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Colchicine to decrease NLRP3-activated inflammation and improve obesity-related metabolic dysregulation

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ABSTRACT

Obesity is a major risk-factor for the development of insulin resistance, type 2 diabetes, and cardiovascular disease. Circulating molecules associated with obesity, such as saturated fatty acids and cholesterol crystals, stimulate the innate immune system to incite a chronic inflammatory state. Studies in mouse models suggest that suppressing the obesity-induced chronic inflammatory state may prevent or reverse obesity-associated metabolic dysregulation. Human studies, however, have been far less positive, possibly because targeted interventions were too far downstream of the inciting inflammatory events. Recently, it has been shown that, within adipose tissue macrophages, assembly of a multi-protein member of the innate immune system, the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, is essential for the induction of this inflammatory state. Microtubules enable the necessary spatial arrangement of the components of the NLRP3 inflammasome in the cell, leading to its activation and propagation of the inflammatory cascade. Colchicine, a medication classically used for gout, mediates its anti-inflammatory effect by inhibiting tubulin polymerization, and has been shown to attenuate macrophage NLRP3 inflammasome arrangement and activation *in vitro* and *in vivo*. Given these findings, we hypothesize that, in at-risk individuals (those with obesity-induced inflammation and metabolic dysregulation), long-term colchicine use will lead to suppression of inflammation and thus cause improvements in insulin sensitivity and other obesity-related metabolic impairments.

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1. Introduction

Obesity is the third most common cause of preventable death in the US, and is a major risk factor for the development of insulin resistance (IR), type 2 diabetes (T2DM), dyslipidemia, non-alcoholic fatty liver disease (NAFLD), and cardiovascular disease (CVD) [1–3]. Although the link between obesity and its comorbid conditions appears to be multifactorial, inflammation plays a prominent role through its effects in adipocytes, pancreatic islet cells, and vascular smooth muscle [4–8].

To date, most strategies for improving obesity and its resultant maladies have focused on weight loss. Lifestyle modification alone has been shown to decrease weight and reduce cardiovascular risk factors [9]; however, sustained success rates are insufficient in community programs, weight regain is frequent, and even intensive behavioral programs have not been shown to reduce long-term cardiovascular mortality [10,11]. Bariatric surgery, which may be effective, is an expensive, invasive procedure and is not without morbidity. Moreover, up to 50% of individuals may have at least some long-term weight regain and a return of metabolic abnormalities [12,13]. Few medications are currently FDA approved for weight loss in obesity, and none have been shown to reduce mortality [14].

Additional medical treatment options for preventing the complications of obesity are therefore urgently needed. Our increasing understanding of the underlying inflammatory mechanisms that link obesity, metabolic dysregulation, and cardiovascular disease allow us to propose a novel therapeutic strategy that may help break the link between obesity and its metabolic complications.

2. The hypothesis

We hypothesize that interfering with the inflammatory cascade activated by obesity will help uncouple obesity from its comorbid conditions. Therefore, disrupting the assembly of the NOD-like receptor, pyrin domain containing 3 (NLRP3) inflammasome using the ancient medication colchicine will improve metabolic

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dysregulation via suppression of obesity-related, NLRP3-induced inflammation among obese adults with metabolic syndrome (MetS).

3. Evaluation of hypothesis

3.1. Inflammation is associated with metabolic dysregulation

Inflammation likely leads to metabolic dysregulation through several concomitant pathways. In skeletal, adipose, and hepatic tissues, inflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor α (TNFα) activate serine kinases Jun N-terminal kinase (JNK), inhibitor of kappa B kinase (IKK-β), and protein kinase C (PKC) [15–17]. These kinases in turn serine-phosphorylate and deactivate insulin receptor substrate-1 (IRS-1), an important downstream mediator of insulin action, leading to insulin resistance (Fig. 1) [18–21]. The IKK complex also activates NF-κB, further promoting inflammatory gene expression (15). Additionally, the expression of suppressor of cytokine signaling (SOCS) proteins, which target IRS-1 and IRS-2 for ubiquitin-mediated degradation [22], are induced.

