A BAFF/APRIL axis regulates obesogenic diet-driven weight gain

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The impact of immune mediators on weight homeostasis remains underdefined. Interrogation of resistance to diet-induced obesity in mice lacking a negative regulator of Toll-like receptor signaling serendipitously uncovered a role for B cell activating factor (BAFF). Here we show that overexpression of BAFF in multiple mouse models associates with protection from weight gain, approximating a log-linear dose response relation to BAFF concentrations. Gene expression analysis of BAFF-stimulated subcutaneous white adipocytes unveils upregulation of lipid metabolism pathways, with BAFF inducing white adipose tissue (WAT) lipolysis. Brown adipose tissue (BAT) from BAFF-overexpressing mice exhibits increased Ucp1 expression and BAFF promotes brown adipocyte respiration and in vivo energy expenditure. A proliferation-inducing ligand (APRIL), a BAFF homolog, similarly modulates WAT and BAT lipid handling. Genetic deletion of both BAFF and APRIL augments diet-induced obesity. Lastly, BAFF/APRIL effects are conserved in human adipocytes and higher BAFF/APRIL levels correlate with greater BMI decrease after bariatric surgery. Together, the BAFF/APRIL axis is a multifaceted immune regulator of weight gain and adipose tissue function.
Obesity is a major and unabated public health problem\(^1\)–\(^3\). Obesity stems from an imbalance between energy intake (e.g., food intake, microbiome, nutrient absorption), energy expenditure (EE) (e.g., basal metabolism, thermogenesis), and energy turnover (lipolysis, lipogenesis)\(^4\)–\(^5\). Brown adipose tissue (BAT) and white adipose tissue (WAT) are key contributors to controlling body weight\(^6\)–\(^7\). BAT, a highly energy-consuming tissue consisting of numerous mitochondria, is capable of removing lipids from circulation to activate adaptive thermogenesis\(^8\). WAT can assimilate calories through storage of lipids into adipocytes. WAT lipolysis facilitates the breakdown of lipids into functional consumable units including glycerol\(^9\). Persistent imbalances of caloric intake and EE leads to the pathological expansion of adipose tissue (AT) that contributes to the propagation of obesity-associated low-grade inflammation. This maladaptive obesity-associated inflammation underlies the pathogenesis of many obesity-associated sequelae, including type 2 diabetes mellitus, atherosclerosis, and non-alcoholic fatty liver disease (NAFLD)\(^10\)–\(^11\). In light of the clinical and public health challenges, leveraging a better understanding of the pathogenesis of weight gain may lead to new innovative therapeutic approaches\(^12\)–\(^13\).

Obesity is associated with an increase in circulating Toll-like receptor (TLR) ligands (e.g., lipopolysaccharide [LPS])\(^14\). However, the contribution of TLRs, including TLR4, to obesity pathogenesis remains controversial with both beneficial\(^15\) and detrimental\(^16\)–\(^19\) roles reported. TLR4 signaling in myeloid cells is negatively regulated by radioproteic 105 kDa protein (RP105; CD180)\(^20\), a molecule originally thought to be B-cell-specific and facilitate B-cell proliferation\(^20\)–\(^21\) in response to LPS. Examination of paradoxical findings that RP105 has dichotomous cell type-specific effects on TLR4 signaling identified that RP105-mediated regulation of B-cell proliferation was not owing to B-cell intrinsic RP105 expression\(^22\). Rather, chronic upregulation of B-cell activating factor (BAFF) expression was revealed as the mechanism underlying suppressed B-cell proliferation in response to LPS in RP105-deficient mice\(^22\). Despite its known actions as a negative regulator of TLR-driven inflammation, RP105-deficiency dampens AT inflammation and protects mice from obesity-associated metabolic derangements\(^23\). However, the mechanisms underlying protection from diet-induced obesity (DIO) in RP105-deficient mice have not been defined.

Both TLR4 and RP105 regulate the production of inflammatory mediators including the tumor necrosis factor (TNF) superfamilys members (e.g., TNF, BAFF)\(^22\)–\(^24\). TNF can induce white adipocyte lipolysis\(^25\)–\(^26\) and may modulate brown adipocyte thermogenesis\(^27\)–\(^30\). Although recent studies highlight the ability of immune-modulators to regulate AT function\(^31\)–\(^34\), the link between immune mediators, specifically TNF superfamily members, weight gain, and BAT/WAT function remains poorly understood. BAFF and its close homolog A proliferation-inducing ligand (APRIL) are regulators of B-cell maturation and survival\(^35\). BAFF and APRIL are produced by diverse myeloid, lymphoid, and non-hematopoietic cell types\(^36\), including adipocytes\(^37\), astrocytes\(^38\), and gut epithelial cells\(^39\). Human and mouse adipocytes express all known BAFF and APRIL receptors including BAFF receptor (BAFF-R), transmembrane activator and CAML interactor (TACI), and B-cell maturation protein (BCMA)\(^37\). Although BAFF can bind all three receptors, APRIL only binds to TACI and BCMA\(^35\). Recent reports indicate that BAFF is a regulator of atherosclerosis, glucose dysmetabolism and BAFF-R and TACI regulate obesity-associated metabolic sequelae\(^40\)–\(^44\). Importantly, whether and how a BAFF/APRIL axis contributes to the regulation of weight gain in DIO models has not been examined.

Our exploration of mechanisms regulating protection from DIO in RP105-deficient mice led to the serendipitous discovery of a critical role of BAFF and APRIL in the regulation of weight gain. Here, our data indicate that multiple transgenic mouse lines with increase in BAFF expression associates with protection from weight gain. BAFF induces upregulation of lipid metabolism pathways in subcutaneous white adipocytes and is sufficient to augment WAT lipolysis in vivo and in vitro. BAFF is also sufficient to enhance brown adipocyte respiration in vitro and energy expenditure (EE) in vivo. APRIL, a close homolog of BAFF, also modifies both BAT and WAT lipid handling. Lack of both BAFF and APRIL robustly augments DIO in mice. BAFF/APRIL effects are conserved in human adipocytes and higher systemic BAFF/ APRIL levels correlate with a greater BMI decline in post-bariatric surgery. Our collective data highlight a novel pathway of immune-mediated regulation of AT/adipocyte lipid handling and may represent novel targets for the treatment of obesity.

