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Title: **From Wasting to Obesity, the Contribution of Nutritional Status to Immune Activation in HIV Infection**

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Abstract

The impact of HIV infection on innate and adaptive immune activation occurs in the context of host factors which serve to augment or dampen the physiologic response to the virus. Nutritional status, and in particular body composition, affects innate immune activation through a range of conditions including the loss of mucosal barrier protections and microbiome dysbiosis in malnutrition to the pro-inflammatory contribution of adipocytes and stromal vascular cells in obesity. Similarly, T cell activation, proliferation, and cytokine expression are reduced in the setting of malnutrition and increased in obesity, potentially due to adipokine regulatory mechanisms restraining energy-avid adaptive immunity in times of starvation and exerting a paradoxical effect in overnutrition. The response to HIV infection is situated within these complex interactions between host nutritional health and immunologic function, which contribute to the varied phenotypes of immune activation among HIV patients across a spectrum from malnutrition to obesity.

Key words: HIV, malnutrition, adipose tissue, obesity, inflammation, immune activation

Introduction

Following the introduction of effective antiretroviral therapy (ART) in resource-rich, developed countries, the incidence of HIV-associated wasting in advanced disease has declined while the proportion of overweight and obese HIV-infected individuals on long-term treatment has steadily risen [1, 2]. In contrast, due to the geographic overlap of high HIV prevalence and chronic food insecurity, new infections frequently occur against a backdrop of chronically insufficient macronutrient intake (hereafter referred to as malnutrition) [3]. Host nutritional status affects innate immune activation through a variety of mechanisms from altered mucosal barrier defenses and microbiome in malnutrition to pro-inflammatory cytokine expression by stromal vascular cells and hypertrophied adipocytes in obesity. Similarly, nutritional status modulates T cell activation, proliferation, and function, in part via endocrine mechanisms thought to act on T cell surface receptors. Here, we review the interaction of nutrition and the immune response to HIV across the spectrum of nutritional status ranging from malnutrition to obesity (summarized in the Figure).

Part 1: HIV and Malnutrition in Resource-Rich and Resource-Limited Contexts

The young, emaciated patient with advanced AIDS is an enduring image of the early HIV epidemic, and can unfortunately still be found with alarming frequency in many resource-limited settings where HIV testing and treatment have not become universally available or accepted. However, a low body mass index (BMI, a marker of generalized malnutrition) in the setting of HIV infection should be divided into two frequently overlapping phenotypes. The first, cachexia, is a wasting phenotype characterized by a dangerous cycle involving profound loss of adaptive immune system protection (i.e., CD4+ T cell depletion), increased basal metabolic rate (due in part to a persistent inflammatory response), and increased protein catabolism with accelerates the loss of lean body mass [4-10]. The second phenotype arises from the simultaneous presence of clinical malnutrition due to insufficient caloric intake and

concomitant HIV infection in varying stages of immunosuppression. Global surveys estimate that over 800 million individuals have chronically insufficient caloric intake, with the highest prevalence in sub-Saharan Africa and Southern Asia [11]. The prevalence of low BMI can be substantial in African HIV patient populations; in a study of HIV-infected adults at clinics across Lusaka, the capitol of Zambia, one-third were malnourished (BMI <18.5 kg/m^2) at the time of ART initiation [12]. Frequently these phenotypes overlap. In resource-rich settings progressive weight loss with untreated HIV leads to low BMI and its associated organ system dysfunction and immune deficits, while in resource-limited settings the immune deficits accompanying a low BMI are exacerbated by the acquisition of HIV infection.

Malnutrition, enteropathy and microbial translocation

The combined effects of environmental factors, nutrient deficits, and HIV infection on gastrointestinal mucosal barrier defenses and microbiome composition (discussed below) contribute to increased translocation of microbes and microbial proteins into the bowel wall and circulation in malnourished, HIV-infected individuals [13-16]. Microbial translocation, as measured by circulating lipopolysaccharide (LPS; a component of the bacterial cell wall), anti-endotoxin IgM and IgG antibodies, soluble CD14, and other biomarkers is associated with accelerated HIV disease progression and a higher risk of mortality in untreated HIV infection [17, 18], though the prognostic value of these biomarkers is less clear after ART initiation [19, 20]. The loss of barrier defenses against microbial translocation in HIV infection also has consequences for adaptive immune activation. In Italian HIV patients, serum LPS levels predicted disease progression independently of age, CD4+ T-cell count, viral load, or duration of infection, and higher circulating LPS levels after ART initiation were associated with greater CD4+ and CD8+ T cell activation and poor CD4+ T cell recovery [17, 21].

