences in glucose clearance and eWAT weight were observed (Fig. 2C, 2D). Although hepatocyte ballooning was visualized via H&E staining, no differences in histological features were observed between either group (Fig. 2E). Altogether, our data suggest that hepatic IL-17RA signaling does not directly regulate systemic glucose metabolism or adiposity. Therefore, IL-17RA signaling in other sites such as the intestine may be more relevant.

Altered hormone and cytokine levels are present in Il17raΔIEC mice

To characterize what factors may be promoting impaired glucose clearance in Il17raΔIEC mice, Luminex analysis was performed on serum from HFD-fed Il17raΔIEC and Il17rafl/fl
mice to analyze the levels of relevant hormones. We analyzed circulating levels of connecting-peptide (c-peptide), a short amino acid sequence that maintains the proinsulin molecule, and insulin. Compared to their littermate controls, Il17raΔIEC mice displayed a trend toward having decreased levels of c-peptide and displayed significantly decreased levels of insulin (Fig. 3A) which coincides with their impaired glucose clearance. We also analyzed the levels of adipokines that are indicative of metabolic disorder. Differences in the levels of resistin were not observed, but Il17raΔIEC mice displayed decreased leptin (Fig. 3B). Finally, Luminex analysis was used to assess levels of circulating cytokines. Levels of IL-17A were below the minimum detectable range (data not shown). However, we observed increased levels of IL-22, another Th17 cytokine, in Il17raΔIEC mice (Fig. 3C). Injection of IL-22 induces hepatoprotective effects, including protecting against fatty liver (19, 20). Although leptin traditionally inhibits insulin production, IL-22 has been shown to increase insulin sensitivity, which may explain why Il17raΔIEC mice display decreased serum insulin despite displaying reduced leptin levels (20, 21). Additionally, IL-22 and leptin both activate STAT3 and initiate similar effects such as promoting wound healing, the release of antimicrobial proteins, and the secretion of mucus (22). Therefore, enhanced levels of IL-22 may be produced by Il17raΔIEC mice to compensate for lower levels of leptin. To test whether IL-22 or leptin mediate the elevated hepatic expression of Fasn in Il17raΔIEC mice, we stimulated HepG2 cells with IL-22.Fc or rhLeptin. However, we observed that neither stimulation influenced Fasn expression (Supplemental Fig. 1C). Likewise, although injection of mice with 80 μg of IL-22.Fc increased the expression of Saa1/2, an IL-22 inducible inflammatory gene, it did not influence hepatic Fasn expression (Supplemental Fig. 1D). Therefore, levels of IL-22 alone may not be sufficient to mediate Fasn expression, and additional factors during HFD may help mediate these effects. Altogether, our data suggest that intestinal IL-17RA signaling influences systemic hormone and cytokine levels.

**HFD-induced microbiota changes partially drive metabolic function**

Obese individuals possess a microbiota that promotes impaired glucose metabolism and increased insulin resistance, and certain commensal bacteria and bacterial metabolites may increase insulin sensitivity (23). The intestinal microbiota has also been shown to influence adipokine levels. Germ-free mice exhibit decreased serum levels of leptin than conventionalized mice when fed a chow diet, but it has also been shown that mice treated with prebiotics and fed a HFD display greater leptin sensitivity than their nonprebiotic-treated counterparts (24, 25). Therefore, we investigated whether the microbiota regulates metabolic functions of Il17raΔIEC mice.

Il17raΔIEC and Il17raΔIEC mice possess increased levels of SFB, which exacerbate obesity-induced liver damage (10, 11). As expected, increased levels of SFB were detected in fecal samples of Il17raΔIEC mice before HFD treatment; interestingly, SFB levels were substantially reduced (and no longer significantly different) in both groups after prolonged HFD treatment (Fig. 4A). Because SFB have been shown to induce Th17 responses, including levels of IL-17A, this decreased level of SFB after HFD treatment may explain why serum levels of IL-17A were below the minimum levels of detection (8–10). Although 16S rRNA sequencing of fecal bacteria DNA did not reveal major phyla-level changes in microbiota composition (Supplemental Fig. 2A), family-level changes including
significantly decreased levels of Sutterellaceae and Erysipelotrichaceae were observed in \( \text{Il17ra}^{\Delta \text{IEC}} \) mice (Fig. 4B). We also observed species-level changes in \( \text{Il17ra}^{\Delta \text{IEC}} \) mice, including elevated levels of \textit{Bacteroides uniformis} (Supplemental Fig. 2B) which has been associated with decreased serum insulin and leptin after HFD (26).