### Results

**BAFF modulates diet-induced weight gain.** High-fat diet (HFD)-fed RP105\(^{-/-}\)- mice exhibited robust protection from DIO, altered subcutaneous (inguinal [iWAT]) and visceral (epi-duodrenal [eWAT]) adiposity, obesity-associated metabolic sequelae, and enhanced EE as compared with WT counterparts (Fig. 1a–f; Supplementary Fig. 1a–b), in agreement with a previous report\(^23\). Exploration of direct underlying mechanisms driving unexpected resistance to DIO in RP105\(^{-/-}\)- mice revealed that the effect was not associated with differential energy intake/metabolism, fat absorption, leptin sensitivity, or intestinal microbiome composition (Supplementary Fig. 1c–f). Employment of bone marrow transfer studies suggested that lack of RP105 expression in the non-hematopoietic compartment was critical for protective effects of RP105 genetic ablation (Supplementary Fig. 2a–b). Generation and utilization of an effective conditional RP105 deletion (RP105\(^{lox/lox}\) mice; Supplementary Fig. 2c) in total immune cells (Vav1\(^{cre}\), B cells (CD19\(^{cre}\)), skeletal muscle (MLC\(^{cre}\)), or central/peripheral nervous system (Nestin\(^{cre}\)) failed to fully reveal a primary RP105-expressing cellular locus responsible for observed differential weight gain (Supplementary Fig. 2d–g). Collectively, these findings suggested the possibility of an indirect mechanism underlying protection from DIO in RP105\(^{-/-}\)- mice.

Given the functional relevance of BAFF in B-cell responsiveness in RP105\(^{-/-}\)- mice\(^22\), we hypothesized that BAFF, which can be induced by multiple proinflammatory means (e.g., TLR signaling)\(^36\), may also affect DIO in RP105\(^{-/-}\)- mice. Deletion of BAFF in RP105\(^{-/-}\)- mice (RP105\(^{-/-}\)/BAFF\(^{-/-}\)) was sufficient to reverse the protection from HFD-driven weight gain, fasting glucose, glucose tolerance, and fat pad distribution as compared to WT controls (Fig. 1b–e; Supplementary Fig. 3a). Deletion of BAFF in RP105\(^{-/-}\)- mice was likewise sufficient to dampen EE (Fig. 1f; Supplementary Fig. 3b–c) without alteration to locomotor activity (Supplementary Fig. 3d). These observations indicated that BAFF may regulate weight homeostasis in RP105\(^{-/-}\)- mice.

To define whether the effect of BAFF on weight gain extends beyond the RP105 system, we utilized multiple model systems that enhance the expression of BAFF. Although RP105\(^{-/-}\)- mice exhibit marginally increased BAFF expression (~20% increase), B-cell-deficient mice (\(\mu\)MT) manifest a two log-fold secondary increase in BAFF, and BAFF-Tg mice manifest a three log-fold increase in BAFF (Fig. 1g). Enhanced systemic BAFF levels were associated with increased resistance to HFD-driven weight gain in an approximate log-linear dose fashion (Fig. 1g–h). HFD-fed BAFF-Tg (Fig. 1i–m; Supplementary Fig. 4) and \(\mu\)MT (Supplementary Fig. 5) mice were protected from weight-driven glucose dysmetabolism (e.g., fasting glucose and GTT) and NAFLD progression (hepatic triglyceride levels and alanine transaminase [ALT]).
Overall, these findings suggest that increased circulating BAFF levels may broadly regulate DIO-driven weight gain.

**BAFF modifies subcutaneous adipocyte lipolysis.** Obesity pathogenesis/weight gain is regulated, in part, by adipocyte lipid handling. Inflammatory mediators (e.g., TNF) regulate lipid processing. BAFF, a TNF superfamily member, and all three known BAFF receptors (e.g., BAFF-R, TACI, BCMA) are expressed by both mouse and human adipocytes. Thus, we examined the ability of BAFF to modulate adipocyte function.
Utilization of an unbiased RNA-seq analysis revealed that recombinant BAFF (rBAFF), compared with saline-treated counterparts, robustly upregulated lipid handling pathways, including metabolism of lipids and lipoproteins, regulation of lipid metabolic process, neutral lipid metabolic process, and cellular lipid metabolic process (Fig. 2a) in primary mouse subcutaneous (inguinal) adipocytes. In line with these observations, visceral (epididymal) WAT from RP105−/− and BAFF-Tg mice had significantly increased mRNA expression of adipose triglyceride lipase (Pnpla2) and hormone-sensitive lipase (Lipe), both critical regulators of lipolysis (Fig. 2b). Enhanced mRNA expression of Pnpla2 and Lipe correlated with increases in systemic BAFF levels (Figs. 1a and 2b). We next tested the direct impact of BAFF on subcutaneous white adipocyte lipolysis. rBAFF was sufficient to augment the presence of free glycerol in adipocyte supernatants, which did not synergize with non-epinephrine (Fig. 2c), a major lipolysis-inducing hormone. Further, rBAFF was sufficient to enhance the mRNA expression of both Pnpla2 and Lipe in vitro and in vivo (Fig. 2d, e) and amplify protein expression of phosphorylated hormone-sensitive lipase (HSL: aka Lipe; Fig. 2f). Endotoxin was undetectable in utilisable rBAFF (Supplementary Fig. 6a) and heat-inactivated rBAFF did not augment the presence of free glycerol in subcutaneous white adipocytes (Supplementary Fig. 6b). Genetic deletion of BAFF-R, TACI, or BCMA alone abrogated rBAFF-driven subcutaneous adipocyte lipolysis in vitro (Fig. 2g). Similarly, rBAFF did not augment lipolysis in BAFF−/−/BAFF-R−/− or BAFF−/−/TACI−/−/BCMA−/− subcutaneous adipocytes (Supplementary Fig. 7). rBAFF-driven lipolysis was specific to white, but not brown adipocytes (Supplementary Fig. 8a). Combined, these findings indicate that BAFF is capable of modulating WAT/subcutaneous white adipocyte lipolysis.