Malnutrition enteropathy is characterized by bowel wall edema, reduced nutrient absorption and bowel transit time, reduced secretory IgA production, and changes in mucosal surface morphology resulting in villous blunting, increased permeability, and local inflammation [22, 23]. Environmental enteropathy, thought to result from a combination of recurrent, transient infections with pathogenic bacteria and altered intestinal microbiota, is common in tropical regions with poor sanitation and is also characterized by villous blunting, reduced nutrient absorption, and accelerated bowel transit [16, 24-26]. Lastly, HIV enteropathy is characterized by mucosal T cell depletion in conjunction with impaired cellular tight junctions between epithelial cells [27-29]. The ensuing inflammatory response produces villous changes similar to malnutrition enteropathy, which reduces nutrient absorption [30, 31]. In resource-limited settings, the gastrointestinal system of malnourished HIV-infected individuals can be affected by all three conditions simultaneously, and treatment of one condition (e.g., with ART initiation) may not reduce inflammation and microbial translocation due to concomitant conditions.

Impaired gastrointestinal mucosal integrity and microbial translocation do not appear to be present during acute HIV infection, and the temporal course of systemic inflammation attributed to microbial translocation does not correspond entirely with markers of mucosal integrity or damage [15, 32]. Despite the initiation of ART and plasma viral suppression, defects in junctional complex expression, the presence of bacterial products in the lamina propria, and reduced IL-17 and IL-22 producing cells persist in treated HIV infection [28, 33], and even the early initiation of ART shortly after infection does not fully normalize gastrointestinal mucosal dysfunction markers [34]. These findings suggest the changes in mucosal integrity accompanying HIV infection involve permanent changes in gastrointestinal cellular function, including the loss of IL-17 and IL-22 producing cells and altered epithelial gene expression, which require time to emerge. While most studies of microbial translocation and innate immune

activation in HIV infection are from developed countries, similar findings are reported from resource-limited settings [13, 14].

Malnutrition, HIV, and the microbiome

The centrality of the human gastrointestinal microbiome to the maintenance of host energy homeostasis and metabolism was recognized decades ago, but more recent evidence points to an important role modulating mucosal and systemic immune activity [35-37]. The human microbiome is composed of an estimated 10^{14} microbes, comprising approximately 1000 species that include archaea, bacteria and eukaryotes, but predominantly constituted by the five bacterial phyla of *Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria* and *Verrucomicrobia* [38]. Quantitation of the relative proportions of each phyla, and more specific taxonomic ranks, have identified consistent phenotypes present in the setting of HIV infection, malnutrition, and states of persistent systemic inflammation and adaptive immune activation.

An altered gastrointestinal microbiome appears to occur early in the course of HIV-infection and may contribute to, or is at least correlated with, mucosal inflammatory activity, mucosal CD4+ T cell depletion, and peripheral CD8+ T cell activation [39-41]. The microbiome alterations, and the accompanying local and systemic immune effects, persist following the early stages of infection and do not revert with ART treatment, possibly due to a persistent presence of HIV virus at the mucosal surface or the lasting depletion of gastrointestinal CD4+ T cells and other immune effectors despite effective suppression of plasma viremia [42, 43].

In a study of rectosigmoid biopsies from HIV-infected subjects not yet on ART, ART-treated subjects, and HIV-negative controls, those with untreated HIV were found to have a marked dysbiosis of mucosal-

