

Is Adipose Tissue a Reservoir for Viral Spread, Immune Activation, and Cytokine Amplification in Coronavirus Disease 2019?

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Coronavirus disease 2019 (COVID-19), the worst pandemic in more than a century, has claimed >125,000 lives worldwide to date. Emerging predictors for poor outcomes include advanced age, male sex, preexisting cardiovascular disease, and risk factors including hypertension, diabetes, and, more recently, obesity. This article posits new obesity-driven predictors of poor COVID-19 outcomes, over and above the more obvious extant risks associated with obesity, including cardiometabolic disease and hypoventilation syndrome in intensive care patients. This article also outlines a theoretical mechanistic framework whereby adipose tissue in individuals with obesity may act as a reservoir for more extensive viral spread, with increased shedding, immune activation, and cytokine amplification. This paper proposes studies to test this reservoir concept with a focus on specific cytokine pathways that might be amplified in individuals with obesity and COVID-19. Finally, this paper underscores emerging therapeutic strategies that might benefit subsets of patients in which cytokine amplification is excessive and potentially fatal.

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Introduction

Coronavirus disease 2019 (COVID-19) has now infected over 2 million people worldwide, with a death toll of more than 125,000 people. Emerging risk factors for poor outcomes in this disease include age, male sex, and cardiovascular comorbidities, including hypertension, prior cardiovascular disease, diabetes, and, more recently, obesity (1). The Centers for Disease Control and Prevention (Atlanta, Georgia) has now reported a threefold increase in death in New Orleans compared with New York, and speculation has grown as to whether these worrying mortality statistics might in part be attributable to higher levels of morbid obesity (2). In this paper, we develop a theoretical framework that describes why individuals with obesity may be at increased risk of poor outcomes compared with counterparts without obesity (3). We propose a mechanism for adverse consequences of virus seeding to adipose tissue (AT), with potential for prolonged viral shedding and extended cytokine activation in a voluminous and richly vascularized organ that is already perturbed in a metabolic and inflammatory sense in human individuals with obesity (4). We present a rationale for testing this concept in patients with COVID-19 through prospective studies of individuals with and without obesity with accessible AT and plasma to determine whether or not an inflammatory and cytokine signature presages a systemic cytokine storm and clinical decline.

Evidence for Association of Obesity with Worse COVID-19 Outcome

Obesity was not specifically reported in the initial cohort studies of COVID-19 from Wuhan, China (5), but regional epidemiological data

from the United States suggests that at least 25% of patients who die of this disease have obesity, which is similar to reported rates of cardiovascular disease in the same high-risk group (21%) (6). More recently, a small retrospective study of 85 individuals with COVID-19 identified obesity as a risk factor for admission to the intensive care unit, with patients requiring increased medical attention (3). Moreover, in the influenza A subtype H1N1 pandemic, obesity was also strongly associated with a worse disease outcome and death (7). Together, these data raise the questions of whether there is a mechanistic link between obesity and disease survival and whether obesity over and above its endocrine or cardiometabolic associations might independently contribute to COVID-19 risk.

Likely Mechanisms Involved in Poor Outcomes in Individuals with Obesity

It is clear that obesity could contribute to both diabetic and cardiovascular COVID-19 risk, and these elements of risk, in addition to thrombosis more recently, have been well described in the scientific literature (8-12). Moreover, obesity is an independent risk factor for hypoventilation syndrome in patients in the intensive care unit (13) and could thus contribute to respiratory failure in patients with acute respiratory distress syndrome (14). Here, we propose additional unheralded pathophysiologic aspects of increased AT burden in morbid obesity that may amplify the pro-inflammatory response to extensive viral infection. AT should be viewed as a highly active organ interfacing immune, endocrine, and metabolic homeostasis throughout the body (15). In individuals with obesity, there is marked

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dysregulation of myeloid and lymphoid responses within AT, with associated dysregulation of cytokine profiles (15). Intrinsically bound to this are endocrine and metabolic derangements, including insulin resistance and adipokine dysregulation with dysfunctional lipid and fatty acid metabolism (16). In highly vascularized AT, endothelial and smooth-muscle cells, as well as resident macrophages, exhibit additional perturbations in response to an activated renin angiotensin system at a local level, with attendant depletion and dysfunction of the counterregulatory angiotensin converting enzyme 2 Mas receptor system (17,18). This makes AT, particularly in visceral distributions, pro-immunogenic, metabolically active, and highly integrated into the cardiovascular system, with the capability to drive acute disease through augmented inflammation at an organ level in the heart, vasculature, pancreas, liver, and kidneys (19). This “preactivation state” of AT in obesity makes this organ a potential target for further immune amplification by external pathogens such as viruses.