**BAFF modifies Ucp1 expression and EE in BAT.** BAT/brown adipocyte thermogenesis is a key regulator of lipid utilization and weight control. We examined whether BAFF could impact BAT thermogenic capacity. Uncoupling protein 1 (Ucp1) promotes adaptive thermogenesis in BAT45. BAT from RP105−/− and BAFF-Tg mice, compared with WT controls, exhibited increased Ucp1 mRNA expression (Fig. 3a). BAT mitochondria from these mice had significantly enhanced respiration as compared with WT controls (Fig. 3b). Congruently, rBAFF was sufficient to amplify Ucp1 mRNA expression (Fig. 3c), enhance Ucp1-dependent respiration in primary brown adipocytes in vitro (Fig. 3d) and raise the threshold of norepinephrine-driven thermogenic activity (Fig. 3e) in WT mice in vivo. rBAFF enhancement of Ucp1 mRNA expression was specific to brown adipocytes and not subcutaneous adipocytes (Supplementary Fig. 8b). Subcutaneous WAT from RP105−/−, BAFF-Tg, RP105−/−/BAFF−/−, BAFF−/− mice exhibited similar mRNA expression of Ucp1-dependent thermogenic/beige markers (e.g., Pgc1a, Ucp1, Dio2, Cidea, Elovl6) as compared with WT counterparts (Supplementary Fig. 9). We additionally assessed the sufficiency of exogenous BAFF to modify whole-body thermogenic parameters in vivo. In contrast to pre-treatment (Supplementary Fig. 10a), short-term exogenous rBAFF in CD-fed mice transiently amplified EE, specifically in the dark cycles (Supplementary Fig. 10b–c) but did not alter the respiratory exchange ratio or body weight (Supplementary Fig. 10d–e). Congruently, rBAFF administration to HFD-fed mice enhanced total EE (Fig. 3f, g). Collectively, these findings indicate that BAFF, in parallel to its effects on subcutaneous WAT/adipocytes, is capable of modulating BAT/brown adipocyte Ucp1 expression and in vivo EE.

**APRIL similarly modulates white and brown adipocyte lipid handling.** Examination of HFD-fed BAFF−/− mice revealed no alteration in weight gain (Fig. 4a), WAT or BAT weight (Supplementary Fig. 11a), slightly increased total body weight (Supplementary Fig. 11b), similar fasting glucose (Supplementary Fig. 11c), and protection from hepatocellular injury (Supplementary Fig. 11d)—findings consistent with previous reports46,47. Cumulatively, these findings hinted the possibility that other BAFF-like molecules may similarly modulate weight gain to potentially compensate for such effects in the absence of BAFF. BAFF−/− mice exhibited enhanced circulating levels of APRIL (Fig. 4b), a close homolog of BAFF35. Congruent with similarities of BAFF and APRIL function on immune cells, an unbiased RNA-seq approach revealed significant gene overlap (52% of BAFF-induced genes) between BAFF or APRIL-treated primary subcutaneous white adipocytes (769 genes; Fig. 4c). Pathway analyses revealed that APRIL stimulation of subcutaneous white adipocytes also upregulated pathways associated with lipolysis including regulation of lipid metabolism, fatty acid metabolism, PPAR signaling, and the glucocorticoid receptor regulatory network (Fig. 4d). rAPRIL utilized did not exhibit endotoxin contamination (Supplementary Fig. 12). Like BAFF, APRIL was sufficient to augment subcutaneous white adipocyte mRNA expression of Lipe and Pnpla2 in vitro and in vivo (Fig. 4e, f) and p-HSL (aka p-Lipe) protein levels in vitro (Fig. 4g). Lack of TACI or BCMA, but not BAFF-R, prohibited APRIL-driven subcutaneous white adipocyte lipolysis in vitro (Fig. 4h)—as would be expected from the pattern of receptor usage by APRIL35. Likewise, rAPRIL-mediated lipolysis was blunted in BAFF−/−/BAFF-R−/− and BAFF−/−/TACI−/−/BCMA−/− subcutaneous white adipocytes (Supplementary Fig. 13). Examination of APRIL’s effects on brown adipocytes demonstrated sufficiency to augment Ucp1 mRNA expression and enhance mitochondrial respiration (Fig. 4i, j). However, exogenous rAPRIL administration did not amplify EE in lean or obese mice (Supplementary Fig. 14). Together, these findings indicate that APRIL, similar to BAFF, is capable of modulating both subcutaneous white and brown adipocyte lipid handling. Although close homologs with some overlapping function, deeper examination of our unbiased RNA-seq analyses displayed
a large number (2535) of genes modulated by APRIL, as compared with BAFF in primary subcutaneous white adipocytes (Fig. 4c). APRIL specifically downregulated inflammatory pathways including IL6-mediated signaling, TLR signaling pathway, and inflammation-mediated by chemokine and cytokine signaling pathway (Supplemental Fig. 15a). Screening of obesity-associated proinflammatory cytokines and chemokines in subcutaneous white adipocytes demonstrated that BAFF, but not APRIL, upregulated the mRNA expression of multiple proinflammatory mediators, including Tnf, Cxcl1, Ccl2, and Ccl3 (Supplemental Fig. 15b–e). Thus, these findings suggest that despite similar effects to modify adipocyte lipid handling, APRIL, unlike BAFF, may not induce an inflammatory response in subcutaneous white adipocytes.