adherent bacteria characterized by increased *Proteobacteria* and reduced *Bacteroidetes*, which was accompanied by increased mucosal CD4+ and CD8+ T cell activation, increased circulating CD8+ T cell activation, and, among ART-treated participants, increased circulating IL-6 [44]. In particular, the mucosal community was enriched for *Proteobacteria* genera including *Salmonella, Escherichia*, *Serratia, Shigella,* and *Klebsiella* species, all of which can act as pro-inflammatory pathobionts. A similar shift in gastrointestinal microbiome was seen in a subsequent study of colon biopsies of untreated HIV-infected persons, which found increased *Proteobacteria*, reduced *Firmicutes*, and alterations in the relative composition of the *Bacteroidetes* phyum compared to HIV-negative controls. Furthermore, the HIV-associated changes in *Bacteroidetes* members, primarily an increase in *Prevotella*, were associated with both mucosal and circulating CD4+ and CD8+ T cell activation [45]. Similar associations between microbiome composition and systemic immune activation were observed in the fecal microbiome, including a potentially a potentially beneficial effect of fecal *Lactobacillales* (phylum *Firmicutes*) to promote circulating CD4+ T cell recovery and lower CD8+ T cell activation on ART [46, 47].

The preponderance of studies of HIV-negative, malnutrition-associated microbiome alterations enrolled children rather than adults, but despite this limitation the observed commonalities with HIV-associated gastrointestinal dysbiosis bear consideration. A link between kwashiorkor and a predominance of *Staphylococcus aureus* and coliform bacteria of the phylum *Proteobacteria* in gastric juice and rectal swabs was identified as early as 1958 [48]. Later studies of malnourished children and well-nourished controls in Bangladesh found poor nutritional status was associated with enrichment of *Proteobacteria*, including a 174-fold and nine-fold increase in *Klebsiella* and *Escherichia* respectively, and depletion of *Bacteroidetes* [49]. In Indian children, nutritional status was negatively correlated with the proportion of *Proteobacteria* (including *Escherichia, Shigella,* and *Enterobacter*) and positively correlated with the proportion of anaerobic *Firmicutes* (including *Roseburia, Faecalibacterium,* and *Butyrivibrio*) [50]. This

pattern of enriched *Proteobacteria* and depleted *Bacteroidetes* and *Firmicutes* accompanying malnutrition has also been observed in other case-control pediatric studies [51, 52].

At the phylum level, malnutrition is accompanied by gastrointestinal microbiome alterations similar to those observed in untreated and ART-treated HIV-infected persons. While additional studies are needed to confirm the dysbiosis observed in underweight children is also present in malnourished adults, it seems reasonable to assume that adult malnutrition is accompanied by some degree of enrichment of *Proteobacteria* and a depletion of *Bacteroidetes* and *Firmicutes*. To explore this further, we propose two areas as research priorities: first, to investigate commonalities in mucosal immune dysfunction leading to similar dysbiosis phenotypes in HIV infection and malnutrition; second, to determine the extent to which a high degree of persistent immune activation in malnourished, HIV-infected individuals can be attributed to compounding or synergistic effects of HIV and nutritional factors on the gastrointestinal microbiome.

Food insecurity

Food insecurity, or a lack of consistent access to a sufficient quantity of affordable, nutritious food, is associated with a higher likelihood of viral non-suppression in HIV-infected persons, with resultant effects on disease progression and immune activation [53, 54]. In the United States and Europe, food insecurity is more common among HIV patients with substance abuse, mental illness, and those living in poverty, while in resource-limited settings food insecurity is often endemic in areas with high HIV prevalence [55-57]. Food insecurity, and the frequently attendant economic privations, have adverse effects on clinic attendance, obtaining medication refills, and taking ART at the frequency and dosages prescribed, all of which lead to loss of virologic suppression, increased inflammation and cellular immune activation, and higher likelihood of ART regimen failure and resistance [58-60]. Food assistance

may have a role in incentivizing patients to attend clinic visits and collect medications as scheduled [57, 61, 62].

A second aspect of food insecurity and immune activation is dietary quality, particularly in resource-limited settings where HIV-infected individuals may be reliant on carbohydrate-rich staple foods (e.g., ground maize) with a high glycemic index. A recent systematic review of glycemic index and glycemic load dietary intervention studies suggests high carbohydrate staple foods increase IL-6, CRP, and other inflammation biomarkers [63], which may present an opportunity for properly-constituted food assistance to reduce chronic immune activation in addition to improving clinic attendance and ART adherence.