Low-grade systemic inflammation can also impair pancreatic islet cell functioning and insulin secretion. In early stages of β-cell dysfunction, inflammatory cytokines disrupt the proper intracellular calcium storage and flux necessary for adequate insulin secretion [6,7]. Chronically, inflammatory signaling via the NF-κB and mitogen-activated protein kinase (MAPK) pathways leads to mitochondrial stress, reactive oxygen species (ROS) formation, and eventual β-cell apoptosis [23,24]. It is this eventual islet cell depletion that moves the at-risk individual from obesity to insulin resistance to frank diabetes [25,26].

3.2. Obesity is associated with inflammation

Many mouse and human studies have demonstrated the association of obesity with inflammation. Obesity results when mice are fed a high-fat diet (HFD), and such HFD mice demonstrate increased levels of insulin resistance and inflammatory cytokines and chemokines, including IL-1β, IL-6, IL-18, TNFα, monocyte chemoattractant protein-1 (MCP-1), plasminogen activator inhibitor-1 (PAI-1), and macrophage migration inhibitory factor (MIF), as compared to lean mice [27–32]. Similarly, in cross-sectional human studies, circulating markers of inflammation have been associated with increasing obesity [33–37], and this pro-inflammatory phenotype is already present among children with obesity [38–40]. Weight loss, either by lifestyle modification or bariatric surgery, decreases this inflammatory state [21,38,41–43].

The sources of the chronic low-grade inflammation of obesity arise from multiple organs, but it appears that adipose tissue itself is a large contributor to the inflammatory process. Adipose tissue consists of adipocytes and the stromal vascular fraction (SVF), which includes fibroblasts, vascular tissue, and adipose tissue macrophages. In lean individuals, macrophages comprise less than 10% of the cells in adipose tissue [44]. The adipocytes themselves are small in volume and secrete high levels of adiponectin and omega-3 fatty acids, which predispose the macrophages to differentiate to an anti-inflammatory phenotype. Such M2 macrophages release immunosuppressive cytokines, such as IL-10 and TGFβ, which act on fat and muscle tissue to enhance insulin sensitivity [45]. However, in obese individuals, adipocytes are both greater in volume and number, leading to inefficient triglyceride storage and increased levels of circulating fatty acids and ceramides [46]. These molecules bind to pattern recognition receptors in adipose tissue macrophages and stimulate differentiation to a pro-inflammatory M1 phenotype [47]. Chronic inflammation ensues, with an increased release of chemokines and cytokines including TNFα, IL-1β, IL-6, IL-18, plasminogen activator inhibitor-1 (PAI-1), monocyte chemoattractant protein-1 (MCP-1), and reactive oxygen species (ROS) [47–49]. These factors further augment adipose tissue inflammation by recruiting additional adipose tissue macrophages as well as B and T cells [30].

3.3. Proof of concept studies that reducing inflammation can improve obesity-related metabolic dysfunction

If inflammation is a significant cause of obesity-related metabolic dysregulation, then anti-inflammatory agents should reverse