**BAFF/APRIL axis is a contributor to diet-induced weight gain.**

As APRIL modifies subcutaneous white adipocyte lipid handling, we next tested the effect of genetic deletion of APRIL on DIO. Surprisingly, APRIL−/− mice likewise exhibited augmented levels of systemic BAFF suggesting that deletion of BAFF or APRIL alone drives compensatory increases of the reciprocal family member (BAFF−/− Fig. 4b; APRIL−/−; Fig. 5a). Consistent with the overarching observation of increased BAFF association with body weight control (Fig. 1e, f), APRIL−/− mice were resistant to DIO (Fig. 5b) with decreased pWAT and BAT weight (Fig. 5c), despite similar food intake (Fig. 5d), as compared with WT controls. This protection was associated with higher EE (Fig. 5e) and decreased metabolic sequelae, including glucose dysmetabolism (Fig. 5f–i) and hepatocellular damage (Fig. 5j).
Given potential compensatory mechanisms between BAFF and APRIL, we next examined the contribution of the BAFF/APRIL axis to HFD-driven weight gain. Combined deletion of BAFF and APRIL in mice led to robust amplification of weight gain, above the levels of BAFF−/−, APRIL−/−, and WT mice (Fig. 6a). Further, BAFF−/−/APRIL−/− mice fed HFD exhibited increased amounts of subcutaneous (iWAT) and visceral (eWAT, pWAT) AT (Fig. 6b). Lack of both BAFF and APRIL was associated with protection from glucose dysmetabolism (Fig. 6c–e) and a similar degree of hepatocellular damage (Fig. 6f).

We subsequently sought to initially interrogate the role of the BAFF/APRIL receptor(s) in HFD-driven weight gain. Mice lacking a single BAFF/APRIL receptor (BAFF-R−/−, TACI−/−, BCMA−/−) were protected from weight gain (Supplementary Fig. 16a). The protection from weight gain was associated with increased systemic levels of BAFF (Supplementary Fig. 16b). Notably, only TACI−/− and BCMA−/− mice were protected from HFD-driven, glucose dysmetabolism, and hepatocellular damage compared with WT controls (Supplementary Fig. 16c–e). In contrast, BAFF-R−/− mice exhibited similar glucose dysmetabolism and hepatocellular damage compared with WT controls (Supplementary Fig. 16c–e).
Subcutaneous WAT from BAFF-R<sup>−/−</sup>, TACI<sup>−/−</sup>, or BCMA<sup>−/−</sup> mice exhibited similar mRNA expression of Ucp1-dependent thermogenic/beige markers (e.g., Pgc1α, Ucp1, Dio2, Cidea, Elovl6) as compared with WT counterparts (Supplementary Fig. 16f–j). Combined, these data indicate a role for the BAFF/APRIL axis in the development of obesity (Figs. 1h and 6a, Supplementary Fig. 16a).

**BAFF/APRIL axis effects are conserved in humans.** To determine whether BAFF and APRIL’s effects were conserved in humans, primary human white adipocytes were isolated from omental WAT of a pediatric cohort of severely obese individuals undergoing surgical treatment (Supplementary Table 1). Consistent with our findings in mice, rBAFF and rAPRIL treatment resulted in enhanced human white adipocyte-free glycerol release.
(Fig. 7a) and induced the mRNA expression of LIPE and PNPLA2 (Fig. 7b–c). At baseline, persons with severe obesity had lower levels of circulating BAFF but increased levels of APRIL as compared to lean controls (Fig. 7d, e; Supplementary Table 2). BAFF, but not APRIL, was negatively correlated with increased BMI (Fig. 7f, g). Notably, 1-year after bariatric surgery and after substantial weight loss (Supplementary Table 1), BAFF and APRIL levels were positively correlated with a greater decrease in BMI (Fig. 7h, i). Collectively, these findings suggest that the BAFF/APRIL axis potentially exerts conserved effects on human adipocytes and may act as a potential surrogate marker for the degree of weight loss after bariatric surgery.

**Discussion**

The traditional perception of inflammation in the context of obesity has focused on the detrimental impact of inflammatory mediators on the pathogenesis of secondary metabolic derangements associated with obesity. Although inflammation undoubtedly exacerbates obesity-associated sequelae, the direct relevance to the regulation of weight gain remains underdefined. In this study, we used a reductionist approach to tease apart the role of the BAFF/APRIL axis in obesity. Our findings highlight the beneficial effects of the BAFF/APRIL axis in regulation of weight gain by altering AT metabolic function and energy homeostasis (Fig. 8). These observations add to the growing number of reports invoking a potentially dichotomous relationship presented by in

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**Fig. 5 APRIL-deficient mice are protected from obesity development.** a–j WT and APRIL−/− mice were fed HFD for 20 weeks. a Systemic BAFF concentration quantified by ELISA. b Weight gain. c Tissue weight distribution as indicated at time of harvest. d Food Intake. e Combined energy expenditure at 16 weeks of HFD analyzed by TSE Phenomaster system. f Fasting glucose at 20 weeks of HFD. g GTT at 14 weeks of HFD. h AUC. i Fasting insulin at 20 weeks of HFD. j Systemic ALT at 20 weeks of HFD. k Weight gain of WT, BAFF−/−, APRIL−/−, or BAFF−/−/APRIL−/− mice fed HFD for 20 weeks. a–j Representative of three independent experiments, n = 6–7/condition. a–j For bar and line graphs, data represent mean ± SEM. a, c–f, i–j Unpaired two-tailed Student’s t test. *p < 0.05, **p < 0.01, ***p < 0.001. Source data are provided as a Source data file.
In agreement with our findings (Supplemental Fig. 11b), published studies harnessing BAFF−/− mice also revealed increases in body weight and weight gain. Published evidence suggests that BAFF may modulate obesity-associated glucose dysmetabolism in a sex-dependent manner. The potential impact of this finding is bolstered by the observed sexual dimorphism in normal human BAFF levels. Traditionally, female mice, unless genetically (ob/ob; db/db) or hormonally manipulated, are protected from severe DIO and consequent pathogenesis of obesity-associated sequelae. Congruently, female cohorts in these studies gained ~1 g of body weight on an HFD (total body weight of 19 g after 4 weeks on a HFD) (Fig. 6a). The limitations of this approach make it difficult to draw robust conclusions regarding gender-specific mechanisms. Thus, future in-depth interrogation of how the BAFF/APRIL axis impacts obesity-associated glucose dysmetabolism, the relevance of gender-specific mechanisms would be warranted, possibly through the exploitation of the thermoneutral housing model. Likewise, the impact of aging, a well-appreciated modifier of AT homeostasis and biology, on gender-specific mechanisms and BAFF/APRIL axis modulation of weight gain would be highly pertinent.