Malnutrition and T cell function

While there is a paucity of data from HIV-infected individuals, malnutrition is associated with broad suppression of antigen-specific immunity, including reduced T cell output, maturation, proliferation, and cytokine expression. The preponderance of these studies, by far, are in children or adolescents <18 years old and are summarized in a recent systematic review [64]; the findings should be extrapolated to adults with some caution. Compared to the well-nourished, malnutrition is associated with reduced T cell proliferative responses, reduced T cell expression of activation and memory surface markers [65, 66], and greater T_H2 polarization with concomitant decreased T_H1 cell IFN-γ and IL-2 production [66, 67]. Malnutrition is also accompanied by a lower likelihood of skin test conversion after Bacillus Calmette–Guérin vaccination and reduced dermal delayed type hypersensitivity responses to *Candida*, phytohemagglutinin and other common recall antigens [68]. Lastly, while total IgG and other antibody levels were comparable between malnourished and well-nourished subjects in most prior studies, reduced seroconversion rates or antibody titers were reported after typhoid, diphtheria, tetanus,

hepatitis B, measles and other vaccinations in severe malnutrition, though this does not appear to be as uniform a finding for moderate and mild malnutrition [64]. While these deficits likely impair an efficient response to pathogens, it is important to note the changes appear reversible and nutritional rehabilitation of malnourished individuals is associated with an improvement in adaptive lymphocyte proliferative responses, chemotaxis, and cytokine production [69].

Part 2: Adipose Tissue and Immune Activation in Comorbid HIV and Obesity

Adipose tissue represents one of the largest organs in the body and comprises a range of cell types with diverse energy storage, metabolic regulation, neuroendocrine, and immunologic functions. HIV infection and ART treatment cause alterations to adipose tissue distribution and biology with broad effects on cytokine and hormone expression, lipid storage, and the composition of adipose-resident immune cell populations. The resultant changes have important consequences for innate and adaptive immune responses and chronic immune activation.

Obesity prevalence in the HIV population

The proportion of overweight and obese individuals in high- and middle-income countries has increased steadily over the past three decades, affecting all race/ethnicity, sex, and age groups to varying degrees, and more recently obesity rates have increased in low-income countries [70]. More than one-third of adults in the United States are overweight (BMI 25-29.9 kg/m^2) and a similar proportion are obese (BMI >30 kg/m^2) [71]. Obesity is also becoming more prevalent in the HIV population. In an analysis of over 14,000 HIV-infected persons in the United States and Canada, the percentage of patients who were obese at ART initiation increased from 9% to 18% between 1998 and 2010, and 22% of individuals with normal BMI (18.5-25 kg/m^2) at treatment initiation had become overweight after three years of ART, and 18% of those overweight at initiation had become obese. Compared to age-matched National

Health-Nutrition Examination Survey (NHANES) controls from the general population, HIV-infected white women had a higher BMI after 3 years of ART as compared to controls, while no difference in BMI after 3 years of ART was observed for HIV-infected white men and non-white men and women compared to controls [2].

HIV infection alters adipose tissue distribution and metabolic characteristics

Older ART agents, particularly the thymidine analogues zidovudine (AZT) and stavudine (d4T), were associated with a high prevalence (up to 50% in some studies) of peripheral lipoatrophy of the limbs, face, and buttocks; lipohypertrophy of the visceral, cervical, and dorsocervical area (i.e., the "buffalo hump"); or a combination of these changes [72, 73]. The accumulation of ectopic adipose tissue in a variety of organs, particularly epicardial, hepatic, and muscle bundle fat infiltration, contributes to local inflammation and end-organ disease [74-76]. Subcutaneous fat biopsies from individuals with HIV-associated lipoatrophy demonstrate reduced mitochondrial DNA (mtDNA) and structural changes characterized by increased fibrosis, apoptosis, and formulation of lipogranulomas, while the adipocytes demonstrate reduced expression of several transcription factors necessary for cellular differentiation and fatty acid uptake, but higher TNF-α and IL-6 expression [77-81]. Taken together, these findings indicate a shift to a pro-inflammatory, profibrotic, and dysregulated metabolic state within the fat tissue of HIV patients. While the prevalence of lipodystropy has declined with the introduction of newer ART agents, the presence of HIV viral particles and latently HIV-infected, adipose-resident CD4+ T cells within adipose tissue may still contribute to impaired lipid metabolism and storage [82, 83].

