Countering the Effects of Chronic Inflammation in Obesity

Nikhil V. Dhurandhar*
Pennington Biomedical Research Center, Louisiana State University System, LA, USA

Obesity is linked with a chronic low grade inflammation of non-infectious origin, typically marked by a preponderance of inflammatory cytokines, and a reduced presence of anti-inflammatory cytokines in adipose tissue or circulation. Inflammation in obesity is strongly linked with insulin resistance. However, approaches such as weight loss or anti-inflammatory therapy have not been adequate in countering insulin resistance linked with inflammation and obesity. Hence, a better understanding of the origins of obesity-associated inflammation is needed to design more effective approaches. Also, novel approaches that can improve insulin resistance independent of body weight or inflammation may be highly desirable. These two aspects are briefly described here. It is postulated that a preponderance of pro- and anti-inflammatory cytokines associated with body adiposity may in fact be a mechanism to maintain body fat stores within a desirable range. Excessive fat accumulation may derail this regulation, leading to a chronic state of inflammation associated with obesity and diabetes. Recognizing this underlying physiological basis for inflammation may help in understanding why anti-inflammatory therapy has not achieved significant improvement in insulin sensitivity. Finally, a novel strategy to potentially improve glycemic control is discussed. This strategy relates to a template offered by Ad36, a human adenovirus, and its E4orf1 protein, which can enhance glucose disposal even in the presence of inflammation and obesity, and without weight loss. Overall, a different perspective about the origin of chronic inflammation, and a novel treatment strategy may help in countering the effects of chronic inflammation in obesity.

Key words: Insulin resistance, Insulin sensitivity, Glycemic control, Metabolic syndrome, Adiposity, Adenovirus Ad36, E4orf1

BACKGROUND

Research in the past two decades has firmly established that obesity is linked with a chronic low grade inflammation of non-infectious origin.1,2 Typically, such an inflammatory state is marked by a preponderance of inflammatory cytokines such as Tumor necrosis factor alpha (TNF alpha), C-reactive protein (CRP), Interleukine (IL) 6, and a reduced presence of anti-inflammatory cytokines such as adiponectin or IL10 in adipose tissue or circulation. Initially recognized as adipose tissue infiltration by activated macrophages3, which promote the release of inflammatory cytokines, the involvement of other tissues such as brain, liver and heart, and cell types such as lymphocytes, natural killer cells is now reported.4 Nonetheless, adipose tissue has remained a primary focus of research to understand obesity-related inflammation.4 In fact, inflammation in obesity is strongly linked with insulin resistance. Consequentially, considerable research is underway to better understand the upstream contributors of such inflammation, in the hope of designing strategies to ameliorate insulin resistance.

Weight loss can ameliorate obesity, and obesity-related inflammation and insulin resistance.5,6 However, achieving a meaningful and sustained weight loss is highly challenging for most individuals. Many high visibility weight loss trials involving lifestyle changes show that majority of the participants could not lose even 5% of the body weight, and a long term average weight loss to be less than 5% of the starting body weight.7 While a few available obesity drugs and bariatric surgery procedures may produce greater and more sustained weight loss, these approaches are not yet widespread, due to various reasons, including potential (and sometimes exaggerated) concern for adverse effects, high treatment costs, and limited insur-

Corresponding author Nikhil V. Dhurandhar
John Henry Hernandez Endowed Professor in Health Promotion, Infection and Obesity Laboratory, Pennington Biomedical Research Center, 6400 Perkins Road, Baton Rouge, LA 70808, USA
Tel +1-225-763-2741 Fax +1-225-763-3030 E-mail Nikhil.Dhurandhar@PBRC.EDU

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ance coverage. Overall, although sound in theory, weight loss is not a realistic approach for many individuals to reduce inflammation and improve insulin sensitivity.

