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TNF-α/NF-κB mediated upregulation of Dectin-1 in hyperglycemic obesity: implications for metabolic inflammation and diabetes

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Abstract

Background Dectin-1, a key innate immune receptor, plays a critical role in cellular responses and is implicated in chronic inflammation and metabolic syndromes. This study addresses a pivotal gap in elucidating the regulatory mechanism governing Dectin-1 expressionin obesity and diabetes, hypothesizing that hyperglycemia and TNF-α synergistically upregulate Dectin-1 in adipose tissue (AT), thereby exacerbating inflammatory responses and contributing to metabolic dysfunction.

Methods The study included 95 overweight and obese Kuwaiti individuals, categorized into prediabetic (HbA1c < 6.5%) and diabetic (HbA1c ≥ 6.5%) groups. Anthropometric and clinical measurements were recorded. AT biopsies were obtained for RNA extraction and immunohistochemistry. Pre-adipocytes from lean and obese individuals were cultured, differentiated into adipocytes, and treated with TNF-α under normal or high-glucose conditions to assess Dectin-1 expression. Chromatin immunoprecipitation (ChIP) assays analyzed NF-κB binding to the Dectin-1 promoter. Wildtype and TNF-α-/- mice were used to evaluate TNF-α's effect on Dectin-1 expression in AT.

Results Our data demonstrate that hyperglycemic obesity significantly induces Dectin-1 expression in AT through the TNF-α/NF-κB signaling pathway. In a cohort of 95 obese individuals, subdivided into prediabetics (HbA1c < 6.5%, $n = 49$) and diabetics (HbA1c ≥ 6.5%, $n = 46$), a strong positive correlation was observed between AT Dectin-1 transcripts and plasma HbA1c levels exclusively in diabetic participants, underscoring the specificity of Dectin-1 upregulation in hyperglycemic conditions. Elevated Dectin-1 expression was consistently associated to increased inflammation markers. Immunohistochemical analysis revealed co-localization and concurrent upregulation of Dectin-1 and TNF-α proteins in hyperglycemic AT. Functional assays in TNF-α deficient mice and human adipocytes further validated that TNF-α and hyperglycemia act cooperatively to regulate Dectin-1 expression. Mechanistically, we demonstrated that NF-κB directly binds to the Dectin-1 promoter, mediating its transcriptional activation in response to glucose and TNF-α.

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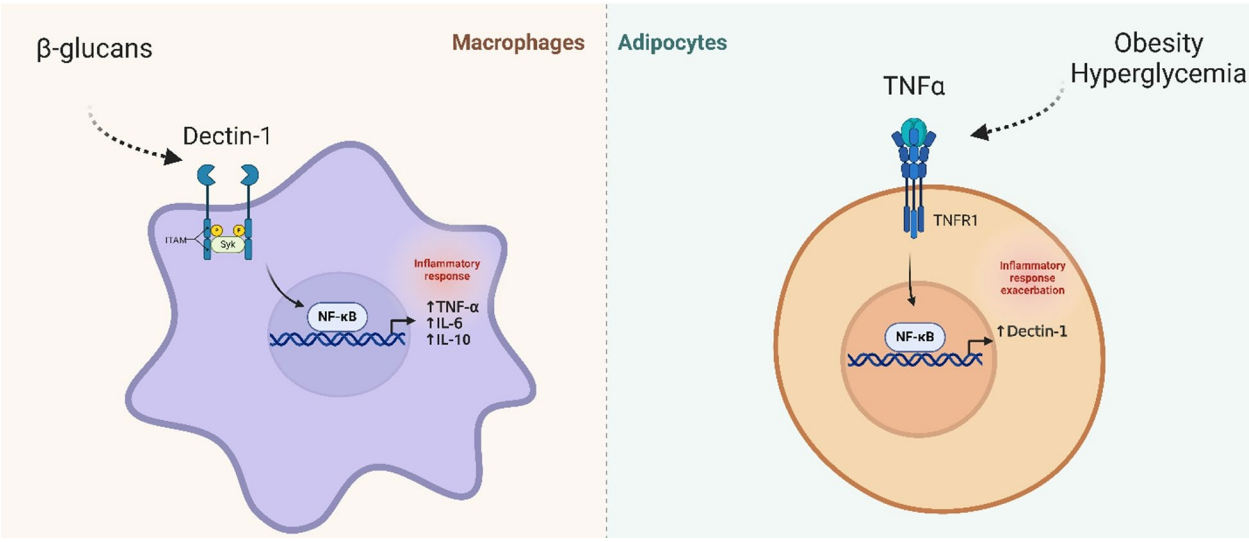
Conclusion This study significantly advances the understanding of upregulation Dectin-1 in metabolic inflammation, filling a crucial niche in diabetes research and suggesting new therapeutic targets for obesity-related metabolic disorders.

One-sentence summary

In hyperglycemic obese individuals, TNF-α upregulated dectin-1 expression through a direct binding of NF-κB to its regulatory region, a process that elevates metabolic inflammation in adipose tissue.

Graphical Abstract



Keywords Dectin-1, AT, TNF-α, NF-κB, HbA1c, Diabetes

Introduction

The global increase in obesity prevalence remains a significant public health concern, leading to adverse outcomes such as diabetes and cardiovascular diseases [1–4]. The pathophysiology of obesity primarily involves systemic and chronic low-grade inflammation, disrupting cellular and tissue homeostasis [5, 6], with the molecular mechanisms to be fully understood [7, 8].

Diabetes is closely linked with obesity, and glycated hemoglobin A1c (HbA1c) serves as a key diagnostic and control marker [9]. HbA1c levels of ≥6.5% indicate diabetes, while levels between 5.7% and 6.4% suggest an increased risk of developing diabetes, known as prediabetes [10]. A longitudinal retrospective study conducted by Nakajima and Sawa (2015) found that approximately 50% of obese individuals with normal HbA1c levels develop diabetes or prediabetes within eight years [11]. Consequently, excess body weight poses a challenge to glycemic control due to its pro-inflammatory effects.

Adipose tissue (AT) is a diverse cellular compartment containing adipocytes, progenitor cells, residential macrophages, neurons, and vascular cells [12, 13]. The interplay between these cell types is crucial for AT homeostasis and adaptation to environmental changes [14]. In obese individuals, fat accumulation in AT leads to hypoxia and dysfunction of AT cells, triggering inflammatory responses [15, 16]. Dysfunctional AT releases high levels of free fatty acids, adipokines, and pro-inflammatory cytokines/chemokines into the bloodstream, adversely affecting the function of other organs [17]. Importantly, the secretion of inflammatory markers is strongly associated with the stage of obesity and its complications [18, 19].

Dectin-1 has emerged as a biomarker for obesity and insulin resistance [20]. It belongs to the type II transmembrane family of C-type lectin receptors expressed in leukocytes and antigen-presenting dendritic cells [21, 22]. Dectin-1 primarily functions in fungal recognition and modulation of innate immunity [23–25]. Studies using Dectin-1 knockout (KO) mouse models have linked it to fungal infections, in particular, Pneumocystis carinii infection [26]. Dectin-1 activation triggers the production of pro-inflammatory mediators and stimulates phagocytosis [27].