Obesity, HIV, and the microbiome

As discussed above in the sections on malnutrition, HIV-infection can be accompanied by a marked dysbiosis of fecal and mucosal-adherent bacteria characterized by increased *Proteobacteria* and reduced

Bacteroidetes and *Firmicutes*, and these changes are associated with mucosal T cell activation, circulating T cell activation, and serum markers of innate immune activation [44, 45]. Independent of HIV infection, obesity is accompanied by characteristic changes in the gastrointestinal microbiome characterized by lower levels of *Bacteroidetes* and proportionately higher levels of *Firmicutes* in several studies [84-86], which are postulated to enhance dietary nutrient absorption [87]. In animal models, stool characterized by this phylum-level shift was shown to 'transmit' obesity when inoculated into lean animals [84], suggesting that alterations of the gastrointestinal microbiome by conditions such as HIV infection could alter energy uptake and the metabolic balance. Colon biopsies of untreated, HIV-infected persons show reduced *Firmicutes* but little change in *Bacteroidetes* at the phylum level compared to HIV-negative controls (however, the relative composition of *Bacteroidetes* at the genus level did shift) [45]. Based on prior animal and human studies, this alteration in the *Bacteroidetes* : *Firmicutes* ratio in untreated HIV would appear to be protective against obesity. However, many patients gain weight after ART initiation, particularly in the first 12 months, and the potential contribution of microbiome changes after ART initiation to weight gain is one area for further study [2].

Obesity is associated with increased serum inflammatory markers in HIV-infected persons

As observed in the general population, serum levels of CRP are higher among HIV-infected adults with greater adiposity [88-91]. In the Fat Redistribution and Metabolic Change in HIV Infection (FRAM) cohort, each twofold increase in visceral adipose tissue was associated with 17% higher serum CRP, while a similar increase in subcutaneous adipose tissue was associated with 21% higher levels [88]. Circulating levels of IL-6, TNF-α receptor 1, and macrophage inflammatory protein-1α also rise in proportion to fat mass in HIV-infected persons, likely due to greater expression from stromal vascular cells and hypertrophied adipocytes [91, 92]. The enlargement of adipose tissue depots is primarily due to adipocyte hypertrophy, rather than hyperplasia, and increases in adipocyte size result in

disproportionate increases in IL-6 and TNF-α expression [93-95]. It is estimated that adipose tissue-derived IL-6 constitutes up to 35% of circulating levels in obese individuals and is a substantial contributor to CRP production [96]. This raises the question of whether the reported association between CRP or IL-6 levels and adverse health outcomes in studies of predominantly non-obese populations should be extrapolated to obese HIV-infected individuals, as in the obese a higher proportion of these biomarkers may emanate from adipose tissue as opposed to other sites of inflammation [97-99].

Obesity and adipose tissue immune cell profiles

Immune cell infiltration of adipose tissue accompanies progressive weight gain and contributes to both *in situ* and systemic inflammation. Adipose tissue from obese humans and animal models shows a striking increase in CD8+ T cells and T_H1 and T_H17-polarized CD4+ cells, a decrease in T regulatory cells, and an increase in M1-phenotype (TNF-α, IL-12, IL-23-producing) pro-inflammatory macrophages [100-103]. CD8+ T cell infiltration into adipose tissue is an early and necessary step preceding M1-phenotype macrophage recruitment in mice, and antibody-induced CD8+ T cell depletion results in reduced M1-phenotype macrophage adipose tissue infiltration [100]. Adipocyte hypertrophy is associated with increased production of macrophage chemotactic protein-1 and macrophage inflammatory protein-1α, which promote macrophage infiltration, and increased IL-8, which promotes neutrophil chemotaxis [104-106].

Recent studies highlight an important role for T_H17 cells, a subset of CD4+ effector T cells defined by their production of IL-17, in promoting adipose tissue inflammation and metabolic disease [107, 108]. T_H17 cells are central contributors to the maintenance of mucosal barriers, pathogen clearance at the mucosal surface, and the defense against fungi and extracellular bacteria [109, 110], but loss or