Another seemingly obvious approach to improve inflammation-related insulin resistance is to use anti-inflammatory agents to counter inflammation. Proof of concept studies that transgenically reduce or eliminate specific inflammatory cytokines in animal models have successfully improved insulin sensitivity.\(^6,9\) However, outcomes of human trials that used pharmacological agents to reduce inflammation and improve insulin sensitivity have yielded only modest benefits.\(^10\) These clinical trials have used antagonists or antibodies to known inflammatory cytokines, or anti-inflammatory agents such as salicylate. Some trials improved glucose levels, or HbA1c, but did not improve insulin sensitivity in most cases. A careful analyses of a compilation of 22 such studies that used anti-inflammatory agents to improve insulin resistance\(^10\) reveals that although all trials reduced inflammation, only the studies using non-diabetic subjects, but not diabetic individuals, improved insulin sensitivity (unpublished observation). The studies using non-diabetic individuals were mainly focused on using anti-inflammatory agents to improve rheumatoid arthritis. Thus, neither weight loss nor anti-inflammatory therapy significantly helps improve insulin resistance to obese diabetic individuals.

Hence, a better understanding of the origins of obesity-associated inflammation is needed to design more effective approaches. Also, novel approaches that can improve insulin resistance independent of body weight or inflammation may be highly desirable. These two aspects are briefly described below.

1. Origins of obesity-associated inflammation

While the characteristics of obesity-associated inflammation have been described in considerable detail, relatively less information is available about the precise causes of inflammation. Some of the suggested theories are as follows:\(^1\): A) the possibility that nutrients themselves, obtained from food, are a source of inflammation. Therefore, it is suggested that excess of nutrients may lead to excessive stimulation of inflammation. B) Another possibility is that in response to food intake, intestinal permeability increases to allow systemic uptake of nutrients, which also allows the entry of endotoxin (lipopolysaccharide, LPS) secreted by the gut microbes into circulation. LPS has pro-inflammatory ability and chronic excessive food intake may lead to chronic and exacerbated inflammatory response. C) Another theory proposes that excess of nutrients are treated by the immune system as invading microbes in a case of ‘mistaken identity’ triggering off an immune response and inflammation.

While these theories may be vetted out and substantiated in future, a new concept ascribes a physiological role of ‘body fat maintenance’ to obesity-related inflammation. As previously outlined elsewhere\(^10,11\), it is proposed that when adipose tissue expands in response to demands for energy storage, the peripheral tissue, in particular, experiences hypoxia. This hypoxia in adipose tissue signals inflammatory cytokine response, which in turn, promotes angiogenesis and modified extracellular matrix of adipose tissue.\(^12,13\) This collectively increases the blood supply to meet the demand for oxygen of the tissue and facilitates the growth of the tissue. However, the inflammatory response also exerts additional effects, including the induction of insulin resistance, decrease in adipogenesis, and an increase in lipolysis and thermogenesis. Taken together, this appears to be a response of the body to attenuate excess fat gain, in multiple ways.\(^10\) A) Minimizing nutrient influx: by reducing glucose uptake via the induction of insulin resistance, B) Reducing stored fat: by increasing metabolic rate, lipolysis, and reducing adipogenesis. C) Reduction of pro-adipogenic effects: Anti-inflammatory cytokines such as adiponectin can increase insulin sensitivity, adipogenesis and reduce lipolysis, and counter some pro-inflammatory influences. However, along with a preponderance of inflammatory cytokines, obesity is associated with a reduction in anti-inflammatory cytokines, which further reduces pro-adipogenic effects.

A proof of this known interplay of counter-regulatory influences of pro- and anti-inflammatory cytokines was recently demonstrated in an in-vitro experiment that exposed 3T3-L1 preadipocytes to a decreasing concentration of pre-inflammatory cytokine TNF-alpha, and an increasing concentration of anti-inflammatory cytokine adiponectin.\(^12\) As expected, the exposure of cells to pro-inflammatory cytokine reduced glucose uptake, adipogenesis and lipid accumulation, and increased lipolysis. These effects were countered with increasing concentration of adiponectin.