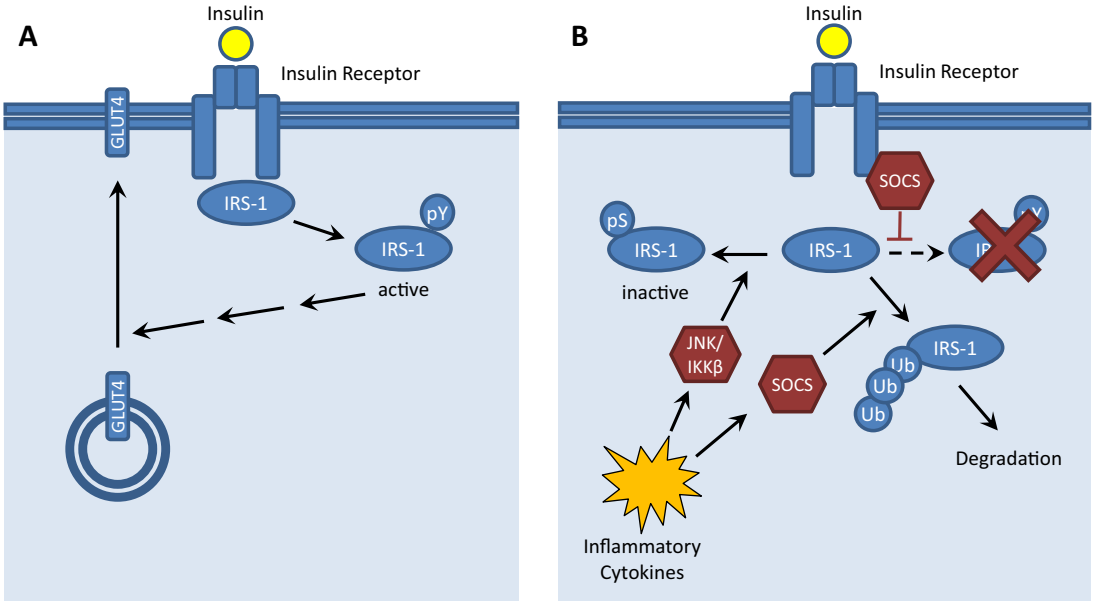

Fig. 1. (A) Insulin binding to its receptor stimulates tyrosine phosphorylation and activation of IRS-1 in adipocytes and myocytes. The resultant signaling cascade leads to GLUT4 sequestration vesicle trafficking to the plasma membrane, allowing for glucose entry into the cell. (B) Inflammation promotes insulin resistance by interfering with this pathway. Specifically, JNK and IKKβ inactivate IRS-1 via serine phosphorylation, while SOCS proteins prevent IRS-1 tyrosine phosphorylation and promote IRS-1 ubiquitination and subsequent degradation.

obesity's inflammation-related complications. This has been the case for some mouse models of obesity. HFD mice treated with a neutralizing IL-1β antibody demonstrate significant improvement of glycemic control and beta cell function [23], with no effect on weight gain or food intake [50]. Given prophylactically, IL-1β antibody treatment helps prevent the onset of fasting hyperglycemia and insulin resistance in HFD mice. IL-1 receptor antagonist (IL-1Ra) treatment of DIO mice leads to similar results, with decreased β-cell apoptosis, increased β-cell proliferation, and improved glucose-stimulated insulin secretion [50,51]. Studies inducing blockade of other inflammatory cytokines or pathways in mice, including TNFα [52], IL-6 [53], MCP-1 [54], MIF [31], and NF-kB [55] have also demonstrated favorable metabolic results from reduction of inflammatory processes.

However, such robust results have not been replicated in human clinical trials. Blockade of TNFα had no effect on metabolic parameters of participants with T2DM [52] and only marginal impact in subjects who were non-diabetic but insulin-resistant [56]. Similar findings were seen when using aspirin or the anti-IL-1β antibody canakinumab [57–59]. A different monoclonal anti-IL-1β antibody, gevokizumab, led to improvements in HbA1c, but not beta-cell secretory function or insulin sensitivity in preliminary trials [60]. Anakinra, a recombinant human IL-1Ra, improved beta-cell secretory function and decreased Hemoglobin A1c in subjects with diabetes [61], but not in those with impaired glucose tolerance [62]. Furthermore, anakinra did not affect insulin sensitivity as measured by insulin clamp or HOMA-IR in either study.

Although these results are disappointing, they are not wholly unexpected. The machinery involved in obesity-induced chronic inflammation is complex. Cytokines may play complementary, if not redundant, roles in promoting human inflammation. Chemokines and endothelial adhesion molecules play integral roles in the sustained inflammatory response by facilitating chemoattraction, diapedesis, and migration of additional leukocytes to adipose and pancreatic tissues. For this reason, eliminating a single cytokine may not be sufficient to cause significant changes in human glucose homeostasis. Notably, high-dose salicylate therapy, which exerts its anti-inflammatory effects through several concomitant pathways [63–65], did lower hemoglobin A1c and triglyceride concentrations in subjects with T2DM [66]. However, concerns over long-term safety and tolerability have precluded its use for metabolic dysregulation in routine clinical practice.