BAFF and APRIL are traditionally perceived as regulators of B-cell maturation and survival. BAFF impacts the effector capacity of multiple immune cells (e.g., macrophages, B cells, T cells). Various immune cells infiltrate AT in obesity. We hypothesize that this interplay between BAFF/APRIL, immune cells, and AT is likely an important contributor to the overall AT biology and function. Hence, detailed interrogation of how BAFF/APRIL axis-driven modulation of immune cells shapes adipocyte biology and weight gain represents key future studies. B cells infiltrate AT in the context of obesity and have been suggested to contribute to the downstream metabolic derangements of obesity. Our data indicate that multiple lines of mice with increased systemic BAFF, including RP105−/− or BAFF-Tg (augmented B-cell numbers) and μMT, APRIL−/−, BAFF−−/−, TACI−/−, or BCMA−−/− mice (devoid or decreased number of mature B cells) are similarly protected from DIO and the subsequent downstream metabolic sequelae (Fig. 1, Fig. 5, Supplementary Fig. 16). The consistent unifying factor among these different transgenic lines is elevated systemic BAFF levels, invoking that observed BAFF effects in our studies may be independent of B cells. In contrast to our findings, existing reports indicate that μMT mice gain similar weight to WT counterparts. Nonetheless, reported protection from obesity-associated glucose dysmetabolism is congruent with our findings. As B cells contribute to the establishment of the gut microbiota (e.g., IgA regulation), it is plausible that lack of B cells directs differential gut microbial colonization within μMT mice harbored at different facilities.

The microbiome modulates body weight. Our examination of the microbiome in one model of increased circulating BAFF, RP105−/− mice, revealed a similar, HFD-driven, microbiome in WT and RP105−/− mice (Supplemental Fig. 1f). These findings would suggest that the microbiome may not play an integral role in the reversal of weight gain in RP105−/−/BAFF−−/− mice. However, these findings could be limited to the RP105−/− system. We posit that analyses of multiple mouse lines with varying levels of systemic BAFF (RP105−/−, RP105−/−/BAFF−−/−, BAFF−−/−, μMT, BAFF-Tg, APRIL−/−, BAFF−−/−/APRIL−/−, BAFF-R−−/−, TACI−−/−, BCMA−−/−), would likely reveal differential intestinal microbiome composition. However, the possible differences may not correlate with different BAFF levels in these mice, but rather may be dependent on direct alteration of key biological processes in these specific settings such as BAFF-driven modulation of...
Further, BAFF-Tg mice exhibit enhanced, commensal-flora dependent, systemic IgA levels. Whether and how systemic IgA and presence of plasma cells in the gut, in the setting of the BAFF axis, affects intestinal homeostasis and consequently contribute to obesity and weight gain remains unknown. Future, in-depth interrogations including, but not limited to, microbiome depletion, microbiome transfer studies, and analysis of these mice under germ-free conditions would provide further clarity to our understanding of the microbiome within the BAFF/APRIL axis in the regulation of body weight.

Despite similar capacities of BAFF and APRIL to modify subcutaneous white and brown adipocyte lipid handling, we demonstrate that BAFF−/− or APRIL−/− mice do not exhibit increased obesogenic diet-driven weight gain (Figs. 4a and 5b). However, BAFF−/− mice exhibit ~1.5-fold (48%) increase in APRIL, whereas APRIL−/− mice similarly exhibit approximately 2-fold (59%) increase in systemic BAFF (Figs. 4b and 5a)—likely stemming from the activation of compensatory mechanisms. Our findings that lean individuals have higher levels of BAFF than obese individuals, but lower APRIL levels, suggest possible reciprocal regulation between BAFF and APRIL. Inflammatory mediators within the same family (e.g., IL-17a and IL-17f) can regulate each other’s expression. Thus, examination of the interlink between BAFF and APRIL expression is warranted. As

Fig. 7 Effect of BAFF and APRIL axis is conserved in human adipocytes. a–b Human primary adipocytes isolated from persons undergoing bariatric surgery. Adipocytes were treated with saline (NS), rBAFF (500 ng/ml), or rAPRIL (500 ng/ml) for 24 hours as indicated. a Supernatant free glycerol quantified by colorimetric assay. b LIPE mRNA expression quantified by qPCR, normalized to NS. c PNPLA2 mRNA expression quantified by qPCR, normalized to NS. d BAFF and e APRIL systemic concentration in lean or obese persons quantified by multiplex. f BAFF and g APRIL systemic concentration correlation with BMI. h BAFF and i APRIL systemic concentration correlation with decrease in BMI 1-year after bariatric surgery. a–c Representative persons, n = 9–10/condition. d–g Representative patients, n = 4 lean and n = 30 obese. h–i Data combined, n = 22 persons undergoing bariatric surgery. a–c For bar and line graphs, data represents mean±SEM. d–e For box plots, the midline represents the mean, boxes represent the interquartile range and whiskers show the full range of values. a–e Unpaired two-tailed Student’s t test. a, c, d *p < 0.05, ****p < 0.0001. f–i Linear regression. Source data are provided as a Source data file.
lack of both BAFF and APRIL brought about robust DIO-driven weight gain (~50% increase Fig. 6a), this suggests that the BAFF/APRIL system likely works in concert to regulate weight gain.