Intestinal IL-17RA signaling regulates the microbiota by altering the expression of antimicrobial genes (10). Likewise, HFD and microbial dysbiosis have been associated with impaired intestinal barrier integrity to promote the dissemination of microbial factors that influence systemic metabolism (3). Because we detected no differences in the expression of colonic intestinal barrier or antimicrobial genes (Supplemental Fig. 2C, 2D), we analyzed if ileal IL-17RA signaling regulates gene expression after HFD. After HFD feeding for 1 wk, \( \text{Il17ra}^{\Delta \text{IEC}} \) mice did not display differences in ileal tight junction or \textit{Reg3}-associated antimicrobial gene expression (Supplemental Fig. 2E, 2F). However, we observed decreased expression of \textit{Nox1} (produces hydrogen peroxide) and a trend towards reduced \textit{Pigr} (transcytosis of secretory IgA) which have been shown to regulate the microbiota (Supplemental Fig. 2F). This coincides with data we previously published regarding naive chow diet fed \( \text{Il17ra}^{\Delta \text{IEC}} \) mice (10). Ileal tissues from \( \text{Il17ra}^{\Delta \text{IEC}} \) mice also displayed significantly decreased expression of \textit{Saa1/2} (Supplemental Fig. 2E). Although we previously observed reduced SFB colonization in the terminal ileum of naive \( \text{Il17ra}^{\Delta \text{IEC}} \) mice, no difference in ileal SFB levels were observed after 1 wk of HFD (Supplemental Fig. 2G). Thus, ileal IL-17RA signaling may play a greater role in regulating microbiota changes.

To characterize if IL-17A-driven microbiota changes influence systemic glucose metabolism, we placed \( \text{Il17ra}^{\Delta \text{IEC}} \) and \( \text{Il17ra}^{\text{fl/fl}} \) mice on HFD and treated them with an antibiotics mixture for 4 wk to deplete their microbiota. Depletion was confirmed by RT-PCR of bacterial DNA from feces (data not shown). No significant differences in weight gain were observed (Fig. 4C). As expected, microbiota depletion resulted in an overall reduction of blood glucose values. \( \text{Il17ra}^{\Delta \text{IEC}} \) and \( \text{Il17ra}^{\text{fl/fl}} \) mice had comparable levels of glucose (Fig. 4D). Therefore, IL-17RA signaling-driven changes in microbiota composition mediate glucose metabolism. Microbiota depletion did not greatly diminish the previously observed difference in eWAT weight, and hepatic histology did not noticeably differ between groups (Fig. 4E, 4F). ORO analysis revealed slightly less lipid droplet accumulation in the livers of antibiotics-treated \( \text{Il17ra}^{\Delta \text{IEC}} \) mice (Fig. 4G). We also analyzed liver tissues for changes in metabolic genes; interestingly, while elevated expression of \textit{Fasn} was present in the livers of nonantibiotics-treated \( \text{Il17ra}^{\Delta \text{IEC}} \) mice, this trend was not observed after antibiotics treatment (Supplemental Fig. 3A). We also observed that the difference in serum insulin levels was eliminated between mice after antibiotics treatment, but consistent with the non-antibiotics treated group we observed a trend for decreased leptin in the antibiotics treated \( \text{Il17ra}^{\Delta \text{IEC}} \) mice (Supplemental Fig. 3B). This indicates that IL-17RA signaling-driven microbiota changes may also mediate systemic lipid metabolism and hormone levels.

