Diabetes and inflammation: Fundamental aspects and clinical implications

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Abstract

Aim. – The aim of this paper is to provide the fundamental background of the inflammation theory associated with type 2 diabetes, to discuss the clinical consequences of low-grade inflammation, particularly in terms of cardiovascular risk, and to infer some clinical therapeutic strategies deriving from drugs that already exist or are in development.

Methods. – This non-exhaustive work is the result of a Pubmed® research, based on requests including the following keywords: diabetes, inflammation, innate immunity, obesity, reticulum endoplasmic stress, cytokines, endothelial dysfunction.

Results. – Obesity and type 2 diabetes are linked with a low-grade inflammation state that reflects the activation of innate immunity where metabolic, environmental and genetic factors are implicated. The role of endoplasmic reticulum stress and unfold protein response is underlined. Inflammation markers are predictive for the risk to develop diabetes, and are associated with an increased cardiovascular risk. While lifestyle modifications are followed by an improvement in inflammation markers, treatments inferred from the inflammation theory are of great interest, although quite moderate effects on glycaemic control have been observed with some of them.

Conclusion. – The development of molecules targeting different inflammatory mechanisms could lead in diabetic patients to improvement of both glycaemia and cardiovascular prognosis.

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Keywords: Diabetes; Inflammation; Innate immunity; Obesity; Endoplasmic reticulum stress; Review

Résumé

Diabète et inflammation : aspects fondamentaux et implications cliniques.

But. – Le but de ce travail est de fournir les notions fondamentales de la théorie inflammatoire associée au diabète de type 2, ainsi que d’aborder, d’une part, les conséquences cliniques de l’inflammation de bas grade, notamment en termes de risque cardiovasculaire et, d’autre part, d’en déduire des stratégies thérapeutiques à partir des traitements actuellement disponibles ou en développement.

 Méthodes. – Le contenu de ce travail, nécessairement non exhaustif, est le résultat d’une recherche bibliographique sur PubMed®, à partir des mots-clés : diabète, inflammation, innée, obésité, stress du réticulum endoplasmique, cytokines, dysfonction endothéliale.

 Résultats. – L’obésité et le diabète de type 2 sont liés à une inflammation de bas grade, témoin de l’activation de l’immunité innée où interviennent des facteurs métaboliques, environnementaux, et génétiques. Le rôle du stress du réticulum endoplasmique et de la réponse aux protéines mal conformées est souligné. Les manœuvres de l’inflammation sont prédicatifs du risque de diabète, et sont associés à un risque accru d’événements cardiovasculaires. Si les modifications du mode de vie font évoluter les paramètres de l’inflammation dans le bon sens, plusieurs thérapeutiques issues de la théorie inflammatoire sont également intéressantes, même si pour certaines l’effet sur le contrôle de la glycémie semble modeste.

 Conclusion. – Le développement de molécules ciblant différents mécanismes de l’inflammation est susceptible de conduire à l’amélioration non seulement de la glycémie, mais aussi du pronostic cardiovasculaire des sujets diabétiques.

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Mots clés : Diabète ; Inflammation ; Immunité innée ; Obésité ; Stress du réticulum endoplasmique ; Revue

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1. Introduction

It is now commonly accepted that diabetes is associated with low-grade inflammation. In this overview, the fundamental mechanisms that lead to inflammation and diabetes and, in particular, the pathophysiology of innate immunity activation, and the roles of obesity, endothelial dysfunction and reticular stress, are discussed. The clinical implications of the inflammation theory, including the relevance of inflammation markers as predictors of type 2 diabetes in clinical studies, and the potential treatments of diabetes, inferred from the pathophysiology, are also reviewed.

2. Fundamental links between diabetes and inflammation

2.1. Innate immune-system activation

The innate immune response is considered an adaptive mechanism designed to restore homoeostasis during and after external threats [1]. This rapid, first-line defence mechanism is based on non-lymphoid tissue components [2]. The innate immune system, through germline-encoded receptors called ‘pattern-recognition receptors’ (PRRs), recognizes the conserved components of microorganisms known as ‘pathogen-associated molecular patterns’ (PAMPs). For example, toll-like receptor 4 (TLR4) senses lipopolysaccharide (LPS), a component of the Gram-negative bacterial wall [3]. Macrophages are mononuclear phagocytes involved in immunological and inflammatory processes, the function of which is to provide an immediate defence against foreign organisms [4,5]. Thus, they also play a role in the low-grade inflammation of obesity and diabetes.

The acute-phase response is part of the innate immune response, and comprises a series of specific physiological reactions aimed at containing foreign organisms, repairing tissue damage and recruiting host defence mechanisms [6]. Sentinel cells such as macrophages, endothelial cells and adipocytes detect environmental threats and release inflammatory cytokines, which stimulate the production of acute-phase proteins (APP) [7]. C-reactive protein (CRP) and serum amyloid A (SAA) are the major upregulated APPs in these circumstances [6]. Fibrinogen, tissue factor, complement components, plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA) also tend to increase, but to a lesser extent [6]. Sialic acid, very low-density lipoproteins (VLDLs), lipoprotein activator (tPA) also tend to increase, but to a lesser extent [6]. In addition, stress can cause contraction of the splanchnic vessels of the gastrointestinal tract, resulting in gut ischaemia, which promotes the entry of LPS into the portal system, leading to inflammatory cytokine production by Kupffer cells and, possibly, by hepatocytes and hepatic endothelial cells as well [6].