Tumor necrosis factor-alpha (TNF-α) plays a multifaceted role in the pathophysiology of obesity and diabetes. In obesity, TNF-α is primarily secreted by AT and acts as a pro-inflammatory cytokine, contributing to the chronic low-grade inflammation characteristic of obesity [28]. It promotes AT dysfunction by impairing insulin signaling

pathways, inducing insulin resistance, and inhibiting adipocyte differentiation (reviewed in [29]). Additionally, Hotamisligil and colleagues showed that AT TNF-α is elevated in obese diabetic rodents and is a mediator of obesity-related insulin resistance and type 2 diabetes (T2D) [30]. In diabetes, TNF-α exacerbates pancreatic β-cell dysfunction and apoptosis, leading to impaired insulin secretion and worsening hyperglycemia [31]. Furthermore, TNF-α contributes to the progression of diabetic complications such as cardiovascular disease and nephropathy through its pro-inflammatory effects [32]. Overall, TNF-α serves as a critical mediator linking obesity-related inflammation and insulin resistance to the pathogenesis and progression of T2D.

In the context of obesity and insulin resistance, the upregulation of Dectin-1 in AT isolated from obese individuals correlates positively with clinical indicators of obesity, such as body mass index (BMI) and body fat percentage, as well as pro-inflammatory cytokines [33]. In patients with T2D, peripheral blood mononuclear cells exhibit differential Dectin-1 gene expression [34]. However, the precise signaling pathways driving Dectin-1 upregulation in adipocytes of adults with obesity-associated diabetes remains poorly understood. Here, we demonstrate that under hyperglycemic conditions, TNF-α/NF-κB signaling significantly upregulates Dectin-1 expression, contributing to the observed elevation in metabolic inflammation within the AT of obese individuals.

Results

Demographic and clinical characteristics of the study population

This study included 95 overweight and obese participants, with their physiological and clinical properties summarized in Table S1. We were interested to understand the differences between those who were developing diabetes (prediabetics) and those who had already been diagnosed (diabetics). So, we divided our cohort into two significantly distinct groups based on their HbA1c levels, a marker for a long-term blood sugar control. We found a clear divide ($p < 0.0001$): prediabetics (obese without diabetes) had HbA1c levels 5.59 ± 0.43%, while obese diabetics averaged HbA1c 8.41 ± 1.44%. While all participants were between 32 and 61 years old, a notable difference materialized, patients with higher glycemia were significantly older ($p = 0.001$). Interestingly, despite these differences the two groups had similar height, weight and BMIs (Table S1). Diabetic participants were presented with higher waist circumference and plasma triglyceride levels, indicating a difference in body distribution and metabolism (Table S1). Surprisingly, prediabetics exhibited significantly elevated levels of plasma cholesterol, HDL, and LDL ($p \leq 0.03$) compared to diabetics (Table S1). Conversely, the diabetic group showed a significantly higher FBG and HOMA-IR that were 1.6-fold and 2.7-fold higher, respectively, compared to the prediabetics ($p \leq 0.0001$; Table S1), respectively. These observations set a stage to further investigate the impact on Dectin-1 gene expression as a continuation for our previous study [33].

Dectin-1 gene and protein expression associated with elevated Hb1Ac

Building on previous work from our lab and others, which suggested that Dectin-1 gene expression increases in mesenteric and subcutaneous AT isolated from obese individuals [20, 33, 35], we examined its RNA and protein levels in the AT isolated from our cohort subgroups. In obesity-induced diabetic group, AT *DECTIN-1* gene expression was showing a significantly positive correlation with their plasma Hb1Ac levels ($r = 0.454$, $p = 0.001$; Fig. 1A). This relation was not observed in the prediabetic group, where a non-statistically significant negative correlation was noted (r = -0.05, p = NS). Further analysis using RT-qPCR confirmed that individuals with HbA1c ≥ 6.5% (the diabetic group) had a significantly higher levels of Dectin-1 transcripts ($p = 0.0218$, Fig. 1B), than those with HbA1c < 6.5% (the prediabetic group). This trend was consistent at the protein level, as shown by immunohistochemistry (IHC) analysis of AT sections (Fig. 1C). Quantitative assessment of IHC-FFPE sections of AT demonstrated a significant increase in Dectin-1 protein levels in diabetic patients relative to those with HbA1c < 6.5% ($p = 0.0034$, Fig. 1D).

Increased AT Dectin-1 expression is associated with inflammatory signatures

Driven by the observation of elevated Dectin-1 in diabetics, and to delineate a prospective mechanism regulating its gene expression in response to hyperglycemia, we analyzed the relationship between Dectin-1 and various inflammatory markers (Table S2). Consistent with our findings in a separate obese cohort [33], we found that Dectin-1 expression was positively correlated with several inflammatory molecules including interleukins IL-8 and IL23A ($r \geq 0.445$; $p \leq 0.002$), and cytokines CCL5 and CCL20 ($r \geq 0.377$; $p \leq 0.014$), and their receptors CCR1, CCR2, and CCR5 ($r \geq 0.411$; $p \leq 0.005$), regardless of the glycemic status (Table S2). However, certain inflammatory markers showed group-specific associations. In prediabetics, Dectin-1 mRNA was significantly linked to IL-18, CCL3, and CCL18 ($r \geq 0.457$; $p \leq 0.002$). In contrast, in diabetics, Dectin-1 was associated with IL-1β, CCL2, and CCL7 ($r \geq 0.407$; $p \leq 0.004$, Table S2). No associations were observed between Dectin-1 expression and IL-5, IL-6, IL-12 A, IL-13, CCL8, CCL11 or CCL15. Together, this suggested that Dectin-1 might be involved