3.4. NLRP3 mediates inflammatory activation in obesity

A member of the innate immune system, the NOD-like receptor family, pyrin domain containing 3 (NLRP3) inflammasome has been implicated as a major source of the chronic inflammation seen in obesity. NLRP3 (also known as cryopyrin or NALP3) is primarily expressed in monocytes and macrophages, with little production seen in other leukocytes or adipocytes [21,67]. Many danger-associated molecular pattern (DAMP) molecules commonly seen in obesity, such as monosodium urate (MSU) crystals [68], cholesterol crystals [69], islet amyloid polypeptide [70], oxidized low density lipoprotein (LDL) [71], or saturated free fatty acids [72], can induce NLRP3 inflammasome formation. NLRP3 does not bind with DAMPs directly, but rather is stimulated through a second-messenger [73]. Upon activation by DAMPs, the endoplasmic reticulum-based NLRP3 comes into apposition with the mitochondrially-bound adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC; also known as PYCARD) and procaspase-1 to form a multiprotein inflammasome complex (Fig. 2) [74–76]. Microtubules serve as the roadways for the subcellular transport of these molecules within the macrophages and are necessary for proper cytosolic localization and activation of the inflammasome components [76].

The formation of this inflammasome leads to cleavage of procaspase-1 to its active form, caspase-1, which in turn cleaves inactive pro-IL-1 and pro-IL-18 into their active forms [77]. IL-1β initiates the acute phase response and triggers the production and secretion of other pro-inflammatory cytokines, such as TNFα, IL-6, and MCP-1, resulting in a progressively amplified cytokine network [78]. IL-18, meanwhile, induces the production of interferon gamma (IFNγ), IL-10, toll-like receptor 4 (TLR4), Fas ligand, and vascular cell adhesion molecule-1 (VCAM1) [79,80].

In mouse models, *Nlrp3* and *IL1b* expression in adipose tissue is positively correlated with body weight and adiposity, and food restriction results in significant decreases in their expression (21). Importantly, knocking out components of the NLRP3 inflammasome (e.g. $Nlrp3^{-/-}$, $ASC^{-/-}$, $caspase\text{-}1^{-/-}$ and $lL1b^{-/-}$) in mice prevents the development of insulin resistance and inflammatory cytokine expression during a protracted high fat diet (HFD) state [21,27,81,82]. Additionally, ablation of the NLRP3 inflammasome results in markedly smaller adipocytes, elevated levels of adipose GLUT4 and adiponectin, and decreased inhibition of IRS-1 [21,83]. Caspase-1 knockout mice demonstrate increased fat oxidation and gain significantly less body weight than wild-type mice during a 10-week HFD regimen, despite similar food intake [27].

In human trials, NLRP3 activation has also been positively correlated with adiposity and metabolic dysregulation. Obese individuals with MetS have greater levels of NLRP3 activity and *IL1b* expression, increased numbers of adipose tissue macrophages, and decreased numbers of regulatory T cells in their visceral adipose tissue than either metabolically healthy obese or lean individuals [84]. Among obese subjects with T2DM, weight loss leads to decreased mRNA expression of *IL1b* and *NLRP3* and enhanced insulin sensitivity [21].

3.5. Colchicine can inhibit NLRP3 inflammasome activation

As noted above, microtubules serve as the mechanism for the subcellular transport of ASC and NLRP3 within macrophages and are necessary for proper cytosolic localization and activation of the inflammasome components [74,76]. Thus, molecules that affect microtubular function can potentially alter NLRP3 inflammasome assembly and function.

Colchicine, a potent inhibitor of tubule polymerization, has been used for centuries to treat inflammatory disorders such as gout, and more recently Familial Mediterranean Fever [85], Behcet's Disease [86], and pericarditis [87]. Colchicine is a tricyclic alkaloid derived from the flowering plant *Colchicum autumnale* [88], whose mechanism of action is to bind tubulin in a poorly reversible manner. At lower doses, this interferes with microtubule formation and elongation, and at higher concentrations it promotes microtubule depolymerization [89].

Classically it was thought that colchicine's microtubule disruption led to anti-inflammatory effects by inhibiting neutrophil chemotaxis, diminishing release of lysosomal enzymes during phagocytosis, and inhibiting the expression of adhesion molecules on the surface of endothelial cells and leukocytes [90–92]. However, recent *in vitro* and *in vivo* studies have suggested that a more important anti-inflammatory mechanism is colchicine's ability to hinder NLRP3 and ASC intracellular transportation and spatial arrangement, thereby inhibiting inflammasome activation within macrophages (Fig. 2B) [68,76]. Martinon, et al., demonstrated that culturing human monocytes with MSU or calcium pyrophosphate dihydrate (CPPD) crystals stimulated the production of activated IL-1β and IL-18 in an inflammasome-based manner. However, adding colchicine to the cultures blocked this IL-1β stimulation. Misawa, et al., showed that colchicine and nocodazole, another