2.1.1. Role of toll-like receptors (TLRs)

The data suggest that gut microbiota can be considered an environmental factor involved in the control of body weight and energy homeostasis [9,10]. TLRs are important factors in the pathophysiology of chronic low-grade inflammation mediated by gut flora. Mice fed a high-fat diet (HFD) for 2 to 4 weeks exhibit a significant increase in plasma LPS, which is thought to be due to its increased absorption from gut flora during fat digestion. This metabolic ‘endotoxaemia’ can contribute to low-grade inflammation [11]. Moreover, subcutaneous infusion of LPS results in hyperglycaemia, obesity, steatosis, macrophage infiltration of adipose tissue (AT), hepatic insulin resistance and hyperinsulinaemia, while mice deficient in CD14 (the receptor for LPS) are protected against these metabolic consequences [11].

Among their different functions, TLRs are able to sense pathological levels of lipids. Indeed, TLR4 is involved in macrophage cytokine production in response to saturated fatty acids in mice. Shi et al. [12] demonstrated that nutritional saturated fatty acids are potent stimulators of interleukin (IL)-6 and tumour necrosis factor (TNF)-α mRNA expression in macrophage-like cells, whereas food-derived polyunsaturated fatty acids inhibit saturated fatty acid-induced TNF-α mRNA expression [12]. An increase in TNF-α levels was also induced by a single high-fat meal in both patients with the metabolic syndrome and control subjects, with a significant correlation with endothelial dysfunction [13]. Also, macrophages isolated from TLR4-deficient mice have shown blunted cytokine expression in response to saturated fatty-acid treatment.

Kim et al. [14] published the first report documenting the key role of TLR4 in the mechanisms whereby diet-induced obesity induces vascular inflammation and insulin resistance. Their study, conducted with endothelial cells derived from samples of mouse thoracic aorta with HFD-induced obesity, demonstrated that, in the presence of LPS, TLR4 mediates the activation of IL-6 and tumour necrosis factor (TNF) mRNA expression [12]. An increase in TNF-α levels was also induced by a single high-fat meal in both patients with the metabolic syndrome and control subjects, with a significant correlation with endothelial dysfunction [13]. Also, macrophages isolated from TLR4-deficient mice have shown blunted cytokine expression in response to saturated fatty-acid treatment.

2.1.2. Role of stress

Stress in its most common definition is associated with the secretion of hormones such as corticosteroids, catecholamines, glucagon and growth hormone (GH). It is also associated with inflammation: high blood pressure has a proinflammatory effect on endothelium, which can produce IL-6 [6]. Excess corticoid secretion associated with chronic stress can promote visceral obesity: abdominal fat contains high levels of glucocorticoid receptors, and the glucocorticoid-receptor complex binds to the lipoprotein lipase gene promoter, which enhances fatty-acid uptake [6]. In addition, stress can cause contraction of the splanchnic vessels of the gastrointestinal tract, resulting in gut ischaemia, which promotes the entry of LPS into the portal system, leading to inflammatory cytokine production by Kupffer cells and, possibly, by hepatocytes and hepatic endothelial cells as well [6].

2.1.3. Genetic predisposition

Many studies suggest that there is a genetic predisposition to chronic low-grade inflammation. Gene polymorphisms in PRRs can affect the innate immune response. The polymorphism Asp299Gly of the TLR4 gene is known to inversely affect innate immune function and atheroma by attenuating recep-
tor signalling, and is also associated with a decreased risk of atherosclerosis [15]. In addition, the polymorphism is associated with reduced plasma CRP levels, and a decrease in the prevalence of angiographic coronary artery disease and diabetes [16].

2.1.4. Type 2 diabetes and innate immune-system activation

The metabolic syndrome and type 2 diabetes share many metabolic disorders, such as lipid profiles and elevated APP, which are also observed during malignancy and infection [1,8]. Pickup et al. [1] hypothesized that the innate immune system might be involved in the pathogenesis of type 2 diabetes, and demonstrated a graded increase in serum sialic acid concentrations, with the lowest levels seen in healthy individuals and the highest levels in type 2 diabetic patients. The same tendency was found for CRP, IL-6 and SAA levels.

However, the inflammation theory is not the only mechanism involved in the initiation of type 2 diabetes, as the condition does not develop in all subjects or conditions that include a long-term stress response. Genetic or acquired individual factors are also probably involved [8], and triggering factors such as overeating, underactivity, increasing age, psychological stress and smoking are also implicated in the activation of innate immunity leading to insulin resistance [7].

2.2. Obesity and low-grade inflammation, insulin resistance and type 2 diabetes

2.2.1. Adipose tissue composition

AT was long considered an inert mass with the sole function of fat storage. However, it is now recognized that AT is an endocrine organ that secretes numerous adipokines, cytokines and chemokines [4]. Two types of AT have been identified—brown AT and white AT—but only the latter significantly persists throughout life (in humans), so all further discussions of AT in this article refer to this type. White AT is heterogeneous, composed of mature adipocytes, and a stromavascular fraction that includes preadipocytes, fibroblasts, endothelial cells, histiocytes and macrophages [4]. Although preadipocytes have been reported to differentiate into macrophages, it appears more likely that the macrophage population originates as a consequence of the recruitment of blood monocytes [17] or bone-marrow-derived precursors [5].