Recent reports suggest that BAFF and APRIL’s receptors, including BAFF-R and TACI, regulate obesity pathogenesis\(^{40-43}\). Although BAFF-R\(^{-/-}\) or TACI\(^{-/-}\) mice gain significantly more weight on HFD\(^{40-43}\), these mice are protected from the downstream glucose dysmetabolism. Our data indicate that mice with a lack of a single receptor (BAFF-R\(^{-/-}\), TACI\(^{-/-}\), BCMA\(^{-/-}\)), regardless of which, are all protected from HFD-driven weight gain (Supplementary Fig. 16a). Similarly, we also show that deficiency of a single receptor is sufficient to induce increased systemic levels of BAFF (Supplementary Fig. 16b). In agreement with published reports, our findings also suggest that BAFF-R\(^{-/-}\), TACI\(^{-/-}\);\(^{-/-}\) mice are protected from HFD-driven weight and TACI\(^{-/-}\) mice are protected from glucose dysmetabolism\(^{41}\). Conversely to our data, existing evidence suggests that BAFF-R\(^{-/-}\) mice are protected from glucose dysmetabolism but exhibit enhanced NAFLD pathogenesis\(^{43}\) and enhanced steatosis\(^{43}\). As BAT activity is linked with the development of steatosis\(^{68-70}\), it is plausible that augmented steatosis in BAFF-R\(^{-/-}\) mice consist of dampened BAT activity or even direct effect of BAFF on hepatocytes via BAFF-R signaling. To our knowledge, our data describing the role of BCMA in HFD-driven obesity represents the first depiction on this topic. Given the intricate complexity of this system, in-depth exploration of the receptor side of the BAFF/APRIL axis is clearly warranted. Further, targeted tissue/cell type manipulation of BAFF/APRIL axis specific receptor(s) is needed and could be potentially exploited as effective preventative and/or therapeutic method for modifying weight gain.

Our findings indicate that BAFF-R impacts BAFF-driven subcutaneous white adipocyte lipolysis, whereas TACI and BCMA modulate BAFF and APRIL-induced lipolysis—findings in line with the selectivity in BAFF and APRIL binding to these receptors (Figs. 2g and 4h). As a single receptor deletion (BAFF-R\(^{-/-}\), TACI\(^{-/-}\), BCMA\(^{-/-}\)), deletion of BAFF-R/BCMA (Supplementary Fig. 7 and 13), or abolishment of all three receptors (Supplementary Fig. 7 and 13) abrogates BAFF or APRIL-driven lipolysis, this suggests the possibility that engagement of multiple receptors on subcutaneous white adipocytes is necessary to provide sufficient signal strength for activation of lipolysis. Hence, closer examination of the critical BAFF/APRIL receptor(s) involved with lipolysis, thermogenesis, and its capacity to control body weight would be of significant future interest.

BAFF/APRIL effects on lipolysis are distinctive in subcutaneous white adipocytes, whereas induction of Ucp1 expression is specific to brown adipocytes (Supplementary Fig. 8). Consistent with this, subcutaneous WAT isolated from mice with increased BAFF exhibited similar mRNA expression of brown/beige thermogenic markers to WT counterparts (Supplementary Fig. 9, Supplementary Fig. 16f). Combined our findings hint that the BAFF axis may be insufficient for subcutaneous white adipocyte browning. However, the full definition of the BAFF/APRIL axis’ ability to modify Ucp1-dependent and Ucp1-independent browning/beiging of subcutaneous white adipocyte is of strong interest. Specifically, like the dependency of Ucp1 deletion on weight gain is uncovered at thermoneutrality\(^{71}\), future examination of BAFF/APRIL axis at thermoneutrality might be warranted. This layered complexity of the BAFF/APRIL system (two molecules and three receptors) invokes the possibility of a highly regulated mechanism(s) to impact function across different tissues/cell types (e.g., WAT, BAT, liver). In addition, although our findings suggest responsiveness by WAT and BAT to BAFF and APRIL, the relevant source of BAFF and/or APRIL in the context of an obesogenic diet remains unknown. Both hematopoietic and non-hematopoietic cells, including adipocytes, can produce BAFF and APRIL\(^{36,37,72}\). Complicating matters further, BAFF and

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**Fig. 8 BAFF and APRIL axis regulates adipose tissue/adipocyte function and body weight gain.** A proposed model of the impact of the BAFF/APRIL axis on white and brown AT/adipocyte lipid handling. BAFF/APRIL regulation of AT/adipocytes function is associated with protection from DIO in mice. The effect of this axis positively correlates with greater loss of body weight in bariatric patients.
APRIL exist as oligomers, homo/hetero multimers and their conformation regulate the capacity of BAFF and APRIL to activate their three receptors. Thus, future exploration of the conformation and relevant sources of BAFF and APRIL in the context of obesity and control of body weight should be investigated. BAFF and APRIL have the capacity to modify DIO in mice (Figs. 1h, 5b, and 6a). In addition, the effects of both BAFF and APRIL are conserved in primary human adipocytes. Novel preliminary findings of a direct correlation between higher BAFF and APRIL levels with greater changes in BMI after bariatric surgery indicate that the BAFF and/or APRIL axis may play a role in human weight regulation after bariatric surgery. Recent GWAS studies have revealed the association of variants within TNFSF13B (the gene encoding BAFF) and near TNFSF13 (the gene encoding APRIL) with lipid metabolism (e.g., HDL-C). As subcutaneous AT lipolysis is an independent contributor to inter-individual variation in HDL-C, these findings further support the applicability of our mechanistic murine data to understanding how these cytokines regulate metabolism and adiposity. In this regard, even a minimal increase in BAFF (~20% increase in RP105−/− mice) are protective of DIO and would likely not cause the deleterious effects observed with log-increases of BAFF76,77 (e.g., autoimmunity, B-cell-driven pathology) invoking therapeutic potential in humans. In contrast to BAFF, APRIL does not appear to induce a proinflammatory program in adipocytes (Supplementary Fig. 15) while maintaining its capacity to modulate white and brown adipocyte lipid handling and play a role in protection from DIO (Figs. 4–7). Thus, an approach harnessing the BAFF/APRIL axis may need careful monitoring of the levels of BAFF/APRIL in order to provide a maximal therapeutic index.