Thus, the greater preponderance of pro-inflammatory cytokine and a suppression of anti-inflammatory cytokines present in obesity appear to attenuate further weight gain. On the other hand, if body fat drops below an optimal level, inflammatory cytokines decline, and pro-adipogenic anti-inflammatory cytokines increase. This ap-
pears to be a mechanism to maintain a minimum level of adiposity. This theory is also supported by animal studies, which show weight gain in response to transgenic attenuation of pro-inflammatory pathways, and a weight loss, if the inflammatory response is enhanced\(^8,14\), (reviewed in\(^{10,11}\)). Similarly, many anti-inflammatory drugs increase body weight in humans (review\(^{20}\)).

Too much or too little body fat is not optimal for species survival and reproduction, as evidenced by comorbidities and adverse effects on fertility and progeny associated with either extremes of body fat.\(^{15-20}\) Therefore, the body appears to employ pro- and anti-inflammatory influences as the proverbial “yin and yang” to maintain body fat in a desirable range. This control could be offset by excessive and continued fat accumulation, leading to chronic inflammation and insulin resistance.

2. Novel approach to improve insulin resistance independent of weight loss or inflammation

Once obesity, inflammation and insulin resistance develop, the treatment options to improve insulin resistance are limited. Weight loss is a corner stone of such a treatment, but challenging to achieve for many individuals. Therefore, for a majority, who are unable to achieve a substantial and sustained weight loss, approaches that improve glycemic control despite obesity or inflammation are needed. As described below, one such promising approach that is under development selectively harnesses the beneficial properties of a human adenovirus.

Ad36, a human adenovirus belonging to subgroup D, was first isolated from fecal sample of a girl suffering from enteritis.\(^21\) In chicken, mice, rats, or non-human primates, experimental infection of Ad36 increases adiposity, and somewhat paradoxically, improves glycemic control and attenuates hepatic lipid accumulation.\(^{22,23}\) Cross sectional and longitudinal studies show that natural exposure of rhesus monkeys and humans to Ad36 infection is associated with obesity and weight gain, better glycemic control and less hepatic steatosis.\(^{23-32}\) While the observational studies in humans cannot prove causation, the studies do suggest such a possibility.

Studies to understand molecular and cellular signaling underlying the action of Ad36 revealed an interesting mechanism. The role of the central nervous system, if any, in Ad36-induced adiposity is unclear. Instead, a significant correlation between virus load in adipose tissue, with the amount of adipose tissue in Ad36-infected animals\(^{33}\) first suggested a peripheral action for Ad36-induced adiposity. Several in vitro and in vivo studies collectively indicate that Ad36 increases cell replication, adipogenic commitment, differentiation and lipid accumulation, in adipose tissue derived stromal/stem cells and other adipocyte progenitors, by enhancing the cAMP/Phosphatidylinositol 3-kinase (PI3K) pathway, and the adipogenic differentiation cascade including the up-regulation of C/EBP\(\beta\), C/EBP\(\alpha\), PPARY\(^{34-37}\) (review\(^{38}\)). In animal studies, Ad36 reduces the abundance of leptin and increases adiponectin—the key adipokines involved in the control of adipose tissue stores via central and peripheral signaling.\(^{25,39,40}\)

In general, leptin attenuates adipogenesis and adiponectin promotes it. It is possible that a reduction in leptin and an increase in adiponectin by Ad36 has an autocrine/paracrine effect, leading to greater adipogenesis.

Reminiscent of the action of thiazolidinediones class of drugs, Ad36-induced adipogenesis is also accompanied by an improvement in glycemic control. In rats, and mice, Ad36, but not Ad2 – a non-adipogenic adenovirus, improved glycemic control compared to the uninfected control group.\(^{25,40}\) Ad36 improves glycemic control in chow-fed or high fat fed mice, and without requiring weight loss. At the cellular level, Ad36 increases glucose uptake in adipocytes and its progenitors, and in myoblasts.\(^{41-43}\) On the other hand, Ad36 reduces glucose output from hepatocytes.\(^44\) Overall, it appears that in vivo, Ad36 enhances glucose uptake by adipose tissue and skeletal muscle, and reduces hepatic glucose output, collectively contributing to a better glycemic profile.