It is highly likely that the inflammatory process associated with an expanded fat mass may be involved in the development of insulin resistance and type 2 diabetes [4] (Fig. 1). Weisberg et al. [5] demonstrated that AT macrophage accumulation is directly proportional to the magnitude of adiposity, and estimated that the percentage of macrophages in AT ranges from < 10% in lean humans to nearly 40% in the obese. It appears that visceral fat is clearly different from subcutaneous fat in terms of composition, macrophage infiltration, hormone secretion and impact on cardiovascular prognosis.

2.2.2. Cytokines and macrophage phenotypes

Hotamisligil et al. [18] described increases in TNF-α following diet-induced obesity in rodents and its effect on insulin sensitivity. An increased adipose mass has been linked to an increase in numerous other inflammatory molecules, including CRP, PAI-1, SAA, migration inhibitory factor (MIF), resistin, inducible nitric oxide synthase (iNOS), IL-6, colony stimulating factor-1 (CSF-1) and monocyte chemoattractant protein-1 (MCP-1) [4,5], thus reflecting activation of innate immunity. AT macrophages appear to have either an anti-inflammatory phenotype (M2, producing anti-inflammatory cytokines such as IL-10) or a proinflammatory phenotype (M1, producing inflammatory cytokines such as IL-1, IL-6 and TNF-α) [19]. Lumeng et al. [20] observed a switch from M2 to M1 phenotype in mice with diet-induced obesity. Furthermore, bypass-surgery-induced weight loss is associated with a decrease in macrophage infiltration in subcutaneous AT in women. The remaining macrophages produce IL-10, suggesting a phenotype M1/M2-like shift after major weight loss [21].

According to Koistinen et al. [22], TNF-α is weakly expressed in both subcutaneous and deep human AT depots, and such expression is not always modified in obesity. This suggests that AT is not directly implicated in the increased circulating TNF-α levels observed in obesity in humans. It is estimated that 25–30% of the circulating IL-6 is derived from AT [6]. Secretion of IL-6 is higher in visceral than in subcutaneous AT, and

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**Fig. 1.** Visceral adipose tissue-induced insulin resistance. Macrophages from the stromavascular fraction produce tumour necrosis factor (TNF-α) and interleukin (IL)-1 and IL-6, thereby leading to liver and muscle insulin resistance, most notably through c-Jun NH2-terminal kinase (JNK) and IκB kinase-beta (IkKβ) activation. Of the many involved adipokines, leptin can stimulate TNF-α production, whereas adiponectin decreases liver and muscle insulin resistance, and cytokine release. Free fatty acids (FFA) can induce insulin resistance through protein kinase C activation.
the larger proportion is produced by cells of the stromavascular fraction [19,23].

The low-grade inflammation observed in obesity is linked with altered levels of several circulating factors, including CRP, TNF-α, IL-6 and other inflammation markers [5,24]. Adipocytes share some properties with macrophages: both can activate complement, and produce inflammatory cytokines, fatty-acid-binding proteins (FABPs) and many other factors. Also, adipocytes store lipids and regulate metabolic homoeostasis whereas, in proatherosclerotic conditions, macrophages can also accumulate lipids to become foam cells [25].

2.2.3. Macrophage infiltration in white adipose tissue

The increase in macrophage infiltration is significantly correlated with body mass index (BMI) and adipocyte cell size [21]. A study of genetically obese mice has shown that macrophages, identified as crown-like structures (CLS), are prevalent in visceral compared with subcutaneous fat, and are seen surrounding dead adipocytes. Adipocyte cell size is correlated with CLS density in both visceral and subcutaneous fat depots. Although subcutaneous adipocytes are bigger than those of visceral fat, subcutaneous AT contains fewer CLS, suggesting a different susceptibility to death between visceral and subcutaneous adipocytes [26].

The C-C chemokine receptor 2 (CCR2) and its ligand MCP-1 are necessary for accumulation of inflammatory macrophages in AT [19]. MCP-1 and CCR2 knockout mice have fewer macrophages in AT and low inflammatory-gene expression [27]. Mature adipocytes, via adipokine production, stimulate the diapedesis of blood monocytes [17]. This phenomenon is complex, and includes tissue infiltration by monocytes, with activation of capillary endothelium, increased expression of adhesion molecules and adhesion of blood monocytes, followed by their transmigration across the endothelium and their differentiation into macrophages [17].

Obesity is associated with increased leptin secretion, mainly produced by differentiated adipocytes [19]. Leptin, in addition to its key role in food intake and energy expenditure, also regulates immune processes [28,29]. Leptin-deficient mice and humans display an altered immune status [30]. Hyperleptinaemia is associated with an increased inflammatory response, and leptin is able to control TNF-α production and macrophage activation [29]. Conversely, leptin can improve insulin sensitivity through AMP-activated protein kinase (AMPK) activation [19].

Another adipokine—adiponectin—displays anti-inflammatory activity by inhibiting the production of TNF-α and IL-6 by macrophages, and by binding LPS [4]. Adiponectin also decreases hepatic gluconeogenesis and increases lipid oxidation in skeletal muscle [31]. Circulating adiponectin levels are decreased in patients with abdominal obesity, type 2 diabetes and/or coronary heart disease. Adiponectin may even play a protective role against atherosclerosis and insulin resistance [19,24].

Other adipokines also implicated in the modulation of inflammation related to AT include visfatin, an inflammatory adipokine that is highly expressed in carotid plaques [32] and associated with unstable lesions in patients with coronary heart disease [19], as well as vaspin and omentin, which are prominently expressed in visceral fat [19].