In summary (Fig. 8), our data demonstrate the impact of the BAFF and APRIL axis as beneficial mediators of white and brown adipocyte lipid handling and body weight control. These observations may highlight a prospective and appealing pathway open to the development of new clinical approaches for body weight modulation.

Methods

Reagents. All cell culture reagents were endotoxin-free to the limit of detection of the Pierce Chromogenic Endotoxin Quantification Assay. Recombinant BAFF was also heat-inactivated via boiling for 30 min and used as a control for free glycerol release studies.

Mouse obesogenic diet model. All experiments utilized male mice, 6–8 weeks old, on a C57Bl/6 background. WT (Jackson), RP105−/− (in house), Vav1tm1(Stefen Burden), RP105−/−/BAFF−/− (in house), BAFF−/− (Jackson), BMMT (Jackson), BAFF-Tg (Blegen Idec), APRIL−/− (Jackson), BAFF−/− (generously provided by Steven Burden), RP105−/−/BAFF−/−/BCMA−/− (in house), BAFF−/−/BCMA−/− (in house), and BAFF−/−/APRIL−/− (in house) mice were bred at Cincinnati Children’s Hospital Medical Center (CHMC) in a specific pathogen-free (spf) facility maintained at 22°C with free access to autoclaved chow diet food (CD; Lab Diet #5010; calories provided by carbohydrates [58%], fat [13%] and protein [29%]) and water. Mice, 6–8 weeks of age, were fed either an irradiated HFD (Research Diets #D12492; 60% of calories from fat) or a CD and food was replaced weekly. For energy absorption studies, fecal pellets were collected at 15 weeks and analyzed by bomb calorimetry. For lipid absorption studies, at 24 weeks, mice were switched to Behenate mouse diet and fecal pellets were collected at day 3 and day 4 for fat absorption analysis. Phylogenetic analysis of the fecal microbiota was performed essentially as previously described via 16S rRNA pyrosequencing of fecal pellets collected after 8 weeks of HFD feeding. Body composition and in vivo metabolic phenotyping were performed as previously described. Prior to glucose metabolism testing or terminal harvest, mice were fasted overnight as previously described. For glucose tolerance tests, fasted mice were subjected to glucose (mice injected 100 µl of a 10% dextrose solution per gram of body weight) and glucose was monitored using the Accu-Check glucometer per manufacturer instructions. Body weight, body mass index, food intake and energy absorption studies were performed according to manufacturer instructions. Mice were subsequently housed in individual axial boxes and acclimated for 2 days before the start of the study. 12-hour light and dark cycles were maintained throughout. Data points were continuously collected for a total of 5–8 days. Gas exchange (O2 and CO2) and locomotor activity were recorded every 15 minutes and EE was calculated according to the manufacturer guidelines. Ambient temperature (22°C) and humidity were maintained via climate-control units in the metabolic chambers. For exogenous rBAFF or rAPRIL studies, rBAFF (2 µg/mouse), rAPRIL (2 µg/mouse), or saline was administered via intraperitoneal injection every 48 hours.

Bone marrow transfer. Bone marrow from WT were transferred into WT or RP105−/− recipient mice as previously described. In brief, femur-derived bone marrow cells (5 × 10⁹) were transferred into whole-body irradiated RP105−/− recipient mice. Flow cytometric analysis of peripheral blood chimerism was performed 10 weeks after bone marrow reconstitution prior to mice being placed on an HFD or CD.

Generation of RP105lox/lox line. An ES Cell clone (D111) containing the correct targeted allele was obtained from the knockout mouse project (KOMP). This clone passed genotyping of RP105, was obtained from the knockout mouse project (KOMP). This clone passed genotyping quality control and was selected for blastocyst injection. ES cells were grown on neomycin-resistant mouse embryonic fibroblast feeders (a gift of M. Flick) and were then injected into C57BL/6-Albino blastocysts (B6(Gry-Tg8–lyt2)-1{jyg} J stock #00058 from Jackson Labs http://jaxmice.jax.org/strain/00058.html) and implanted into pseudopregnant C57BL/6 mice. Genetic chimerism and genotype were established by blastocyst transfer yielding chimeric mice of high chimerism, several of which were capable of transmitting the targeted allele in the germline as confirmed by PCR. The germline-targeted progeny of these chimeras were subsequently bred to FlPe-Deleter mice (B6.Cg-Tg(ActFLPe)2ZbDym/J stock #00573 from Jackson Labs http://jaxmice.jax.org/strain/00573.html) to excise the FRT-flanked knockout-first β-galactosidase reporter/selection cassette. Excision of the cassette was confirmed by PCR for both the neomycin selection cassette and with primers (HI152 and HI154) flanking the FRT cassette. This PCR product was sequenced to confirm the presence of both the FRT and loxP sites. The presence of the Exon 3 distal loxP site was confirmed by PCR using primers complementary to the genomic DNA flanking the loxP site (IHI123 and IHI124). This product was also sequenced to confirm the presence of the loxP site. The Rpp105−/−/APRIL−/− mice thus generated were then bred to C57BL/6Nl mice. Progeny lacking the FlPe-deleter transgene (confirmed via PCR) were selected to establish an Rpp105lox/lox breeding colony after breeding to homozygosity for both the floxed allele and a wild-type Nnt allele. Genotyping of Rpp105lox/lox mice was carried out with the following two primer pairs: FWD: GAAGAGCATTTACC; Rv: TGAGCATTAGTTGCGTGAGG, FWD: GCATTTCCCCATCTATCATCTGAC; REV: TGGATTAAACATGTCGCGTAC. Exon 3 containing the majority of the coding sequence of Rpp105 (GI188), was identified as a likely result in knockin by a prediction algorithm used by KOMP and was successfully targeted in the generation of the germline Rpp105 knockouts.

Mouse primary subcutaneous and brown adipocytes. Isolation and digestion (1 mg/ml Collagenase Type IV, Dispase 2, CAI) of subcutaneous white AT (iWAT) or brown adipocytes (iWAT) were cultured until confluence and differentiated as previously described. In brief, initial media (Growth media [Dulbecco's Modified Eagle Medium (DMEM):F12, fetal bovine serum (FBS), Pen/Strep] was replaced every 10 days and media was changed every 4 days for 2 days and followed by differentiation media (Growth media, Insulin) for an additional 2 days. Differentiated white adipocytes were utilized for downstream processes.