dysregulation of T_H17 cells is also implicated in the pathogenesis of autoimmune and inflammatory conditions [111]. Adipose tissue CD4+ T cells in obese, insulin resistant persons are skewed toward a T_H17 phenotype, and the tissue microenvironment is characterized by high levels of T_H17-promoting IL-1β and IL-6 in addition to the T_H17 markers RORC, IL-17, and IL-23R [107, 112]. M1-phenotype macrophage cytokine expression promotes a cycle of progressive T_H17-polarization and inflammation, with IL-1β and IL-6 promoting the differentiation of T_H17 cells and IL-23 promoting their stabilization and expansion [113, 114]. While circulating IL-17 levels are frequently low or undetectable, *in vitro* IL-17 inhibits skeletal muscle glucose uptake and hepatocyte insulin sensitivity [107]. A recent study describes the role of ATP leakage into the extracellular space, a hallmark of pathologic cellular conditions such as apoptosis, inflammation, or ischemia, in promoting a T_H17-polarizing milieu [115]. The addition of ATP to visceral adipose tissue from metabolically healthy lean subjects enriched the tissue microenvironment for IL-1β, IL-6, and IL-17, and higher CD4+ T cell expression of a characteristic T_H17 cytokine signature [112]. These studies suggest a central role for T_H17 CD4+ cells in propagating adipose tissue inflammation, and further studies are needed to understand whether HIV status alters the distribution and activity of adipose tissue T_H17-polarized cells in obesity.

Adipose tissue also serves as a reservoir of CD4+ T cells harboring latent HIV infection. A recent study found a higher percentage of activated CD4+ and CD8+ T cells in adipose tissue from HIV-infected subjects compared to HIV-negative controls, in addition to the unique presence of latently HIV-infected memory CD4+ T cells [82, 116]. Furthermore, the median copy number of latent HIV DNA in subcutaneous adipose tissue CD4+ T cells was slightly higher than the median copy number in circulating CD4+ T cells, indicating adipose tissue serves as a significant reservoir for latent HIV infection [116]. Similar findings regarding a higher proportion of activated CD8+ and CD4+ T cells, and latently infected

memory CD4+ T cells, in both subcutaneous and visceral adipose tissue have been reported in simian immunodeficiency virus-infected macaques compared to uninfected animals [116].

Obesity and circulating T cell profiles in HIV-infected and HIV-negative persons

Studies from the pre-ART era found a higher BMI was associated with slower disease progression and CD4+ T cell decline [117-119]. However, it is unclear whether the delayed immunosuppression observed among high BMI individuals was due to an effect of greater adiposity versus other factors such as fewer secondary infections or micronutrient deficiencies. Recent studies in the combination ART era found a higher BMI may promote more robust CD4+ T cell recovery on treatment [120, 121]. An analysis of over 14,000 HIV-infected adults in 13 multi-site cohorts found a higher time-updated BMI was significantly associated with greater CD4+ cell count recovery on ART [122]. After 5 years of ART, the mean CD4+ cell count for a hypothetical patient with a BMI of 30 kg/m^2 was 20% higher compared to a patient with a BMI of 22 kg/m^2 (524 vs. 436 cells/μL), and 31% higher for a BMI of 40 kg/m^2 compared to 22 kg/m^2 (572 vs. 436 cells/μL).

A minimum quantity of adipose tissue appears necessary to maintain normal-range lymphocyte subset counts, but assessing the relationship between adiposity and peripheral T cell populations in the setting of HIV infection is confounded by CD4+ T cell depletion, variations in immune recovery on ART, and the effects of HIV-related immune activation. Thus, studies of HIV-negative individuals may be revealing in this area. Overweight and obese HIV-negative women had higher CD4+ and total lymphocyte counts compared to normal weight women in one study [123], while the expression of activation marker CD25 on CD3+ T cells was 3-fold higher in obese subjects compared to non-obese, and the ratio of T_H1 to T_H2 CD4+ lymphocytes was also significantly higher, in another study [124]. Similarly, an analysis of the European CODAM cohort of HIV-negative individuals found greater waist circumference was associated

with higher circulating markers of adaptive immune activation (neopterin and soluble CD25) [125]. Taken together, these data suggest that, irrespective of HIV infection, higher fat stores are associated with higher circulating CD4 T cell populations, greater T_H1 polarization, and expression of surface markers of immune activation.