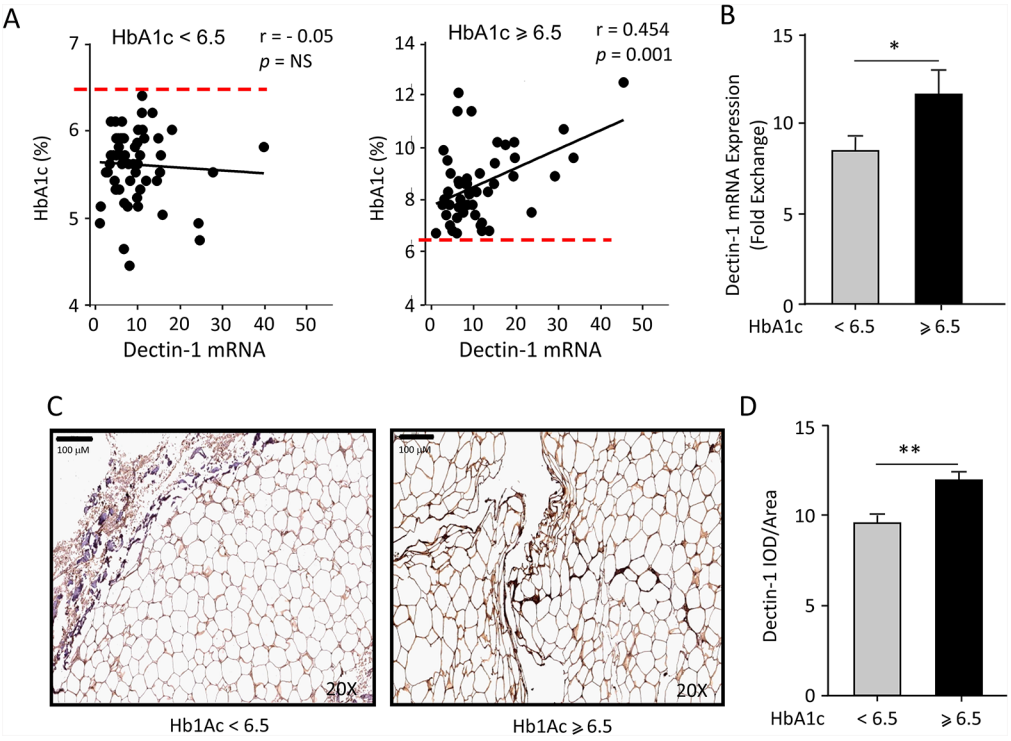


Fig. 1 Elevated Dectin-1 mRNA and protein levels in subcutaneous AT (AT) of hyperglycemic obese individuals. **A** Scatter plots show the relationship between Dectin-1 mRNA expression and HbA1c levels in non-diabetic (HbA1c < 6.5) and diabetic (HbA1c ≥ 6.5) individuals, highlighting a significant positive correlation in the diabetic group. **B** Quantitative RT-PCR analysis reveals significantly elevated Dectin-1 mRNA expression in individuals with HbA1c ≥ 6.5 compared to those with HbA1c < 6.5 (*$p < 0.05$). **C** Representative IHC images of AT show increased Dectin-1 protein expression in individuals with HbA1c ≥ 6.5 compared to prediabetics (HbA1c < 6.5%, Image magnification, 20X). **D** Quantification of IHC staining (IOD/Area) confirms significantly higher Dectin-1 protein expression in the diabetic group (**$p < 0.01$). Scale bars: 100 µM. Data are presented as mean ± SEM values. A two-tailed unpaired Student's t-test was used to determine significance. Abbreviations: AT, adipose tissue; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; IHC, immunohistochemistry; IOD, integrated optical density

in distinct inflammatory pathways depending on diabetes progression stage.

Notably, among the studied pro-inflammatory factors, we observed no correlation between Dectin-1 and IL-6, IL-12 A, IL-13, CCL8, CCL11, or CCL15 (Table S2). This lack of correlation could potentially be attributed to the low expression levels of these factors in AT, regardless of glycemic conditions. Additionally, it is possible that Dectin-1 regulation operates independently of these cytokines and chemokines in AT, supporting the tissue-specific differences in inflammatory signaling pathways. Other factors, such as temporal variations in cytokine expression, or compensatory mechanisms, may also contribute to the observed lack of association. Further studies are needed to investigate these possibilities and to better delineate the regulatory networks involving Dectin-1 in AT.

We further explored the possible role of immune cells residing within the ATin regulating Dectin-1 expression, considering their functional dysregulation in response to obesity and diabetes [36]. Our data revealed that Dectin-1 expression correlated with markers for monocytes and macrophages (CD11c, CD16, and CD68), as well as markers related to their activity (CD86 for M1 macrophages and CD163 for M2 macrophages) in both prediabetic and diabetic groups (Table S2). This pointed to a potential interaction between Dectin-1 and these immune cells in the context of obesity, independent of diabetes status.

Notably, we uncovered a significant positive correlation between Dectin-1 and the pro-inflammatory cytokine TNF-α specifically in the adipose tissue of diabetic individuals ($r = 0.385$; $p = 0.010$), but not in prediabetics (Table S2).

Dectin-1 and TNF-α proteins exhibit co-localization and significant upregulation in hyperglycemic AT

Given the strong association between Dectin-1 and TNF-α, a key player in inflammation and metabolic dysfunction, we investigated their interplay further [37]. Using IHC assays, we found a significant increase in TNF-α protein in AT of the diabetic patients compared to prediabetics ($p < 0.0001$; Fig. 2A and B). Moreover, a significant correlation between Dectin-1 and TNF-α proteins was observed ($p = 0.0204$; Fig. 2C). Using immunofluorescence assays and confocal microscopy, we directly

observed that Dectin-1 and TNF-α were colocalized within AT, both in adipocytes and resident immune cells (Fig. 2D and E). This colocalization, coupled with the increased expression of both proteins in diabetics, strongly suggested a functional interaction between them, particularly in the context of hyperglycemia.

Hyperglycemia and TNF-α induce the expression of AT Dectin-1 in animal models

To explore the dependency of Dectin-1 expression on TNF-α and hyperglycemia, we used animal models, wild-type and TNF-α knockout mice. In initial experiments with chow fed mice, TNF-α injections resulted in increased Dectin-1 expression, particularly in the TNF-α knockout mice, as shown in Figure S1. Notably, wild-type animals did not exhibit a significant response to TNF-α treatment, which could be attributed to the presence of sufficient endogenous TNF-α levels already influencing Dectin-1 expression (Figure S1). Furthermore, Dectin-1 expression in TNF-α KO mice treated with saline was comparable to that of wild-type animals, indicating that other regulatory mechanisms may be involved in the control of Dectin-1 expression. These findings suggest that while TNF-α plays a role in modulating Dectin-1 expression, additional pathways or compensatory mechanisms may be involved, particularly in the absence of TNF-α.

To mimic obesity-induced hyperglycemia, we fed mice a high-fat diet (HFD), with or without added sucrose, and then injected them with either saline or TNF-α. We observed a 40–50% reduction in Dectin-1 expression in TNF-α KO mice compared to WT animals fed the HFD alone (Fig. 3A and B). In wild-type mice on the high-fat, high-sugar diet, Dectin-1 gene expression significantly increased, an effect that was moderated in TNF-α KO mice (Fig. 3A). However, when we gave exogenous TNF-α to mice on HFD, we observed a significant 2.1- and 3-fold increase in Dectin-1 gene expression in both WT and TNF-α KO mice, respectively, compared