It is well established that the binding of insulin to insulin receptor (IR) leads to insulin signaling, including tyrosine phosphorylation of insulin receptor substrate (IRS) and a reduction in serine phosphorylation of IRS, followed by the activation of PI3K, leading to translocation of glucose transporter Glut4 to the membrane, and intake of glucose inside a cell. Interestingly, Ad36 increases glucose uptake independent of insulin, or without recruiting insulin receptor.\(^{41,42,44}\) In fact, Ad36 impairs IRS signaling by reducing tyrosine phosphorylation and increasing serine phosphorylation. This property of impairing proximal insulin signaling is shared by other human adenoviruses.\(^44\) Yet, Ad36 increases glucose uptake by increasing the abundance and activation of Ras, particularly H-Ras, to up-regulate the PI3K/ Glut4 signaling.\(^{41,43,47}\) Thus, Ad36 bypasses the proximal insulin signaling (IR and IRS signaling), and increases cellular glucose uptake.

The E4orf1 (early gene 4, open reading frame 1) gene product of
Ad36 is a 125 amino acid peptide, with a PDZ domain binding motif at N-terminal, which affords functionality to the peptide.\textsuperscript{44} E4orf1 appears necessary and sufficient for the adipogenic and anti-glycemic properties for Ad36.\textsuperscript{47} Ad36 E4orf1 increases cellular glucose uptake independent of proximal insulin signaling, via Ras-mediated up-regulation of the PI3K/Glut 4 signaling.\textsuperscript{47} Based on the \textit{in vitro} studies in hepatocytes, it appears that E4orf1 may reduce lipid uptake in the liver, and promote complete oxidation and export of lipid, which may collectively explain reduced hepatic lipid stores linked with Ad36 infection.\textsuperscript{44}

The role of Ad36 and E4orf1 in enhancing cellular glucose uptake in the presence of chronic inflammation is of particular relevance. Ad36 increases the secretion of inflammatory cytokines by adipocytes and adipose tissue.\textsuperscript{25,49} In fact Ad36 requires the inflammatory cytokine macrophage chemoattractant protein (MCP)-1, for inducing adiposity.\textsuperscript{50,51} Inflammatory cytokines impair insulin signaling by reducing tyrosine phosphorylation and increasing serine phosphorylation of IRS, and impair cellular glucose uptake.\textsuperscript{52} In contrast, although Ad36 increases inflammatory response, and impairs proximal insulin signaling, it enhances cellular glucose uptake. Moreover, Ad36 or E4orf1 increase cellular glucose uptake even in the presence of inflammatory cytokines such as TNF alpha and MCP1.\textsuperscript{53} This is probably because Ad36 and E4orf1 bypass proximal insulin signaling to enhance glucose uptake. Collectively, these data suggest that E4orf1 may offer a template to develop drugs to improve glycemic control independent of weight loss, and insulin, or despite impaired insulin signaling, or chronic inflammation.

In summary, a preponderance of pro- or anti-inflammatory cytokines associated with adiposity may be a mechanism to maintain body fat stores within a desirable range. Excessive fat accumulation may derail this regulation, leading to a chronic state of inflammation associated with obesity and diabetes. Recognizing this underlying possibility may be important in understanding why anti-inflammatory therapy has not achieved significant improvement in insulin sensitivity. Also, while weight loss can improve inflammation and insulin resistance, achieving substantial and sustained weight loss is highly challenging. Therefore, an approach that improves glycemic control independent of weight loss or the presence of chronic inflammation is needed. Ad36 and its E4orf1 protein offers a potential template for future research to design such therapeutic agents.

**CONFLICTS OF INTEREST**


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