Brown preadipocytes were isolated from P1 B6/J neonates, maintained in high-glucose DMEM in incubators at 37°C and 5% CO2 and immortalized with pBABE-puro. The generation of these cells was not described. Brown preadipocytes were subjected to differentiation media (high-glucose DMEM including 10% FBS, 1% antibiotics, 20 nM insulin and 1 nM T3). After 4 days, cells were switched to
induction media (high-glucose DMEM including 10% FBS, 1% antibiotics, insulin, 20 mM, 1 nM T3, 0.125 mM indomethacin, 2 μM dexamethasone, and 0.5 mM 3-iodothyronine (a high concentration of IHBX) for 2 days. Following this, this differentiation media was utilized until maximal differentiation on day 12. Maximally differentiated brown adipocytes were utilized for subsequent downstream studies.

Adipocyte-free glycerol quantification. Murine or human primary adipocytes were cultured in the presence or absence of rBAFF (500 ng/ml) or rAPRIL (500 ng/ml) for 24 h. Free glycerol was quantified by colorimetric assay (Sigma Aldrich) as per manufacturer’s instructions.

qR-PCR. AT and adipocytes were homogenized in TRIzol (Invitrogen) by RNA extraction, reverse transcription to cDNA (Verso cDNA Synthesis Kit; Thermo Scientific) and qPCR analysis (Light Cycler 480 II; Roche)—according to manufacturer’s instructions18,68,73,99. The following primer pairs were used for mouse studies: Batcin For GGCC CAGACGCAAGAGAGGAT Rev GGTGGGTCTTTAGTGTCG Lipe For TCT CTTGGAATGATGCTGACAG Rev GCTCAAACAGGACAGGTC TAGTGTTGCTGTGGTGTTACTG Rev ACGCTTATGGGCTTCCAGTC AGAT GGAGTATGACGCCAAGTG Rev CAGGATTCACATGATCAACAGC CCTTGATGACCCCAAGCC Rev CACCATGACATCCACACACAGCAG Cat #4126) diluted in (5% BSA/PBST). Thereafter, the membranes were incubated for overnight with primary antibodies including

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Total protein was quantified by bicinchoninic acid (BCA) assay (Pierce BCA Protein Assay Kit) according to manufacturer instructions61,86,87,92,99. Differentiated adipocytes were used for downstream applications.

Human primary adipocytes. Isolation and digestion of omental WAT (40 mg/ml Type II Collagenase) collected at the time of surgery102. Filtration of digested tissue was subsequently centrifuged at 250 g and subjected to ACK lysis buffer to isolate the SVF. SVF was cultured in expansion media (DMEM/F12, 15% FBS, 1% antibiotics, insulin, 33 μM strept, 2 mM glutamine, 15 mM HEPES, 10 mg/ml transferrin, 33 μM biotin, 17 μM pantothenate, 0.1 μM dexamethasone, and 0.5 mM 3-iodothyronine (a high concentration of IHBX) for 14-16 days, followed by 7-10d in human adipocyte maintenance media (DMEM/F12, 1% Pen-strep, 2 mM glutamine, 15 mM HEPES, 10 mg/ml transferrin, 33 μM biotin, 0.5 μM insulin). Differentiated adipocytes were used for downstream processes.

Human systemic cytokine quantification. Human BAFF or APRIL plasma concentrations from lean or persons with severe obesity were determined by ELISA using Milliplex® Multiplex kits (MilliporeSigma) according to manufacturer’s protocol. In brief, 25 μL of plasma, plated in duplicate on a 96-well black plate, was incubated with 25 μL of antibody-coated beads. Plates were subsequently washed and 25 μL of secondary antibody was incubated, then 25 μL of streptavidin-RPE. In all, 130 μL of sheath fluid was added to plates that were washed and then read using luminescent technology on a Milliplex Analyzer (milliporeSigma). Data analysis was performed by the Cincinnati Children’s Medical Center Research Flow Cytometry Core.

RNA-seq and gene expression quantification. Gene expression of primary white adipocytes was determined100 by running 50 base pair single-end reads (~30 million reads per sample). Following the removal of barcodes and primers, raw reads were aligned to the mm10 genome using kallisto101, which quantifies transcript abundances of high-throughput sequencing reads. Kallisto pseudomolecules were added as a reference, by identifying transcripts that are compatible with each raw read, which were added by UCAST. The UCASTs identified by UCAST and the pseudomolecules were included for differential analysis. Differential expression was determined by unpaired t tests with a false discovery rate-corrected p value cutoff of 0.05 and a fold change requirement of >2. For pathway analysis, the database at toppgene.cchmc.org was employed, which amasses ontological data from over 30 individual repositories. RNA-seq raw data can be accessed at GSE131298.

Subcutaneous white adipocyte western blot. Differentiated primary white adipocytes were stimulated with saline (NS), rBAFF (500 ng/ml), or norepinephrine (1 μM) for 12 hours and cells were then lysed in a buffer containing 50 mM Hepes, pH 7.4, 120 mM NaCl, 2 mM EDTA, 10 mM sodium pyrophosphate, 10 mM sodium β-glycerophosphate 100 mM CHAPS and protease & phosphatase inhibitors.98 Total protein was quantified by Bradford protein assay and equivalent amounts of total protein from each treatment group was separated using acrylamide/bis-acrylamide gels and transferred to polyvinylidene difluoride membranes. Membranes were incubated for overnight with primary antibodies including α-tubulin (Cell signaling technology Cat #2125) diluted in 5% milk/phosphate-buffered saline with Tween® 20 (PBST) (1:1000), HSL (Cell signaling technology Cat #18381) diluted in 1% BSA/0.5% PBST (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HSL; -5% PBST) (p-HS...
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Additional information

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