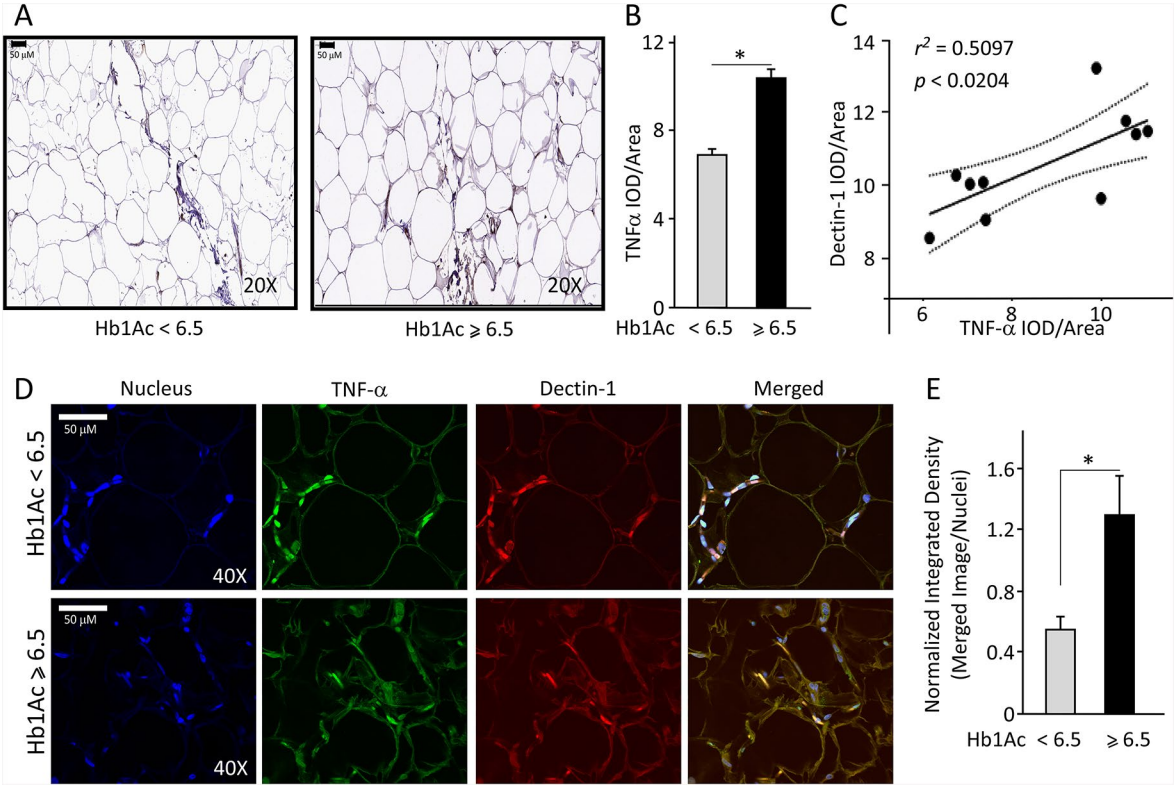


Fig. 2 Co-localization and upregulation of Dectin-1 and TNF-α proteins in hyperglycemic AT. **A** Representative IHC images of AT show increased TNF-α protein expression in individuals with HbA1c ≥ 6.5% compared to those with HbA1c < 6.5 (Image magnification x20, Scale bars: 50 µM). **B** Quantification of TNF-α staining intensity (IOD/Area) reveals significantly elevated TNF-α expression in the diabetic group compared to prediabetics (*$p < 0.0001$). **C** Scatter plot shows a significant positive correlation between TNF-α and Dectin-1 staining intensities. Immunohistochemical analysis was performed on samples from five individuals, and each point represents the average of five different images. Two-tailed unpaired Student's t-test was used to determine significance. **D** Immunofluorescence images display co-localization of TNF-α (Alexa-488 green) and Dectin-1 (Alexa-597 red) using specific primary antibodies in AT, with stronger signals in the diabetic group (HbA1c ≥ 6.5). Image magnification x40, Scale bars: 50 µM. The representative microscopy images from three independent determinations are shown. In the merged images, Dectin-1 and TNF-α proteins were predominantly detected in adipocytes and resident immune cells. Nuclei were stained with DAPI (Blue). **E** Quantification of normalized integrated density (merged image per nuclei) indicates significantly co-localization and gene expression in the diabetic group (*$p < 0.05$, $r^2 = 0.502$). Abbreviations: AT, adipose tissue; TNF-α, tumor necrosis factor-alpha; IHC, immunohistochemistry; IOD, integrated optical density

to their saline-treated counterparts (Fig. 3A). The highest Dectin-1 expression was seen in mice on the high-fat, high-sugar diet that also received TNF-α. These animals showed a 3.5- and 6-fold increase in Dectin-1 transcript levels relative to their counterparts fed with HFD alone and injected with saline (Fig. 3A), respectively. A similar trend was observed at Dectin-1 protein levels in these groups (Fig. 3B and C). This confirmed that TNF-α plays a critical role in regulating Dectin-1 expression in vivo, especially in the setting of high blood sugar.

Induction of Dectin-1 mRNA and protein expression in glucose- and TNF-α-treated human adipocytes via NF-κB binding to the DECTIN-1 proximal promoter

To decipher the molecular mechanisms, we conducted experiments using differentiated human preadipocytes isolated from obese individuals. These adipocytes were treated with TNF-α under normal and high glucose conditions. High glucose alone significantly increase in Dectin-1 expression, and this increase was dramatically amplified when TNF-α was added (5-fold increase in mRNA, and 1.6- fold protein, Fig. 4A, B). Notably, TNF-α alone did not changes in Dectin-1 expression in cells kept in normal glucose concentration (5 mM, Fig. 4A, B). Similar experiments with adipocytes from lean individuals showed only a marginally elevation in response to TNF-α and high glucose (Figure S2). This reinforced the idea that this effect is more pronounced in the context of hyperglycemic obesity.

Concurrently, we examined the NF-κB pathway, which is known to be activated by TNF-α. We found that TNF-α increased NF-κB activation, reduced the levels of its inhibitor (IκBα), and altered the levels of other related proteins (Fig. 4C, D), indicating cellular sensitization. These findings underscore TNF-α's role in modulating Dectin-1 expression via the NF-κB pathway in human adipocytes.

Since Dectin-1 gene expression is TNF-α and activated NF-κB dependent, we conducted in-silico analysis which revealed two adjacent NF-κB binding sites located 189 bp and 485 bp upstream of *Dectin-1* translation start side. These loci are conserved in three species, suggesting true NF-κB binding and potential targets for TNF-α signaling (Figure S3), as further confirmed using chromatin immunoprecipitation assay followed by qPCR (ChIP-qPCR). Notably, a significant enrichment of chromatin fragments, at both NF-κB loci, in cells treated with high glucose and TNF-α (Fig. 4E). Finally, we showed that histone acetylation, a marker of active gene transcription, increased at these loci in cells treated with both high glucose and TNF-α (Fig. 4F). This provided a detailed molecular explanation for how TNF-α regulates Dectin-1 expression in adipocytes under hyperglycemic conditions.

Eleaveted Dectin-1 mediates the induction of Interferon Response Factor-5 (IRF5)

Dectin-1-triggered IRF5 activation has been described following recognition of glucans present on tumor cells [38]. As shown in Fig. 5A, our cohort exhibited a positive correlation between the gene expression of Dectin-1 and IRF5, but not with IRF3. Furthermore, a significant increase in IRF5 proteins were observed in adipose tissue (AT) treated with TNF-α, both with and without high glucose (Fig. 5B-C). Alternatively, IRF3 protein, which is not regulated by Dectin-1 downstream signaling, was