Overall, our study elucidates the relevance of intestinal epithelial-specific IL-17RA signaling in mediating systemic metabolism via regulation of the microbiota. Furthermore, cytokines besides IL-17A may act in the intestine to regulate metabolism.
Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

- **c-peptide**: Connecting peptide
- **eWAT**: Epididymal White Adipose Tissue
- **GTT**: Glucose Tolerance Test
- **HFD**: High-Fat Diet
- **ORO**: Oil Red O
- **SFB**: Segmented Filamentous Bacteria
- **Abx**: Antibiotics cocktail

References


Key Points

- Intestinal IL-17RA signaling mediates systemic glucose and lipid metabolism
- Hepatic IL-17RA signaling does not regulate systemic glucose metabolism
- IL-17RA-driven microbiota changes influence systemic glucose metabolism
Figure 1.
Intestinal IL-17RA mediates systemic glucose and lipid metabolism. A) RT-PCR validating knockdown of Il17ra in the distal colon (n = 6 mice per group). B) Weight gain of Il17ra<sup>fl/fl</sup> (n = 10) and Il17ra<sup>ΔIEC</sup> (n = 7) mice fed HFD for 16 wk. C) GTT of HFD-fed Il17ra<sup>fl/fl</sup> (n = 8) and Il17ra<sup>ΔIEC</sup> (n = 6) mice. D) Relative weight of eWAT after HFD. E) H&E staining of liver tissues from HFD-fed Il17ra<sup>fl/fl</sup> (n = 8) and Il17ra<sup>ΔIEC</sup> (n = 6) mice. F) ORO staining (left) and quantification (right) of liver tissues from HFD-fed Il17ra<sup>fl/fl</sup> and Il17ra<sup>ΔIEC</sup> mice. Data are obtained from at least two separate experiments. Error bars depict the mean ± SEM. *p < 0.05, Mann–Whitney U test and two-tailed, two-way ANOVA.
Figure 2.
IL-17RA signaling in the liver does not mediate systemic glucose metabolism or adiposity. 
A) RT-PCR validating knockdown of IL17ra in the liver (n = 4–5 mice per group). B) Weight gain of HFD-fed Il17raI/O (n = 5) and Il17raΔLiver (n = 4) mice. C) GTT of Il17raI/O (n = 5) and Il17raΔLiver (n = 4) mice fed HFD for 16 wk. D) Relative weight of eWAT after mice were fed HFD for 16 wk. E) Representative H&E staining of liver tissues from Il17raI/O (n = 5) and Il17raΔLiver (n = 4) mice fed HFD for 16 wk. All data are obtained from two separate experiments. Error bars depict the mean ± SEM. Mann–Whitney U test and two-tailed, two-way ANOVA.
Figure 3.
Intestinal IL-17A signaling effects systemic hormone and cytokine levels. A) Levels of serum c-peptide and insulin assessed via Luminex analysis. B) Levels of serum resistin and leptin assessed via Luminex analysis. C) Level of serum IL-22 assessed via Luminex analysis. All data are obtained from two separate experiments. Error bars depict the mean ± SEM. *p < 0.05, **p < 0.01, Mann Whitney U test.
Figure 4.
Intestinal IL-17RA signaling mediates microbiota-dependent changes in glucose metabolism. A) RT-PCR evaluation of fecal SFB levels before (day 0) and after (day 105) HFD treatment. B) Family-level 16S rRNA sequencing data collected from fecal samples. C) Weight gain of Il17ra<sup>fl/fl</sup> (n = 2) and Il17ra<sup>ΔIEC</sup> (n = 3) mice treated with an antibiotics mixture starting week 12 of HFD. D) GTT of Il17ra<sup>fl/fl</sup> (n = 2) and Il17ra<sup>ΔIEC</sup> (n = 3) mice fed HFD for 16 wk and treated with an antibiotic mixture starting on week 12. E) Relative weight of eWAT after mice were fed HFD. F) H&E staining of liver tissues from HFD- and antibiotics-treated Il17ra<sup>fl/fl</sup> (n = 2) and Il17ra<sup>ΔIEC</sup> (n = 3) mice. G) ORO staining (left) and quantification (right) of liver tissues from HFD- and antibiotics-treated Il17ra<sup>fl/fl</sup> (n = 2) and Il17ra<sup>ΔIEC</sup> (n = 3) mice. Data from A and B are obtained from two separate experiments. For graph A, error bars depict the mean ± SEM. For graphs C, D, E, and G (right), error bars depict the mean ± SD. **p < 0.01, Mann Whitney U test and two-tailed, two-way ANOVA.