There is increasing evidence that AT in obesity is hypoxic. Obesity is associated with increased expression of hypoxia-inducible factor-1α (HIF-1α) in both humans and rodents [33,34]. In human subcutaneous fat, the HIF-1α gene is down-regulated after bypass surgery [21].

Prolonged overnutrition and increased fatty-acid intake can increase nutritional endotoxaemia and stimulate the TLR4–NFκB pathway in adipocytes. This mechanism might also be a causal factor in the macrophage infiltration of AT in obesity.

2.2.4. Inflammation-mediated insulin resistance

Obese mice deficient in TNF-α and iNOS are more sensitive to insulin than are obese wild-type mice. TNF-α directly decreases insulin sensitivity while increasing lipolysis in adipocytes [18]. TNF-α is thought to play a major role in the pathophysiology of insulin resistance in rodents through phosphorylation of the insulin receptor substrate-1 (IRS-1) protein on serine residues. IL-1 and IL-6 are also implicated in insulin resistance in type 2 diabetes, as are other cytokines, such as IL-8 and IL-18 [35].

Other kinases also implicated are those involving the NFκB pathway, IkBα, activating protein-1 (AP-1), e-Jun NH2-terminal kinase (JNK) and protein kinase C-theta (PKCθ) [19,24]. All of these pathways could interact with insulin signalling via serum/threonine inhibitory phosphorylation of IRS [24]. The proinflammatory NFκB pathway is clearly implicated in insulin resistance, as selective inhibition of the NFκB function in liver and AT protects against insulin resistance in nutritional and genetic animal models of obesity [36].

There are also data to suggest that the macrophage itself can lead to insulin resistance in obese patients [37]. Phosphorylation of the insulin receptor-β subunit (p-INSR-β) is significantly reduced in mononuclear cells (MNC) from obese subjects compared with those from normal controls. Ghanim et al. [37] found that MNC are also characterized by increased activation of the inflammatory pathway, including protein kinase C-β2 (PKC-β2), and suppression of cytokine-signalling-3 mRNA (SOCS-3), which may contribute to alteration of insulin signal transduction and, thus, induce a state of insulin resistance in MNC.

2.3. Endothelial dysfunction, inflammation and diabetes

The maintenance of balanced vascular pressure and perfusion, inhibition of thrombosis and induction of fibrinolysis characterize normal endothelial function. Conversely, endothelial dysfunction involves many inflammatory processes, reduced vasodilation and prothrombic properties.

Hyperglycaemia is associated with an increase in cytokine production mediated by oxidative mechanisms [38]. Thus, the excess production of reactive oxygen species (ROS), together with the interaction of advanced glycation end-products (AGEs) with their receptors (RAGEs) on endothelium, lead to cytokine production by endothelial cells. Cytokine overproduction is also associated with endothelial dysfunction. TNF-α can accelerate
experimental atherosclerosis mainly through induction of adhesion molecule expression (vascular cell adhesion molecule-1 [VCAM-1], ICAM-1, MCP-1 and E-selectin) in endothelial and vascular smooth muscle cells, resulting in altered endothelium-dependent vasodilation and promotion of endothelial cell apoptosis [19]. In addition, intermittent high plasma glucose concentrations are associated with greater expression of adhesion molecules than is stable hyperglycaemia [39]. Moreover, the deleterious effects of PKC-δ and PKC-δ activation by hyperglycaemia are implicated, and have been previously described by Brownlee in his unifying theory [40].

In addition to glucotoxicity, lipotoxicity is also associated with endothelial dysfunction. Vascular inflammation and impaired insulin responsiveness were evaluated in aortic samples obtained from wild type (WT) mice compared with TLR4−/− mice fed a HFD. The TLR4−/− mice were protected against HFD-induced vascular inflammation. Incubation of either aortic explants from WT mice or cultured human microvascular endothelial cells with saturated free fatty acids (FFA) was followed by altered signal transduction and inhibition of insulin-stimulated NO production [14].

Many studies have indicated a positive correlation between the acute-phase response, endothelial dysfunction and insulin resistance. Serine phosphorylation of IRS-1, induced by cytokines, is one of the implicated mechanisms. It contributes to impairment of the normal insulin response and NO synthesis, and leads to reduced insulin-induced vasodilation. Increased plasma concentrations of soluble cell adhesion molecules have been reported in overweight and obese individuals, suggesting that increased fat mass is associated with early systemic endothelial activation [41].

2.4. Role of endoplasmic reticulum (ER) stress in type 2 diabetes

2.4.1. ER stress and the unfolded protein response (UPR)

In order to exert their specific functions, proteins have to be synthesized and correctly folded. The ER is a membrane-bound organelle that provides a unique environment for oxidative protein-folding and post-translational modification of polypeptides such as disulphide bond formation. Protein-folding in the ER needs molecular chaperones such as binding immunoglobulin (Ig) protein (Bip) and folding catalysts [42–44].

Three key factors act as sensors of unfolded protein accumulation in the ER: protein kinase (PKR)-like ER kinase (PERK); inositol-requiring protein-1α (IRE-1α); and activating transcription factor 6 (ATF6) (Fig. 2). These are collectively activated to control the load of polypeptides entering the ER lumen, the concentration of chaperones and catalysts of disulphide bond formation, and the machinery for degradation of misfolded protein. When the translational load is heavy, proteins are degraded to maintain ER function and preserve the cell against apoptosis. ER-associated degradation (ERAD) of misfolded proteins can be activated in such situations.