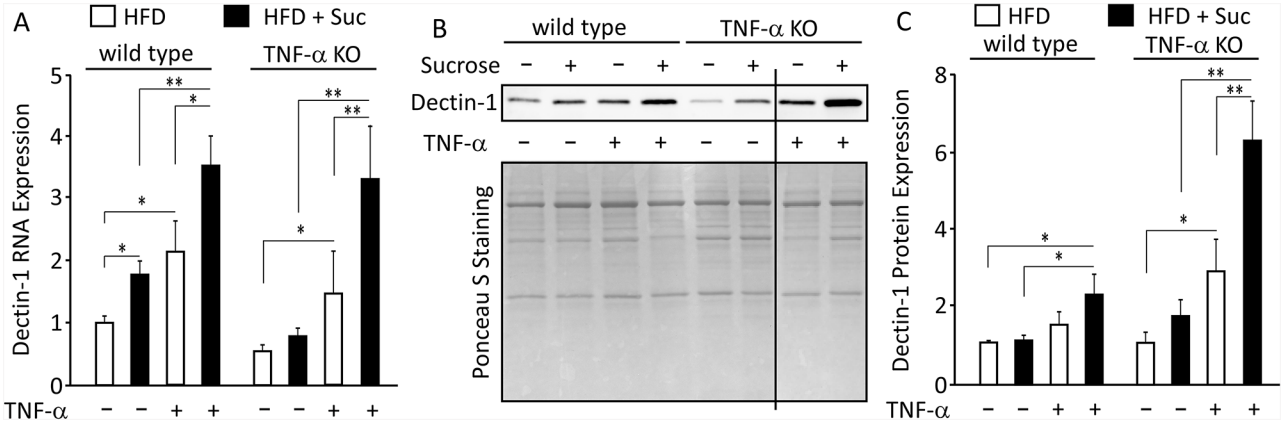


Fig. 3 Hyperglycemia and TNF-α induce the expression of AT Dectin-1 in animal models. **A** Dectin-1 mRNA levels, in AT, were measured by q-RT-PCR in wild-type and TNF-α KO mice fed a high-fat diet (HFD) or HFD supplemented with sucrose (HFD + Suc). Exogenous TNF-α treatment (+) significantly increased Dectin-1 mRNA expression, particularly in TNF-α KO mice (*$p < 0.05$, **$p < 0.01$). **B** Representative Western blot images show Dectin-1 protein levels and TNF-α in AT, with Ponceau S staining indicating equal protein loading (25 mg). **C** Quantification of Western blot data reveals significantly elevated Dectin-1 protein expression in TNF-α KO mice under the HFD + Suc diet with exogenous TNF-α treatment (*$p < 0.05$, **$p < 0.01$). Data are presented as mean ± SEM values obtained from three independent experiments and using 3 animals/group. Two-tailed unpaired Student's t-test was used to determine significance. Abbreviations: TNF-α, tumor necrosis factor-alpha; HFD, high-fat diet; Suc, sucrose

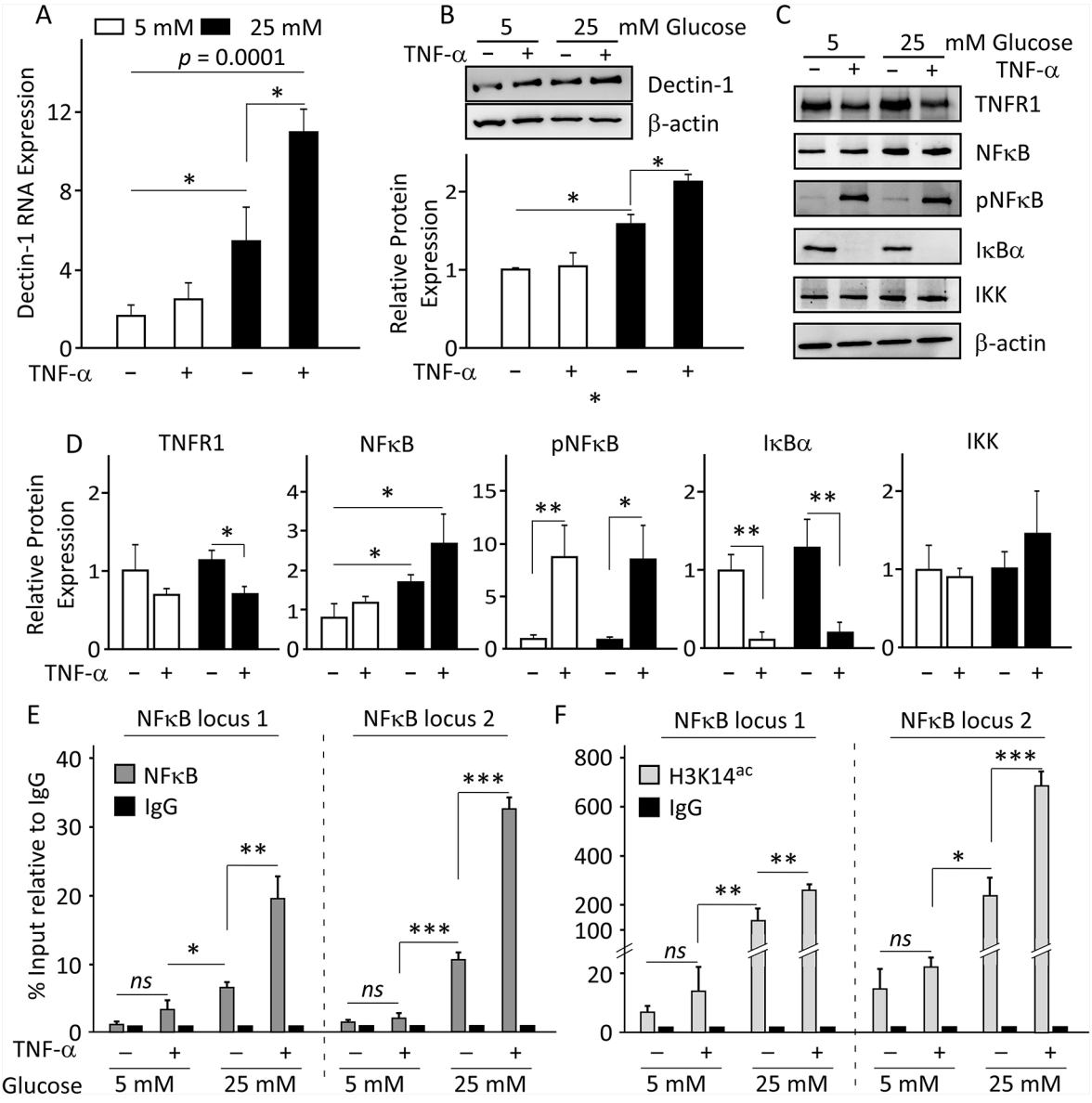


Fig. 4 NF-α and glucose treatments induce Dectin-1 expression and activate NF-κB signaling in differentiated adipocytes derived from obese individuals. **A** Dectin-1 mRNA Expression was measured by q-RT-PCR in differentiated adipocytes from obese individuals treated with or without TNF-α in either normal glucose or high glucose culture conditions. Dectin-1 mRNA expression was significantly upregulated in cells exposed to both TNF-α and high glucose levels. **B** Dectin-1 Protein Expression was determined using Western blot analysis of total protein extracts (25 mg) revealed increased Dectin-1 protein expression in adipocytes treated with high glucose and TNF-α. β-actin was used as a loading control. **C** TNF-α treatment activates the NF-κB signaling pathway in differentiated obese adipocytes. Western blots probed with antibodies against phospho-NF-κB, total NF-κB, IκBα, TNFR1, and IKK demonstrate TNF-α-induced phosphorylation of NF-κB and subsequent degradation of IκBα, indicating activation of the pathway. Additionally, TNFR1 responsiveness to TNF-α signaling diminished after 24 h of treatment, reflecting receptor downregulation. **D** ChIP was performed using antibodies against NF-κB and IgG control, followed by qPCR with primers targeting human DECTIN-1 NF-kB loci 1 and 2. Differentiated obese adipocytes were treated with or without TNF-α and high glucose as indicated. ChIP analysis revealed a significant enrichment of NF-kB binding at the DECTIN-1 loci in cells treated with both TNF-α and high glucose. **E** ChIP was also conducted using antibodies against H3K14^{ac} and IgG control. qPCR targeting DECTIN-1 NF-kB loci 1 and 2 demonstrated increased H3K14 acetylation in adipocytes treated with TNF-α and high glucose. **F** Data are represented as mean ± SEM from three independent experiments. Significance was assessed using a two-tailed unpaired Student's t-test with relative enrichment calculated as the percentage of chromatin input normalized to IgG controls ($n = 3$). Abbreviations: TNF-α, tumor necrosis factor-alpha; TNFR1, TNF-α receptor 1; NF-κB, Nuclear Factor-kappa B; pNF-κB, phospho-NF-κB; IκBα, inhibitor of κB alpha; IKK, IκB kinase. IgG, Immunoglobulin G, H3K14^{ac}, Histone-3 acetyl K14; ChIP, Chromatin immunoprecipitation. (*$p \le 0.05$, **$p \le 0.01$, ***$p \le 0.0006$)