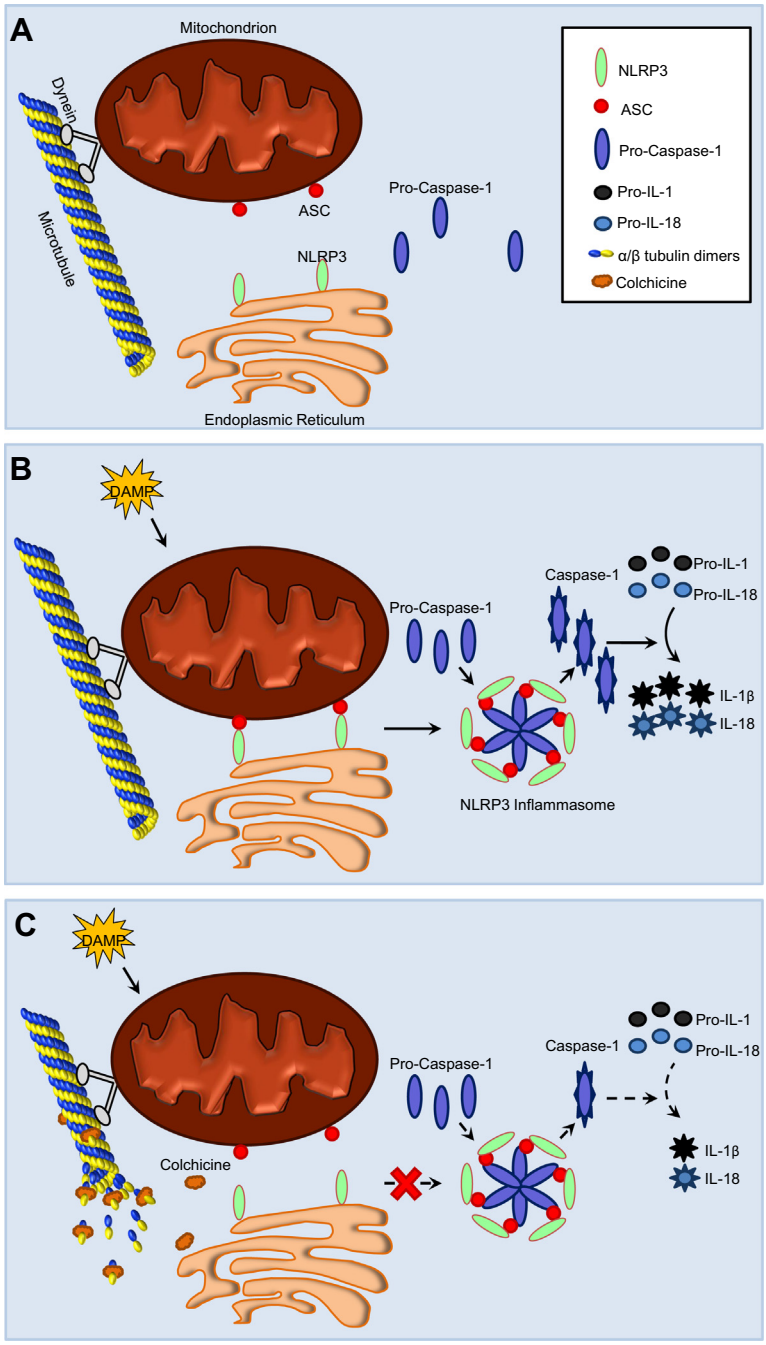

Fig. 2. (A) Components of the NLRP3 inflammasome in a quiescent macrophage. (B) Microtubules mediate NLRP3 inflammasome formation by bringing the mitochondrially-based ASC into apposition with NLRP3, located on the surface of the endoplasmic reticulum. Inflammasome formation cleaves procaspase-1 to caspase-1, which in turn activates IL-1β and IL-18 and initiates the inflammatory cascade. (C) Colchicine blocks NLRP3 inflammasome formation and activation by inhibiting microtubule polymerization.