The chaperone protein Bip is bound to the three sensory factors, which keeps them in an inactivated state. However, when stress is present, Bip is released, leading to activation of the three molecules. PERK and IRE-1 become functional by dimerization,
whereas ATF6 transits forward to the Golgi compartment and is activated by enzymatic cleavage [42–45]. PERK is activated by homodimerization and autophosphorylation, and phosphorylates eukaryotic translation initiation factor-2α (EIF-2α). This reduces the protein-folding load and increases translation of ATF4 mRNA, a transcription factor that activates expression of the genes encoding for new protein chaperones, ERAD machinery and enzymes that reduce oxidative stress, and also functions in amino-acid biosynthesis and transport. Conversely, ATF4 also induces transcription of the C/EBP homologous protein (CHOP), a transcription factor that mediates apoptosis [42–45].

IRE-1 is also activated by homodimerization and autophosphorylation, and this induces endogenous endoribonuclease activity, which is followed by splicing of the transcript for X-box binding protein (XBP1) in a molecule that can be translated in XBP1 protein. XBP1 is a nuclear transcription factor that activates genes encoding for ERAD machinery and chaperones. IRE-1α also has RNase activity, which degrades mRNA to reduce the transcriptional load of newly synthesized proteins that require folding. As IRE-1 can interact with JNK [46] and IκK, it can therefore activate the NFκB pathway that, in turn, promotes apoptosis in response to ER stress [42].

ATF6 activation requires transit to the Golgi compartment, where enzymatic cleavage releases an active subunit that acts as a transcription factor to facilitate ERAD activation and chaperone production [42–45]. ER stress stimuli impair polypeptide folding, and induce adaptive increases in chaperones and catalysts, within the ER lumen through UPR sensor activation. This mechanism is designed to preserve cell homeostasis although, when this response cannot resolve ER stress, the cell becomes subject to apoptosis. Several pathways may be then implicated, including CHOP biosynthesis, Ca2+ release from the ER and/or activation of ER-specific caspases [42].

2.4.2. ER stress and UPR in type 2 diabetes
When stressed ER is localized in a β cell, and as unfolded proteins are proinsulin molecules, it becomes understandable that UPR activation is a key pathophysiological mechanism that might be involved in the initiation of diabetes. Proinsulin requires disulfide bond formation for its correct folding, and Ozcan et al. [47] showed that XBP1-deficient mice develop insulin resistance. Conversely, XBP1s overexpression in β cells isolated from male Wistar rats has been shown to impair glucose-stimulated insulin secretion and to increase β-cell apoptosis [48]. In addition, deletion of the CHOP gene in various mouse models of type 2 diabetes has been followed by improved β-cell function [49]. These findings suggest that CHOP is a fundamental factor that links protein-misfolding in the ER to oxidative stress and apoptosis in β cells under conditions of increased insulin demand [49].

2.4.3. Fundamental mechanisms of β-cell ER stress and diabetes
ER stress can be caused by a number of different stimuli, including heat shock, energy deprivation, hypoxia, metabolic dysfunction, drugs, increased levels of circulating cytokines, FFA, nutrient excess and subsequent activation of the mammalian target rapamycin (mTOR) pathway [45,50]. Oxidative protein-folding in the ER can generate ROS that, in turn, can impede protein-folding through direct protein modification, chaperone inactivation and/or depletion of cellular glutathione, thereby creating a vicious cycle of ER stress and oxidative stress [51]. Periodic increases in proinsulin mRNA translation, induced by hyperglycaemia, can generate UPR activation in β cells. Glucose can also generate ROS through numerous oxidative mechanisms and generation of AGEs, as discussed elsewhere [51].

Using cell cultures and mouse models, Ozcan et al. [47] showed that obesity causes ER stress. This stress, in turn, leads to suppression of insulin receptor signalling through hyperactivation of JNK and subsequent serine phosphorylation of IRS-1. FFA cause ER stress and may lead to pancreatic β-cell apoptosis in type 2 diabetes [52].

Nutrient excess is also associated with ER stress involving PERK, in particular. Oyadomari et al. [53] demonstrated this point by selective impairment of PERK pathway signalling in the liver of transgenic mice. This model results in improved glucose tolerance and reduced hepatosteatosis in animals fed a HFD. Scheuner et al. [54] found that a homozygous mutation of EIF-2α that abolishes its regulatory phosphorylation is associated with hypoglycaemia due to defective neoglucogenesis in neonatal mice. In contrast, heterozygosity for the same EIF-2α mutation predisposes adult mice to obesity and insulin resistance [55]. Furthermore, pharmacological induction of EIF-2α phosphorylation with salubrinal has been shown to potentiate—at least in Wistar rats—the deleterious effects of oleate and palmitate on insulin release [56].

Moreover, cytokines are thought to induce ER stress. Cardozo et al. [57] have described how IL-1β and interferon (IFN)-γ can inhibit expression of the sarco/endoplasmic reticulum Ca2+-ATPase pump (SERCA), thereby depleting ER Ca2+ stores in β cells, and leading to ER stress and apoptosis. Another study by the same group suggested that IFN-γ could worsen ER stress induced by IL-1β [58].

Thus, ER stress might be a common pathway that induces both insulin resistance and β-cell loss, thereby leading to type 2 diabetes [44].

3. Clinical implications of inflammation in type 2 diabetes

3.1. Inflammation markers predictive of type 2 diabetes
It has been extensively documented that plasma concentrations of inflammation markers can predict the risk of type 2 diabetes, so what follows is a general overview of studies that have correlated chronically raised inflammation markers, indices of chronic low-grade inflammation and the risk of type 2 diabetes.