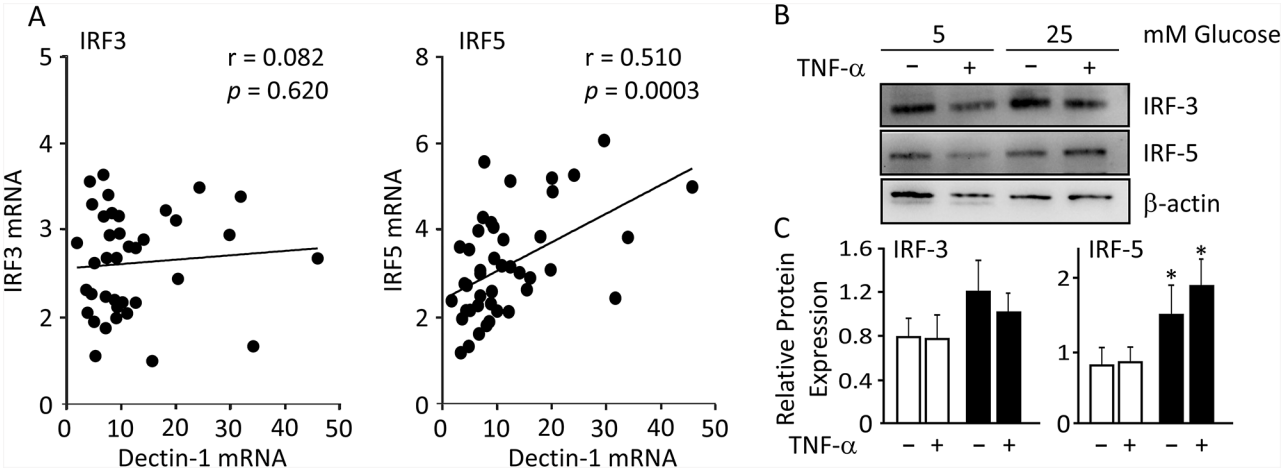


Fig. 5 Eleaveted Dectin-1 mediates the induction of Interferon Response Factor-5 (IRF5). **A** Scatter plots show the relationship between Dectin-1, IRF3 (95% CI, -0.240 to 0.388) and IRF5 (95% CI, 0.254 to 0.699) mRNA expression in diabetic individuals (HbA1c ≥ 6.5), highlighting a significant positive correlation between Dectin-1 and IRF5 mRNA. **B** IRF3 and IRF5 Protein Expression was determined using Western blot analysis of total protein extracts (25 mg) revealed increased IRF5 protein expression in adipocytes treated with TNF-α with and without high glucose. β-actin was used as a loading control. Data are represented as mean ± SEM from three independent experiments. Abbreviations: TNF-α, tumor necrosis factor-alpha. (*$p \leq 0.05$)

moderately elevated under these conditions. These data suggest, at least in part, a functional role for the Dectin-1 signaling pathway in mediating responses to TNF-α under hyperglycemic conditions.

Discussion

Dectin-1 is primarily known for its role in innate immune responses to fungal pathogens. However, recent studies have illuminated its involvement in obesity and diabetes. Our previous research demonstrated that Dectin-1 levels are elevated in the subcutaneous AT of obese individuals compared to lean individuals [33]. Similarly, Castoldi et al. reported elevated Dectin-1 in the mesenteric AT of obese individuals [20].

In this report, we observed a significant increase in *DECTIN-1* gene and protein expression in subcutaneous AT of obese individuals, which positively correlated with HbA1c levels. This finding underscores a potential link between glucose metabolism and Dectin-1 regulation, in alignment with previous findings [20, 39]. Importantly, our research revealed that the upregulation of Dectin-1 in obese individuals is associated with elevated expression of pro-inflammatory mediators, particularly in diabetic individuals. This suggests a broader role of Dectin-1 in mediating inflammation under metabolic stress conditions. Remarkably, the lack of a correlation between AT Dectin-1 and HbA1c in prediabetic individuals may be attributed to the lack of elevated TNF-α levels and the relatively controlled glycemic state - two factors prominently observed in diabetic patients. This hypothesis was mechanistically validated in our study using animal models and cell culture experiments. Moreover, we do not exclude potential elevated reactive oxygen species (ROS) and oxidative stress, which are a characteristic of diabetic conditions and may play a role in enhancing Dectin-1 expression [40]. Further research is required to elucidate the precise molecule pathways involved in these processes.

While, the correlation observations do not establish a causative or consequential role for Dectin-1, existing evidence supports its involvement in the induction of pro-inflammatory cytokines via the spleen tyrosine kinase/Caspase-recruitment domain 9 (Syk-CARD9) signaling pathway [41], a hallmark of metabolic disorders, including obesity, diabetes, and associated complications. Yang et al. reported that dectin-1 mediates diabetic-induced cardiomyopathy by driving inflammatory cytokines production [42]. Upon activation, Dectin-1 triggers downstream Syk signaling, which eventually activates NF-κB, to induce proinflammatory factors [43]. Additionally, Dectin-1 has been shown to active IRF5, amplifying the inflammatory response and activating interferon-beta (INF-β) signaling through the Dectin-1-Syk-IRF5 axis [44, 45]. This dual role in activating both NF-κB and IRF5 underscores its role in metabolic inflammation and its potential contribution to the pathogenesis of diabetes and related complications. In the other hand, pro-inflammatory molecules may also influence Dectin-1 expression indirectly via multiple signaling pathway, including Janus Kinase-Signal Transducer and Activator of Transcription (JAK-STAT) [46], MAPK-ERK [47], and PI3K/Akt [48].

The interplay between Dectin-1 and TNF-α is in fact well established [49]. Consistent with this, we observed co-localization and upregulation of Dectin-1 and TNF-α proteins in hyperglycemic AT, suggesting their interaction in metabolic dysfunction. Our findings show that TNF-α induces Dectin-1 expression in murine AT and differentiated human adipocytes. Haberkamp et al.

recently reported that TNF-α promotes Dectin-2 expression in human macrophages, reinforcing TNF-α's regulatory role on the C-type lectin receptor family [50]. Furthermore, our data demonstrate that glucose and TNF-α treatments induce *DECTIN-1* mRNA and protein expression in human adipocytes, suggesting a direct regulatory effect of these factors. Molecular analysis revealed that NF-κB binds to two loci upstream of the Dectin-1 promoter in response to glucose and TNF-α, providing mechanistic insights into the regulation of Dectin-1 expression in adipocytes. In agreement with our findings, Cortez-Espinosa et al. reported a positive correlation between Dectin-1 expression in monocytes and HbA1c levels (HbA1c > 8.5). However, they observed negative correlations between Dectin-1+/TLR2 + cells and fasting blood glucose (FBG) and HbA1c levels in T cells, B cells, and natural killer cells [51].