inhibitor of tubulin polymerization, suppressed IL-1β production in mouse bone marrow-derived macrophages cultured with various NLRP3-inducers such as nigericin, ATP, silica, and MSU. Inducers of other inflammasomes, such as flagellin (NLRC4) or double-stranded DNA (AIM2), which do not depend on microtubules for activation, continued to demonstrate a robust inflammatory response in the presence of colchicine or nocodazole. Immunocytochemical analysis showed that stimulated bone marrow-derived macrophages cultured with nigericin or MSU resulted in subcellular approximation of mitochondrially-bound ASC to NLRP3, but not NLRC4 or AIM2. Pretreatment with colchicine prevented this co-localization, as evidenced by an *in situ* proximity-ligation assay. *In vivo* studies demonstrated that mice pretreated with colchicine prior to intraperitoneal injection with MSU demonstrated decreased levels of activated IL-1β [76]. Colchicine thus has the potential to modulate numerous inflammatory pathways upstream of the agents previously tested to decrease inflammation in humans with obesity.

Given the complexity of the inflammatory orchestra associated with obesity, with cytokines, chemokines, leukocytes, and endothelial adhesion molecules all working in concert to promote a sustained response, colchicine's ability to affect multiple pathways may be a key characteristic to enable changes in glucose homeostasis and metabolic dysregulation. At doses used clinically

for gout (0.5–1.2 mg daily), colchicine can decrease inflammatory levels in patients with cardiovascular disease [93,94], and prevent recurrent gouty attacks [95], pericarditis [87], and restenosis in bare metal stents [96] in at-risk individuals. Moreover, at higher doses colchicine has been shown to decrease inflammatory levels and vascular endothelial markers in Familial Mediterranean Fever [85,91]. However, to date, there are scant prospective human clinical data focusing on the metabolic effects of using colchicine to inhibit obesity-induced inflammation. Two retrospective trials have demonstrated no long-term metabolic adverse consequences from colchicine use [97,98]; however, neither of these trials specifically examined its effects in those with obesity-induced inflammation. The LoDoCo (Low-Dose Colchicine) trial prospectively examined the cardioprotective effects of colchicine 0.5 mg daily versus placebo in individuals with stable coronary artery disease, of which 30% had T2DM and over two-thirds were using antihypertensive therapy (and thus had components of the metabolic syndrome). The primary outcome – composite incidence of acute coronary syndrome, out-of-hospital cardiac arrest, or noncardioembolic ischemic stroke – was significantly decreased in the colchicine arm (5.3% versus 16.0%, hazard ratio: 0.33, 95% confidence interval 0.18–0.59), lending further credence to colchicine's beneficial effects in MetS. However, neither inflammatory markers, nor BMI, were reported [99].

Colchicine's long-term safety and tolerability has been established in multiple studies and colchicine is FDA-approved for chronic use to prevent recurrent attacks of gout or Familial Mediterranean Fever [88,100,101]. Colchicine should therefore be a relatively safe compound with which to test the hypothesis that reducing inflammation can improve obesity-associated comorbid conditions. Colchicine, taken at the FDA-approved dose of 0.6 mg twice daily, should block NLRP3 inflammasome assembly, reduce markers of inflammation, and improve metabolic dysfunction in obese subjects with MetS and low-level inflammation. Theoretically, lower doses (e.g. 0.6 mg once daily) may also be effective and safer over the long term, but warrants further study. The LoDoCo study has shown that colchicine demonstrates benefit in secondary cardiovascular prevention with a low risk of side effects, even when taken in conjunction with anti-platelet agents and statins [99]. However, it remains to be seen if colchicine, in isolation or in conjunction with other agents, can also be beneficial in the primary prevention of T2DM or CVD in at-risk individuals.

4. Conclusions

It is well established that, as compared to lean individuals, those with obesity have a higher levels of chronic inflammation. This low-level inflammatory state can lead to impaired functioning of metabolic pathways within peripheral, hepatic, and pancreatic tissues, eventually leading to insulin resistance and T2DM. Therapies targeted to inhibit obesity-induced chronic inflammation in mouse models have been successfully shown to prevent or improve metabolic dysregulation, but in humans the results have not been as impressive. A lack of efficacy from targeted immunotherapies may stem from redundancies in the human immune system downstream of the NLRP3 inflammasome. Because colchicine inhibits inflammation more proximally, at the level of NLRP3 inflammasome activation, it presents as an intriguing potential treatment for individuals with metabolic syndrome.

Conflict of interest statement

The authors report no competing interests.

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