The Atherosclerosis Risk in Communities (ARIC) study was a prospective study of 12,330 subjects, aged 45–64 years, from different communities across the USA [59]. After a mean follow-
up of 7 years, 1335 new cases of diabetes were diagnosed. Risk markers were divided into quartiles, and two different adjusted models were applied. After adjusting for age, gender, BMI and waist-to-hip ratio, the odds ratios (OR) for diabetes in the extreme quartile were 1.5 for raised white-cell counts, 7.1 for orosomucoid levels and 2.8 for sialic-acid concentrations [59].

In this cohort and in women only, the same authors reported a significant correlation with other markers, although many were not significant after further adjusting for BMI and waist-to-hip ratio. Only factor VIII was associated with a risk of type 2 diabetes (OR: 1.6), and this association remained significant even after additional adjusting for hypertension, triglycerides and HDL cholesterol [60].

Another study analyzed data from 2924 people enrolled in the Framingham offspring study. Their relative risks of developing diabetes were 1.18 per interquartile range (IQR) for PAI-1 and 1.39 for vWF, which remained significant after adjusting for age and gender, and for other relative risks of diabetes factors, including physical activity, HDL cholesterol, triglycerides, blood pressure levels, waist circumference, homoeostasis model assessment of insulin resistance and inflammation, as assessed by levels of CRP [61].

The Women’s Health Study (WHS), an ongoing US randomized clinical primary-prevention trial initiated in 1992, was the first to establish a correlation between IL-6 and CRP and the risk of diabetes. From a cohort of 27,628 women free of diagnosed diabetes (69% of the initial WHS cohort), cardiovascular disease and cancer at baseline, 188 women developed diabetes over a 4-year follow-up period. They were matched by age and fasting status with 362 disease-free controls [62]. Baseline levels of IL-6 and CRP were divided into quartiles, and were significantly higher among the women who developed diabetes than among the controls. These positive associations persisted even after many adjustments, including BMI. Multivariate relative risks (RR) for the highest vs lowest quartiles were 2.3 for IL-6 and 4.2 for CRP.

The MONICA-Augsburg project, part of the multinational World Health Organization Multinational monitoring trends and determinants in cardiovascular disease (MONICA) study, was designed as three independent cross-sectional surveys carried out in Augsburg, Germany, and its surrounding counties between 1984 and 1995 [63]. A total of 2052 initially nondiabetic men who participated in one of the three MONICA-Augsburg surveys were followed for an average of 7.2 years. A total of 101 new cases of incident diabetes were seen. Men with CRP levels in the highest quartile (CRP = 2.91 mg/L) had a 2.7 times greater risk of developing diabetes compared with men in the lowest quartile (CRP = 0.67 mg/L). However, after adjusting for BMI, smoking and systolic blood pressure, this association became non-significant, thus modifying the strong correlation between CRP and BMI in men [63]. In another analysis of the MONICA-Augsburg cohort, data from 2225 non-diabetic patients were studied [64], including concentrations of CRP and IL-6. After adjusting for age, survey and lifestyle factors, raised concentrations of CRP showed a considerably stronger association with the risk of type 2 diabetes in women (OR for tertile extremes: 7.60) than in men (OR: 1.84). After further adjustment for metabolic risk factors, these associations became non-significant in men, but remained significant in women (OR: 2.57). On the other hand, the OR for IL-6 levels remained significant for both men and women, whatever the adjusted model [64]. There is also evidence of gender differences in the association between diabetes, obesity, endogenous sex hormones and inflammation [65]. High testosterone levels are associated with a higher risk of type 2 diabetes in women, but with a lower risk in men, according to a meta-analysis [66]. This issue is still a matter of debate, however, as many studies have found no such differences [67,68].

The relationship between plasma adiponectin and the risk of type 2 diabetes was unclear until Shanshan et al. [69] performed a meta-analysis that included prospective studies wherein baseline plasma adiponectin levels were measured to predict the incidence of type 2 diabetes. Altogether, 13 prospective studies involving a total of 14,598 participants and 2623 incident cases of type 2 diabetes were included in their analysis. Higher adiponectin levels were clearly associated with a lower risk of type 2 diabetes (OR: 0.72 per 1-log µg/mL increment in adiponectin levels). This inverse relationship was consistently observed in whites, East Asians, Asian Indians, African-Americans and Native Americans, and did not differ according to adiponectin assay, duration of follow-up or gender ratio [70].

The role of endothelial dysfunction has also been associated with the risk of type 2 diabetes. The US Nurses’ Health Study of 121,700 nurses, 32,826 of whom provided blood samples, recorded markers of endothelial dysfunction. In the 737 nurses who developed incident diabetes vs 785 controls, the risk of type 2 diabetes was significantly associated with E-selectin (OR: 5.43) and ICAM-1 values (OR: 3.56) after adjusting for BMI, a family history of diabetes and a number of other factors [70].

Indeed, it appears that many circulating biomarkers of inflammation pathways, such as TNF-α, IL-6, CRP, VCAM-1, ICAM-1, E- and P-selectins, vWF, PAI-1, fibrinogen and adiponectin, may be associated with the development of type 2 diabetes and even with type 1 diabetes, as recently suggested [71]. In addition, a greater frequency of high-sensitivity CRP (hsCRP) levels > 0.5 mg/L was noted in a study including children at risk of type 1 diabetes [72].