On the other hand, Brown et al. (2003) demonstrated that Dectin-1 mediates TNF-α production in response to pathogens [52]. Similarly, Yadav and Schorey showed that blocking Dectin-1 with monoclonal antibodies inhibited TNF-α production by murine macrophages [53]. Shin et al. further demonstrated that dectin-1 is required for efficient bacterial phagocytosis and pro-inflammatory cytokine secretion [53, 54]. Functional analyses have revealed that a mutated form of Dectin-1 exhibits reduced expression, impaired β-glucan binding, and defective cytokine production (e.g., IL-17, TNF-α, and IL-6) by human monocytes, highlighting its critical role in immune response modulation [55]. Taken together, our findings suggest a positive feedback loop in which Dectin-1 upregulates TNF-α expression, while TNF-α and hyperglycemia synergistically enhance Dectin-1 expression in adipocytes. Alternatively, this could represent a cell-specific mechanism where TNF-α and/or hyperglycemia upregulates Dectin-1 in adipocytes, whereas Dectin-1 increases TNF-α expression in immune cells in response to pathogens (see Graphical Abstract).

Adipose tissue is increasingly recognized as an immune organ with critical roles in immune responses [56]. In obesity, altered immune surveillance [57] may inadvertently drive Dectin-1 expression. The observed associations between Dectin-1 and multiple inflammatory markers, particularly TNF-α, suggest that Dectin-1 expression is induced in inflamed AT during early obesity. This induction likely contributes to inflammatory cell infiltration into AT, thereby playing a substantial role in the development of insulin resistance. This aligns with the seminal findings of the Spiegelman group, who first demonstrated the association of TNF-α with AT insulin resistance in obesity using animal models [30].

Conclusion

Dectin-1 plays a crucial role in obesity and diabetes by driving inflammation in AT. Elevated in diabetic-obese individuals, its expression correlates with HbA1c and pro-inflammatory markers, including TNF-α. However, functional studies, including Destin-1 inhibitors, are needed to confirm its causal role. Mechanistically, TNF-α and hyperglycemia upregulate Dectin-1 via NF-κB binding to its promoter region. Dectin-1 amplifies inflammation through Syk-CARD9, NF-κB, and IRF5 signaling, promoting chemokine-driven immune cell infiltration into AT.

These findings highlight Dectin-1 as a potential therapeutic target for metabolic inflammation and its associated complications. While Dectin-1 signaling holds significant promise for therapeutic applications in metabolic inflammation, but further detailed studies, particularly those integrating proteomics and metabolomics, are essential to fully understand the upstream and downstream regulatory mechanisms governing its expression and regulation. Such insights could pave the way for targeted therapies aimed at modulating Dectin-1 in metabolic disorders. Potential strategies for targeting Dectin-1 include the development of pharmacological inhibitors that selectively block its' signaling pathways, thereby reducing chronic inflammation, and the use of gene-editing techniques like CRISPR/Cas9 or RNA interference to downregulate Dectin-1 expression in specific tissues. However, challenges such as achieving tissue specificity and preventing off-target effects are critical, as Dectin-1 is expressed across a range of immune cells, and its role can vary by tissue type. Moreover, there are potential unintended immunological consequences, such as impaired immune responses or immune dysregulation, which could arise from over-inhibition of Dectin-1, underscoring the need for carefully balanced therapeutic interventions. Addressing these challenges will be key to translating Dectin-1 targeting strategies into clinically effective treatments for metabolic inflammation.

The study limitations

While this study provides valuable insights into the Dectin-1 regulation in obesity and diabetes, several limitations must be acknowledged. Although strong correlations were observed, confounding variables, such as environmental and genetic factors, may influence the results. The lack of longitudinal data restricts our ability to assess temporal dynamics and causative relationships. Furthermore, the cohort represents a specific demographic group, potentially limiting the applicability of the results to other populations. Further studies in diverse cohorts are needed to generalize these findings, and validations are needed to strengthen these findings. Technical issues including the use of Ponceau S staining

as a loading control for Western blots, while common, may not detect subtle loading inconsistencies. Further, While the NF-κB pathway is identified as a regulator of Dectin-1, alternative signaling pathways (e.g., JAK-STAT, MAPK) that might also contribute were not investigated. These limitations highlight the importance of cautious interpretation of the data and suggest directions for future research, 987,676 including larger, longitudinally tracked cohorts and more robust technical controls to validate and expand upon these results.

Materials and methods

Study population and anthropometric measurements

This study cohort included 95 Kuwaiti overweight and obese individuals from both sexes (52 males and 43 females) who were recruited in the study at the Dasman Diabetes Institute, Kuwait. The exclusion criteria included pregnant women, patients with pneumonia, cardiovascular diseases, neuropathy, hepatitis, immune complications, cancers, or hematologic disorders as previously described [58–60]. The patient cohort was divided into prediabetics with moderate glycaemia Hb1Ac < 6.5% ($n = 49$; 26 males and 23 females) and diabetics with high glycaemia Hb1Ac ≥ 6.5% ($n = 46$; 26 males and 20 females, Table S1). HbA1c was measured using VARIANT II (Bio-Rad, Hercules, CA, USA). For each category, the sample size was dependent on the sample availability and each participant's decision to be engaged in the research study. Written informed consents were obtained from all study participants in accordance with the ethical guidelines stipulated in the Declaration of Helsinki and approved by the ethics committee of Dasman Diabetes Institute, Kuwait (grant numbers: RA 2010-003; June 2010). Weights and heights were measured using calibrated electronic weighing scales and height-measuring bars. The body mass index (BMI) was determined using the equation [BMI = body weight (kg)/height^2 (m^2)]. Waist circumferences were measured using constant-tension tapes [61]. The IOI 353 Body Composition Analyzer (Jawon Medical, South Korea) was used to determine the whole-body compositions (percent body fat, soft lean mass, and total body water).

Measurement of clinical parameters

Peripheral blood was collected from fasted individuals and evaluated for metabolic and biochemical markers, as has been described previously [62–64]. Fasting blood glucose (FBG) and lipid profiles including plasma triglycerides (TGL), high-density lipoprotein (HDL), low-density lipoprotein (HDL), and total cholesterol (Chol) were measured using a Siemens Dimension RXL chemistry analyzer (Diamond Diagnostics Holliston, MA, USA). Insulin resistance, HOMA-IR, was calculated from basal FBG and insulin concentrations using the following formula: HOMA-IR = fasting insulin (μU/L) × fasting glucose (nmol/L)/22.5. All assays were performed following the instructions of the manufacturers. White blood cell (WBC) count was measured using hematocytometry.

Collection of subcutaneous adipose tissue

As previously outlined [59], human AT biopsy samples (approximately 0.5 g) were obtained from abdominal subcutaneous fat pads adjacent to the umbilicus using standard sterile surgical procedures. Extracted fat tissues were dissected into smaller pieces (50–100 mg), rinsed with cold phosphate-buffered saline (PBS), and preserved in RNAlater for RNA extractions. Alternatively, samples were slice-sectioned using a microtome for immunohistochemistry-paraffin assays. All samples were stored at − 80 °C until use [65, 66].

RNA extraction, cDNA synthesis, and RT-qPCR reactions

RNeasy kit (Qiagen, Valencia, CA, USA) was used to extract the total RNA as described by the manufacturers. The first strand cDNA was synthesized using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems, CA, USA) as previously described [67]. Real-time reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) was performed using specific 20× TaqMan gene expression primers (Applied Biosystems; listed in Table S3) and utilizing the 7900 Fast Real-Time PCR System (Applied Biosystems) as described elsewhere [59]. Relative gene expression to control samples was also calculated using the comparative cycles to threshold (C_T) method, as has been described previously [68]. Results were normalized using glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and means ± standard error of the mean (SEM) are expressed as fold changes in the expression relative to controls as indicated [69].