Adipose tissue hormones alter lymphocyte function

Adipokines are hormones produced by adipocytes which demonstrate a range of metabolic, neuroendocrine, and immunomodulatory properties. Leptin, an adipokine encoded by the *ob* gene and produced roughly in proportion to fat cell mass, was initially characterized as a regulator of appetite but also appears to have a range of local and potentially systemic immune effects [126-128]. Leptin independently induces expression of pro-inflammatory cytokines by macrophages and monocytes [129, 130], and acts directly on hepatocytes to promote CRP expression [131]. Mature CD4+ T cells express the long isoform of the leptin receptor [132, 133], and leptin stimulates T cell proliferative responses *in vitro*, polarizes naïve CD4+ T cell proliferation towards the T_H1 phenotype, and promotes a marked increase in IFN-γ and other T_H1-type cytokines [133-137]. Leptin also enhances *in vitro* expression of activation markers (CD69, CD25, and CD71) on both CD4+ and CD8+ T cells after antigen stimulation in a dose-dependent manner [136, 138]. While the administration of physiologic quantities of recombinant leptin to non-HIV-infected adults with acquired or congenital lipodystrophy increased peripheral CD4+ and CD8+ cell counts, two small trials in HIV-infected individuals have not shown a benefit to CD4+ cell recovery on ART [139-142].

Therapeutic trials to reduce adiposity and immune activation in HIV-infected individuals

Trials of exercise and lifestyle modification have shown reductions in serum CRP, weight loss, and improved cardiorespiratory fitness in HIV persons, though benefits for insulin sensitivity and fasting

glucose are less clear [143-146]. In morbidly obese HIV-infected persons, bariatric surgery appears to be safe and does not affect viral suppression [147, 148].

The accumulation of visceral fat in HIV-infected individuals is accompanied by reductions in endogenous circulating and stimulated growth hormone (GH) levels, a finding also observed in HIV-negative persons with abdominal obesity and independent of age, BMI, and total body fat [149-151]. Inadequate GH levels are associated with reduced bone mineralization, dyslipidemia (characterized by elevated triglycerides and low HDL), elevated blood pressure, reduced vascular health, higher circulating CRP, and a detrimental cycle of further accumulation of visceral adiposity with concomitant progressive reductions in GH secretion [152-155]. Among HIV-infected persons, lower peak levels of GH are associated with higher CRP levels, in addition to higher fasting glucose levels and triglycerides independent of waist circumference [156].

Studies of GH replacement in persons with hypopituitarism demonstrated reductions in visceral adiposity and inflammation, and improved lipid parameters and markers of vascular health, which suggested possible benefits for HIV-infected persons with abdominal obesity [153, 157-159]. However, while trials of recombinant human growth hormone (rhGH) in obese, HIV-infected persons have shown reductions in visceral adipose tissue and hepatic fat [160-162], these benefits must be weighed against the increased insulin resistance observed with rhGH treatment [161-164]. Furthermore, the beneficial effects of GH supplementation on innate immune activation in persons with hypopituitarism are not as evident in HIV-infected individuals. A multi-arm study of rhGH, rosiglitazone, combination rhGH and rosiglitazone, and placebo found no significant difference in a range of serum inflammation biomarkers, including CRP, IL-1, IL-6, TNF-α, and interferon gamma, between study arms after 12 weeks of treatment [160].

Tesamorelin, a synthetic form of growth-hormone-releasing hormone (GHRH), is a FDA-approved treatment to reduce abdominal fat in HIV-infected patients with lipodystrophy. Trials of Tesamorelin demonstrate visceral and hepatic fat reductions, gains in lean body mass, and improved lipid profiles, but without the increase in insulin resistance which limited the clinical utility of rhGH [165-167]. However, despite substantial reductions in visceral fat with Tesamorelin, it is notable that a 26-week randomized trial did not demonstrate a significant effect on CRP levels, and more studies are needed to characterize the effects of Tesamorelin on innate and cellular immune activation [168].

Conclusion

Persistent, chronic innate and adaptive immune activation have been implicated in the pathogenesis of multiple comorbidities in HIV patients and impaired immune recovery on ART. While the etiology of this heightened immune activation is multifactorial, the immunologic effects of HIV infection can be amplified and modulated by host nutritional factors. At the intersection of these nutritional and immunologic processes an opportunity may be present for interventions to mitigate the adverse effects of both malnutrition and obesity on chronic immune activation and improve health outcomes in HIV-infected individuals.