### 3.2. Inflammation markers of type 2 diabetes and cardiovascular risk

Inflammation can lead to diabetes, and inflammation markers are increased in diabetic patients compared with non-diabetic subjects. Furthermore, as inflammation markers are associated with endothelial dysfunction, they can also predict cardiovascular risk, as shown in numerous studies of diabetic and non-diabetic subjects. In an analysis from the Women’s Health Study, baseline levels of CRP, SAA, IL-6 and soluble ICAM-1 were all significantly elevated at baseline in apparently healthy women, who subsequently developed myocardial infarction (MI), stroke or cardiovascular death, or needed coronary revascularization [73]. In addition, in a subset of the US Physicians’
Health Study, a prospective study involving 14,916 apparently healthy men, two groups were defined: 202 participants who subsequently developed MI; and 202 participants matched for age and smoking status who did not report any vascular disease throughout the 6-year follow-up. Individuals with baseline IL-6 levels in the highest quartile had a 2.3-fold increased risk of future cardiovascular events compared with those in the lowest quartile [74].

Some data suggest that inflammation markers could be useful for establishing additional cardiovascular risk in diabetic patients. Pu et al. [75] divided 324 diabetic patients into two groups according to the presence ($n = 241$) or absence ($n = 83$) of coronary artery disease. After multivariate regression analysis, the authors found that hsCRP was an independent predictor of coronary artery disease (CAD). In another study, the same authors found that serum levels of hsCRP and TNF-$\alpha$ were clearly higher in diabetic patients with significant coronary artery stenosis than in diabetic patients with normal coronary angiography [76]. More recently, multivariable regression analysis has revealed that hsCRP and sICAM-1 were independently associated with diffuse CAD in diabetic patients [77].

### 3.3. Therapeutic targets based on the inflammation theory in type 2 diabetes

#### 3.3.1. Lifestyle modifications and metformin

A Mediterranean diet has been shown to improve markers of inflammation and endothelial dysfunction [78]. The Diabetes Prevention Program (DPP) studied the effects of intensive lifestyle interventions or metformin on the progression to diabetes compared with a placebo in 3234 adults with impaired glucose tolerance. After 1 year of follow-up, median CRP values in men and women displayed a 33 and 29% respective decrease with lifestyle modifications, and a 7 and 14% respective decrease with metformin [79].

More recently, the Diet and Exercise for Elevated Risk (DEER) trial, a 1-year randomized trial of men and women with the metabolic syndrome plus high LDL and low HDL cholesterol levels [80], showed that the change in CRP was higher for women in the low-fat-diet group, and in the low-fat-diet plus physical activity group, than in the controls [80].

#### 3.3.2. Insulin

The anti-inflammatory effects of insulin are well documented. Insulin is associated with inhibition of the NFkB pathway, MCP-1 and ICAM-1. Insulin lowers CRP, ROS and FFA release, has antithrombotic effects via PAI-1 inhibition, and induces vasodilation via eNOS stimulation [81]. It has also been recently demonstrated that an insulin infusion can decrease TLR4 mRNA expression in monocytes isolated from diabetic patients [82]. However, in a recent randomized study, the use of either metformin or insulin in patients with recent-onset type 2 diabetes did not reduce inflammatory biomarker levels (hsCRP was the primary endpoint, and IL-6 and TNF-$\alpha$ soluble receptors were secondary endpoints) despite improving glucose control after 14 weeks [83].

#### 3.3.3. Anti-inflammatory drugs

The beneficial effects of salicylate on glycaemia have long been known [84]. Aspirin inhibits phosphorylation in vitro of IRS-1 at Ser307 as well as phosphorylation of JNK and c-Jun [85], and IkK$\beta$ activity, thereby preventing activation of the NFkB pathway, which mediates insulin resistance [86]. This molecule was used at high doses (about 7 g/day) in a study of nine diabetic subjects lasting 2 weeks. The experiment resulted in a 25% reduction in fasting plasma glucose, associated with a 15% reduction in total cholesterol and CRP, and a 50% reduction in triglycerides [87]. However, this drug cannot be used at such high doses without a major risk of gastric ulcer and bleeding.

Salsalate, a non-acetylated prodrug of salicylate, is an equipotent inhibitor of NFkB, but with a lower bleeding risk than aspirin. It has been shown, in 20 at-risk obese non-diabetic subjects, to improve insulin resistance, increase adiponectin by 57%, reduce CRP by 34% and lower glucose levels [88]. Its effects were also evaluated in 40 diabetic patients at a dose of 3 g/day. It appears that the beneficial action of the molecule on glycaemia could be due to its effects on insulin concentration rather than any improvement of insulin action [89]. In the recent Targeting Inflammation using Salsalate in Type 2 Diabetes (TINSAL-2D) study, a decrease in HbA1c levels of up to 0.5% was observed in those treated with the highest dose of 4 g/day [90].

Recent data suggest the presence of IL-1$\beta$ in isolated human islets from type 2 diabetes patients, and many authors have referred to ‘insulitis’ in type 2 diabetes [35,91]. Such local IL-1$\beta$ secretion could play a role in macrophage infiltration via induction of chemokines, and could provide new targets for the treatment of type 2 diabetes [92]. Anakinra is a recombinant IL-1 receptor antagonist (IL-1RA) commonly used in the treatment of rheumatic polyarthritis. When 70 type 2 diabetic patients were randomly assigned to receive either 100 mg/day of subcutaneous anakinra or a placebo, the effects at 13 weeks were moderate, with an average decrease in HbA1c of $-0.46\%$, associated with a reduced level of inflammation markers, but with no significant effect on insulin resistance [93]. In the 39-week follow-up of these patients, starting at the time of drug withdrawal to test the durability of the intervention on $\beta$-cell function [94], although CRP and IL-6 were both significantly reduced, no statistically significant difference was observed in either HbA1c or insulin sensitivity between the two groups.