Cell culture, adipogenesis, and treatments

Pre-adipocytes isolated from lean and obese individuals were purchased from Zen-Bio (USA). Cells were grown in DMEM/F12 media (Invitrogen Corporation, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Invitrogen Corporation) and 100 U/mL of penicillin–streptomycin (Gibco, Carlsbad, CA, USA) and incubated at 37 °C in 5% CO_2. primary pre-adipocytes were differentiated following the supplier's differentiation protocol (Zen-Bio). Briefly, 70% confluent pre-adipocytes were incubated in Adipocyte Differentiation Medium (DM-2) at 37 °C, and 5% CO_2. On day 7, the DM-2 was replaced with Adipocyte Maintenance medium (AM-1) and incubation was continued for another 5–7 days. The media were changed every 2 days. Adipocytes were monitored for lipid droplet appearance in the cytoplasm as previously reported [70]. The differentiation authenticity was confirmed by by adiponectin, Plin2, and PPARg gene

expression, along with immunofluorescence for lipid droplet accumulation using Nile Red staining, as shown in Figure S4. Adipocytes were treated with TNF-α (40 ng/mL, Sigma) or vehicle.

Chromatin immunoprecipitation (ChIP) assays

ChIP assays were performed as described previously [70, 71] using chromatins extracted from pre-adipocytes isolated from lean and obese individuals. Chromatins were immunoprecipitated using anti-NF-κB specific antibodies (L8F6, cell signaling Technology, Danvers, MA, USA), anti-Histone-3 acetyl K14 antibodies ($H3K14^{ac}$, ab176799, Abcam, Cambridge, MA, USA), or non-specific rabbit IgG control antibodies (ab172730, Abcam). Quantitative PCR (qPCR) analyses were performed using the primers flanking the NF-κB locus 1, forward 5'- AG TTTTCCTGAGTCAGCAGTC – 3' and reverse 5'- TG AGTCAAATCATGTGGGCT – 3'; and the NF-κB locus 2, forward 5'- TCCTCATGCCATCCCTCATTTA – 3' and reverse 5'- CATAAACAGGTACTGGATGCCC – 3' primers. For each sample, true association was confirmed using the relative chromatin enrichments to that of a non-specific IgG. Data represent mean ± SEM from three independent biological experiments [72].

Immunohistochemistry assays and western blotting

Immunohistochemistry (IHC) assays were performed as described previously [70, 73]. Processed paraffin-embedded sections of subcutaneous AT were incubated at room temperature overnight with primary antibodies: rabbit polyclonal anti-dectin-1 antibody (ab140039, Abcam) or TNF-α antibody (ab9635, Abcam). After washing process, the slides were incubated for 1 h with goat anti-rabbit conjugated with horseradish peroxidase polymer chain DAKO EnVision Kit (Dako, Glostrup, Denmark), and color was developed using a 3,3'-diaminobenzidine (DAB) chromogen substrate [74]. For image analysis, digital photomicrographs of the entire AT sections (20X; Panoramic Scan, 3DHistech, Hungary) were used to quantify the immunohistochemical staining using ImageJ software (NIH, USA). Dectin-1 AV1 and TNF-α antibody specificity was validated using spleen tissue, as shown in Figure S5.

Western blot analysis was performed as described previously [75]. Briefly, animal tissue or cells were harvested and lysed in RIPA buffer and total proteins were quantified in Quick start Bradford assay (Bio-Rad, USA). Proteins (25 mg) were resolved on 8–12% polyacrylamide gels, transferred to Nitrocellulose membranes (Bio-Rad, Germany). After blocking, the membranes were blotted with the following primary antibodies (Dectin-1, ab140039, Abcam; β-actin, 4970 L; Cell Signaling Technology) and the corresponding horseradish peroxidase-linked secondary antibody (7074P2, Cell Signaling Technology). Proteins were visualized using Super Signal West Femto ECL kit (Thermo-Scientific, USA). Images were captured using the ChemiDoc MP imaging system (Bio- Rad, Germany) as previously described [76]. In Fig. 3, due to differential expression of the housekeeping genes, β-actin and GAPDH at protein levels, at least in part, in response to the different treatments [77], Ponceau S staining was used as proof of equal protein loading (Fig. 3B [78]).

Mice

Male mice (6–7 weeks old; 24 ± 2.7 g) were housed at the Animal Core Facility, Dasman diabetes Institute, Kuwait, in temperature-controlled rooms (22 °C) at 12-h light/dark cycle, with access to food and water *ad libitum.* All experiments on animals were approved by the ethical committee for the use of Laboratory Animals in Teaching and in Research, Dasman Diabetes Institute, Kuwait; in accordance with the guidelines of the Animal Research: Reporting of In vivo Experiments (ARRIVE). Wildtype control mice (B6129SF2/J, Strain #:101045, RRID: IMSR_JAX:101045) and TNF-$\alpha^{-/-}$ mice (B6;129 S-Tnftm1Gkl/J, Strain #:003008, RRID: IMSR_JAX:003008) were purchased from The Jackson Laboratory, USA. Both genotypes were daily injected intraperitoneally with either physiological saline or 100 μg/kg TNF-α. On day 8, mice were scarified, and the subcutaneous AT were harvested, as previously described [70]. AT samples from these mice were obtained, as previously described [79]. Primers used to study murine Dectin-1 RNA levels are listed in Table S3.

Statistical analysis

Statistical analyses were performed using GraphPad Prism software (La Jolla, San Diego, CA, USA) and SPSS for Windows version 19.01 (IBM SPSS Inc., Armonk, NY, USA) as described previously [80]. Data are shown as mean ± standard deviation, unless otherwise indicated. An unpaired Student's *t*-test was used to compare the means between two independent groups. Pearson correlation was used to determine associations between different variables, as has been previously described [80, 81], in all analyses, *p*-value of <0.05 was considered significant, unless otherwise indicated.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-025-06303-x.

Supplementary Material 1: Table S1. Anthropometric measurements and clinical characteristics of the study participants. Table S2. Correlation of Dectin-1 Transcripts, in AT, with the Gene Expression of Various Cytokines/Chemokines, Their Cognate Receptors, and Inflammatory Leukocyte Subpopulations in Diabetic and Prediabetic Cohorts (*p ≤ 0.05, ** p ≤ 0.001). Figure S1: TNF-α induce the expression of AT Dectin-1 in Animal Models. Figure S2: TNF-α and glucose treatment marginally induce Dectin-1 in

differentiated adipocytes isolated from lean individuals. Figure S3: Dectin-1 promoter contains two conserved NF-κB binding sites. Table S3: Primers used in the study. Figure S4: Adipogenesis differentiation authenticity. Figure S5: Dectin-1 AV1 and TNF-α antibody specificity was validated using spleen tissue

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Author contributions
A.A.M., D.H., S.K., R.T., L.M. and P.G. participated in performing experiments and collecting the data; A.A.M., D.H., and R.A. performed data analysis and wrote the manuscript. F.Al-M. reviewed, edited, and provided feedback. A.Al-M., F.Al-M, and R.A. conceived the idea, guided research study, provided material support, procured funds, interpreted data, participated in writing, edited, and approved the manuscript for submission.

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Data availability
The data are available upon request.

Declarations

Competing interests
The authors declare no conflict of interest.

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