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Table: Summary Points on Nutrition and Immune Activation

- Enteropathy due to a confluence of environmental factors, nutrition deficits, and viral effects impairs mucosal barrier integrity and immune defenses, and contributes to both innate and cellular immune activation in malnourished HIV-infected persons.
- A gastrointestinal dysbiosis, characterized by increased *Proteobacteria* and reduced or altered *Bacteroidetes* and *Firmicutes,* is present in HIV patients, and these changes are accompanied by increased mucosal and circulating T cell activation and systemic inflammation. Similar phylum-level changes occur in malnutrition, but the microbiome consequences of comorbid HIV infection and malnutrition are unknown.
- Malnutrition is associated with reduced T cell proliferative responses, reduced T cell expression of activation and memory surface markers, greater type-2 T helper cell (T_H2) polarization, and decreased T_H1 cell interferon-γ and interleukin-2 production, which compound HIV-related immunodeficiency and impair clearance or control of secondary infections.
- Adipocytes constitutively express interleukin-6, tumor necrosis factor-α, and other cytokines, and obese HIV-infected persons have substantially higher circulating levels of inflammation biomarkers. Because these cytokines derive from adipocytes as opposed to other tissues (e.g., blood vessels), obesity may confound previously reported associations between inflammation and health outcomes in HIV-infected persons.
- A higher BMI is associated with more robust CD4+ recovery on antiretroviral therapy, and obesity in is associated with higher circulating T cell counts, increased T cell activation, and CD4+ cell T_H1 polarization in studies of HIV-negative individuals.

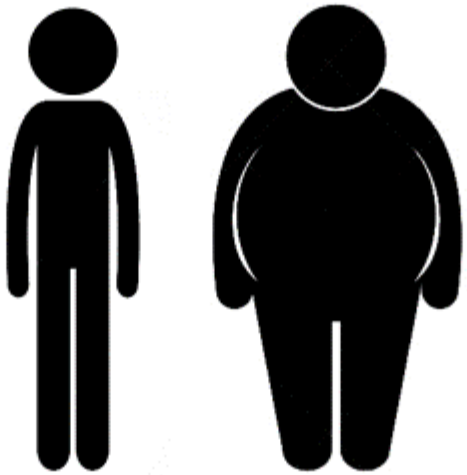
- CD4+ T cells express a receptor for leptin, an adipokine produced by adipocytes, which may have an endocrine function modulating T cell proliferation, activation, and T-helper cell polarization in states of both malnutrition and obesity.
- Clinical trials of growth-hormone-releasing hormone (GHRH) have shown a beneficial effect for reducing visceral and hepatic fat without the added insulin resistance observed in studies of recombinant growth hormone. However, the effect of GHRH on innate and cellular immune activation is still unclear.

MaInutrition and Obesity-related Factors Potentially Affecting Chronic Immune Activation in HIV Infection

Innate immunity:
- Reduced GI mucosal integrity
- Higher microbial translocation
- Villous blunting and local inflammation
- Lower secretory IgA
- Lower eosinophils and NK cells

Adaptive immunity:
- Lower total lymphocytes
- Reduced T cell proliferative response
- Higher T_H2-type CD4+ cell polarization
- Lower T_H1 cell IL-2 and INF-γ expression
- Impaired delayed hypersensitivity response

Potential interventions:
- Food assistance / macronutrient supplements
- Livelihood support / cash transfers
- Clean water & sanitation programs to reduce environmental pathogens
- Expanded HIV testing and earlier treatment



Innate immunity:
- Higher circulating IL-6 and other cytokines produced by adipocytes
- M1 inflammatory macrophage and T_H17 CD4+ T cell polarization in adipose tissue
- Leptin (adipokine) promotes macrophage TNF-α, IL-6 and IL-12 expression

Adaptive immunity:
- More robust CD4+ cell recovery on antiretroviral therapy at higher BMI
- Increased peripheral T cells, T cell activation, and T_H1-type CD4+ cell polarization
- Leptin (an adipokine produced by adipocytes) promotes CD4+ T cell proliferation and T_H1 polarization *in vitro*

Potential Interventions:
- Weight loss and exercise programs
- Gastric bypass
- Growth-hormone-releasing hormone (Tesamorelin)