Polymorphisms of the IL1RN gene might be predictive of a response to this treatment, but other studies need to be performed to confirm these results and to test IL-1RA agents in patients with type 2 diabetes, selected on the basis of their IL1RN SNP genotype [94].

#### 3.3.4. Thiazolidinediones (TZDs)

Data consistent with the inflammation theory are also provided by the efficacy of peroxisomal proliferator-activated receptor (PPAR)-$\gamma$ agonists such as the TZDs, which can delay the development of diabetes despite weight gain. TZDs increase whole-body insulin sensitivity and AT triglyceride storage. Activated macrophages and adipocytes express high levels of PPAR-$\gamma$ [95], whereas TZDs promote adipocyte differen-
tiation and stimulate adiponectin production, suggesting that adipocytes and macrophages are major targets of these compounds. PPAR-γ agonists also suppress macrophage production of TNF-α and IL-6 [96].

In a prospective study, pioglitazone significantly decreased insulinemia, LDL/HDL ratio, hsCRP, matrix metalloproteinase-9 (MMP-9), MCP-1 and carotid intima–media thickness, and increased adiponectin plasma levels [97]. There are also extensive data showing that TZDs can improve many of the factors that aggravate atherosclerosis. Many studies have shown the benefits of TZDs on cardiovascular risk factors, including hypertension and dyslipidaemia, and by reducing microalbuminuria, considered a marker of endothelial dysfunction. Indeed, TZDs might even have kidney-protective actions [98], while the Prospective Pioglitazone Clinical Trial in Macrovascular Events (PROactive) has demonstrated that these agents can improve cardiovascular outcomes [99].

In addition, TZDs can exert marked antioxidant and anti-inflammatory actions similar to those observed with insulin, and these aspects have been recently reviewed [100,101]. However, a controversy over rosiglitazone has emerged since the meta-analysis by Nissen and Wolski was published in 2007. The molecule has been suspected of having an association with a higher risk of myocardial infarction (RR: 1.43) [102]. The results of the study have been widely discussed and are still a matter of debate [103]. The multicentre, randomized, open-label Rosiglitazone Evaluated for Cardiovascular Outcomes in Oral Agent Combination Therapy for Type 2 Diabetes (RECORD) study remains inconclusive as regards the possible effects of rosiglitazone on myocardial infarction compared with a combination of metformin and sulphonylurea in type 2 diabetic patients (RR: 1.14, CI: 0.8–1.63). However, the increasing risks of heart failure and bone fractures (mainly in women) have been confirmed [104].

3.4. Drugs related to the ER stress theory

Interesting results have been recently reported with the use of glucagon-like peptide-1 (GLP-1) agonists, now widely used in the treatment of type 2 diabetes. When rat β cells were exposed to either oleate or palmitate, with or without the GLP-1 agonist exendin-4 or forskolin, the molecules protected β cells against FFA via the induction of the ER chaperone Bip and the anti-apoptotic protein JunB [105].

Many studies have reported on chemical chaperones in the treatment of insulin resistance and type 2 diabetes. The 4-phenylbutyric acid (PBA) and tauroursodeoxycholic acid (TUDCA) were effective in reversing ER stress related to insulin resistance in leptin-deficient ob/ob mice [106]. Normoglycaemia was obtained and maintained for up to 3 weeks with PBA treatment. Treatment with these compounds also resulted in restoration of systemic insulin sensitivity, resolution of fatty liver disease, and enhancement of insulin action in liver, skeletal muscle and AT. In another recent study, the same authors found increased ER stress in hypothalamus extracts from mice fed a HFD compared with those fed a normal diet [50]. The development of obesity could correlate with ER stress in the hypothalamus, and activation of UPR signalling pathways blocks leptin receptor signalling in the brains of obese mice. The use of PBA increased the sensitivity of ob/ob mice to the anorexigenic effects of leptin, and led to a significant decrease in body weight. Interestingly, PBA is also able to increase insulin sensitivity in the db/db obesity model, in which mice are deficient in leptin receptor signalling. TUDCA has been similarly tested and appears to be an even more powerful chemical chaperone than PBA. This study was the first to describe chaperones as leptin sensitizers.

4. Conclusion

Inflammation, diabetes and cardiovascular disease are linked via a number of fundamental mechanisms involving the innate immune system. As the roles of obesity, glucotoxicity and lipotoxicity are now well known, there is growing evidence that ER stress is implicated in β-cell loss and inflammation, leading to impairment of insulin sensitivity and secretion and, eventually, diabetes. Of all the potential therapeutic agents, however, sal-salate and IL-1RA led to moderate decreases in HbA1c levels in clinical trials. Nevertheless, chemical chaperones have shown interesting results in animal models and merit further testing in clinical studies. Also, as TZDs have become widely used in the treatment of type 2 diabetes, selective PPAR-γ modulators are now in development and offer new perspectives. Furthermore, as HbA1c is no longer the only objective in the treatment of type 2 diabetes, drugs that can both correct glycaemia and protect the endothelium should also be developed along the lines of the inflammation theory, as they could lead to a decrease in cardiovascular events in diabetic patients.

Conflict of interest statement

The authors declare no conflicts of interest in the field of this review.

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