Viral Spread to AT and Potential for Activation of Resident Inflammation and Cytokine Pathways

Currently, there is no evidence for direct severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection of AT, although angiotensin converting enzyme 2 receptor expression represents a basis for viral tropism in several cells within this tissue (20), including adipocytes, smooth-muscle cells, and endothelial cells (21). Moreover, many AT-resident cells are proven targets for multiple viruses, including adipocytes [H1N1, type A influenza, and adenovirus 36 (7,22,23)], adipo-stromal cells [adenovirus 36 (24), cytomegalovirus (25)], endothelial cells [SARS-CoV (26)], macrophages [influenza A, SARS-CoV, adenovirus36, human immunodeficiency virus (26,27)], and lymphocytes [SARS-CoV, human immunodeficiency virus (25,26)]. Although SARS-CoV-2 was detected only at low levels in blood in a small human study (28), we cannot exclude hematogenous spread to AT, given the very high virus affinity for its target cell receptor. Alternative routes of SARS-CoV-2 spread to AT include local egress of the virus from organs known to be infected to adjacent visceral fat deposits, such as intrathoracic fat (lungs), epicardial fat (heart), perirenal fat (kidneys), and mesenteric fat (intestines). Finally, shared viral tropism for lung epithelium and AT has already been shown for H5N1 virus infection (29), and AT significantly prolongs the duration of viral shedding in humans with obesity infected with influenza (22). Were similar tropism of SARS-CoV-2 to occur within AT of individuals with COVID-19 and obesity, there exists the potential for prolonged viral shedding in this organ, with extended activation of local “preactivated” immune systems and resident cytokine signaling pathways.

Resident myeloid and lymphoid cells are plentiful in AT, and obesity is associated with macrophage (30) and lymphocyte dysfunction (31). Expansion of distinct memory T lymphocytes within AT can also activate aberrant immune responses with wider tissue damage on viral challenge (31). A recent report from Wuhan suggests that SARS-CoV-2 induces a dysregulated immune response in severely ill individuals with COVID-19 (32), characterized by reduced numbers of circulating memory T lymphocytes, as well as reduced helper/suppressor and regulatory T-cell subtypes. It is tempting to speculate whether already dysfunctional immune responses in individuals with obesity may accentuate this SARS-CoV-2 effect on T-cell function.

Specific inflammatory cytokine programs such tumor necrosis factor (TNF)- α , interleukin (IL)-1, and IL-6 are known to be preactivated in AT in the context of obesity (33), and, thus, viral infection may similarly amplify the already primed organ cytokine response in AT. The cytokine storm identified in multiple respiratory viral infections, including COVID-19, exhibits diverse interferon, IL, chemokine, TNF, and colony-stimulating factor responses, which are beyond the scope of this paper but are comprehensively reviewed elsewhere (34). The intensity of inflammatory lung responses reflect the imbalance between pro-inflammatory cytokines (such as TNF and $IL1\beta$) and their soluble cognate receptors that inhibit cytokine effects in aqueous phase (35). IL-10 produced by macrophages and T lymphocytes (T helper 2 and regulatory T cells) acts as a negative regulator of inflammation, whereas IL-6 and its soluble receptor enhance activity of IL-6 on target cells, providing a mechanism for enhancement of TNF and $IL-1\beta$ activity when these soluble cognate receptors are particularly high (35). Thus, a balance between pro- and anti-inflammatory mechanisms is critical in maintaining lung-tissue homeostasis. It is conceivable that if one or more of these regulatory elements were absent or dysfunctional, then it might contribute to a cytokine storm in the lung or in other tissues such as AT, where aberrant cytokine activation exists. Temporal studies of cytokine dynamics in human “cytokine storm” models show that IL-6 sustains activation of multiple cytokine pathways for many days after the initial immune insult (36). Interestingly, in early COVID-19 studies, IL-6 was a strong independent predictor of mortality (37). Human AT is a major source of IL-6 and its receptor IL-6R (38), and, thus, AT may provide a reservoir for IL-6 activation and cascade signaling in viral infection (Figure 1). Viral spread from affected organs to adjacent AT may take days, with subsequent prolonged viral shedding contributing to the delayed cytokine storm and consequences for tissue injury in patients with COVID-19.

Testing AT Inflammatory Cytokine Reservoir Concept in COVID-19

Initial studies should aim to detect SARS-CoV-2 in AT on autopsy of individuals who have died of COVID-19. Focus should be on analysis of specific cells that have evidence of infection by immunocytotoxic and *in situ* viral detection techniques. Parallel studies should identify whether specific cell types in AT can support SARS-CoV-2 infection and replication *ex vivo*. With respect to the cytokine storm, an integrated-systems biology approach would enable multiple pathways to be assessed simultaneously. In this regard, cytokine and chemokine genomic data analysis in blood and AT would be an important first step. Moreover a weighted gene correlation network analysis of SARS-CoV-2-mediated transcriptional response in infected cells could also be used as a model for human AT analysis downstream (39). Interesting aspects of transcriptional network analysis could then be tested in appropriate animal models to determine pivotal components of the cytokine storm, including key cytokine and chemokine genes that are conserved across species (40). These insights may allow rational diagnostics and therapeutic strategies to be developed. In line with this, IL-6 inhibition has already been proposed as a treatment in COVID-19, and the results of trials of tocilizumab are awaited (41). It would be interesting to examine whether individuals with obesity, who are expected to have higher circulating IL-6 levels compared with lean counterparts, respond more favorably to IL-6 inhibition strategies in COVID-19 in a post hoc analysis of

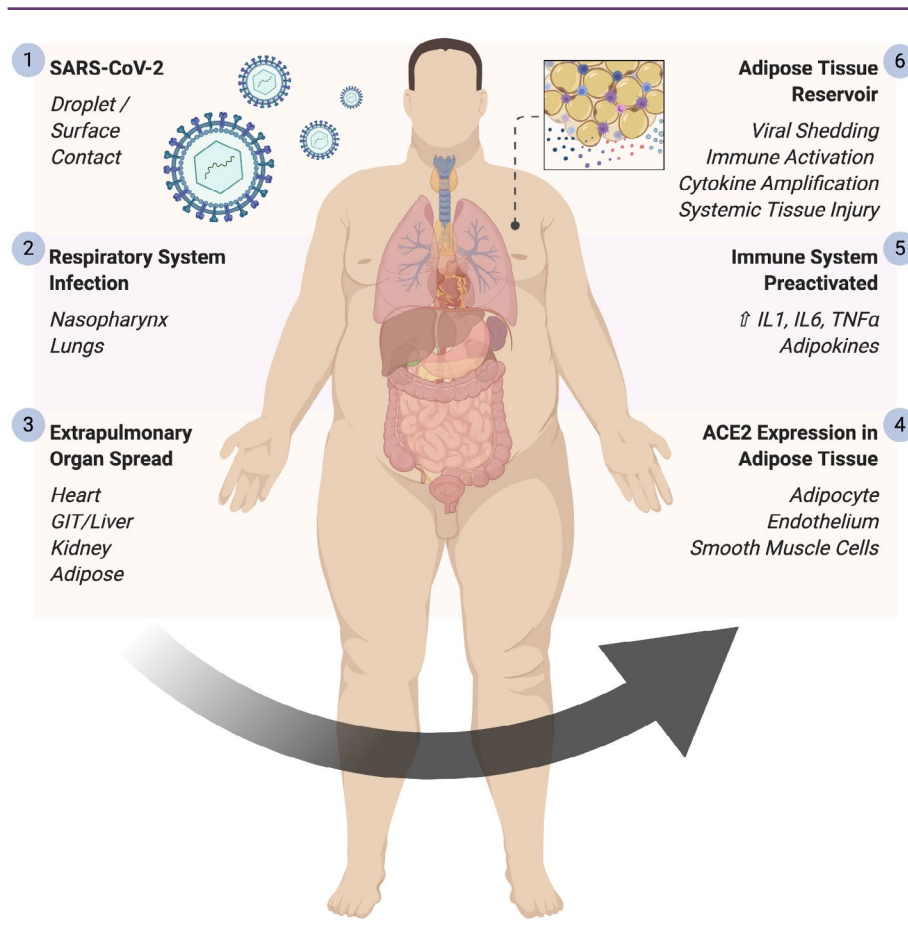


Figure 1 Adipose tissue as a reservoir for SARS-CoV-2 spread, viral shedding, immune activation, and cytokine amplification. Schematic demonstrating the proposed centrality of adipose tissue in the dissemination of SARS-CoV-2 and the ensuing systemic immune activation. Created with Biorender.com. GIT, gastrointestinal tract; IL, interleukin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TNF, tumor necrosis factor.

this randomized controlled trial. Similarly, tissue and systemic analysis of cytokine dynamics may identify likely responders and nonresponders to such therapy.

In summary, we present a rationale for studying the relationship between obesity and COVID-19 disease severity. We provide a theoretical framework whereby viral systemic spread, entry, and prolonged viral shedding in already “inflamed” AT may augment immune responses with consequences for cytokine cascade amplification. We highlight AT as an abundant source for local and systemic enrichment of cytokines, some already independently associated with increased COVID-19 mortality. Finally, we suggest a series of research studies to identify whether a mechanistic link exists among AT, SARS-CoV-2 infection, organ seeding of infection, immune activation, and the delayed cytokine storm known to presage rapid clinical decline in high-risk patients with COVID-19. **O**

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