Obesity-associated low-grade inflammation in type 2 diabetes mellitus: causes and consequences

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ABSTRACT

The epidemic of overweight and obesity is a major problem because of the plethora of health and economic issues that it induces. Key among these is the sharply increasing prevalence of type 2 diabetes (T2D) and cardiovascular disease. The development of T2D is characterised by two processes: 1) insulin resistance, resulting from impaired insulin signalling and leading to an increased demand for insulin, which must be met by increased insulin production by pancreatic β-cells (compensatory β-cell function); and 2) β-cell dysfunction, with T2D developing when the amount of insulin that is produced is insufficient to meet the demand. Overweight and obesity, especially in case of abdominal fat accumulation, are associated with systemic low-grade inflammation. This low-grade inflammation is characterised by, among other things, higher levels of circulating proinflammatory cytokines and fatty acids. These can interfere with normal insulin function and thereby induce insulin resistance, and have also been implicated in β-cell dysfunction. This review focuses on the known and emerging relations between inflammation and T2D. We first discuss current views on the effects of fat distribution on adipose tissue inflammation and adipose tissue dysfunction. Next we focus on the detrimental roles of proinflammatory cytokines and fatty acids on insulin signalling and β-cell function. In the last part of this review we provide some insight into novel players in (the initiation of) inflammation in overweight and obesity, and their effects on T2D and vascular dysfunction.

KEYWORDS

Obesity, insulin resistance, β-cell dysfunction, vascular dysfunction, innate and adaptive immunity

HOW DOES OBESITY CAUSE TYPE 2 DIABETES?

The epidemic of overweight and obesity has caused a dramatic increase in the number of individuals with metabolic abnormalities and premature cardiovascular disease (CVD). The prevalence of diabetes, and especially of type 2 diabetes (T2D), which comprises 80-90% of all individuals with diabetes, also rises sharply with the obesity epidemic. Two processes contribute to the development of T2D. Impaired insulin signalling – also known as insulin resistance – leads to an increased demand for insulin and this increased demand must be met by an increased insulin production by the pancreatic β-cells, a process known as compensatory β-cell function. Thus, obesity-induced insulin resistance will initially lead to higher circulating insulin concentrations but in case of prolonged and/or worsening insulin resistance, β-cells may no longer be able to meet the high demand. This will eventually lead to insufficient hepatic and peripheral glucose disposal, subsequently to higher circulating levels of glucose and eventually to the development of T2D (*figure 1*). In the past three decades, both CVD and diabetes, in particular obesity-induced T2D, have been recognised as inflammatory diseases. The systemic low-grade inflammatory response that is often observed in obesity detrimentally affects both insulin signalling and β-cell function and may thus contribute to the development of T2D.

At the population level, the relative risk of developing T2D rises sharply with an increase in body mass index [BMI], as a measure of excessive body fat. However, within a narrow range of BMI levels, individuals can vary enormously with respect to insulin resistance, and this inter-individual difference has been attributed, to an important extent, to differences in the distribution of fat over the body.[1] In

Figure 1. *Development of insulin resistance and B-cell failure are involved in the development of obesity-associated T2DM*
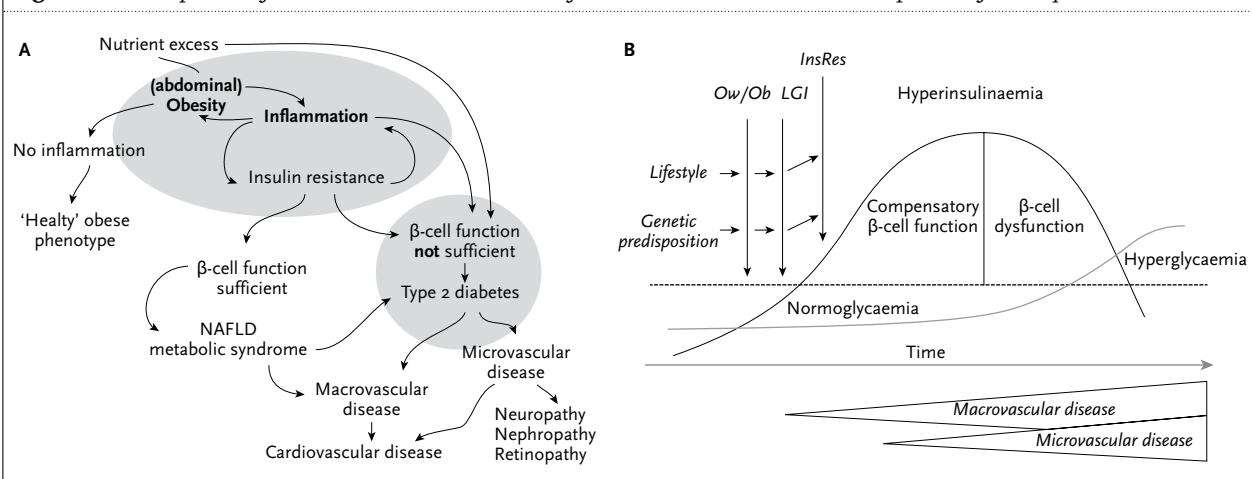
Panel A presents the main events that underlie the development of obesity-associated T2DM and the two main metabolic hubs that are involved, i.e. development of insulin resistance and the development of β-cell failure. Caloric intake in excess of energy expenditure leads to the accumulation of fat. If this fat accumulates primarily in the superficial subcutaneous adipose tissue depot, the low-grade inflammatory response will likely be minimal to absent. If, however, due to genetic and/or lifestyle factors, accumulation of fat is shifted towards the abdominal fat and ectopic depots, a persistent low-grade inflammatory response will develop. This low-grade inflammatory response will lead to cellular insulin resistance and also attract proinflammatory immune cells to adipose tissue, which can worsen the inflammatory response. Insulin resistance increases the demand for insulin, but as long as the pancreatic β-cells can respond with a sufficient compensatory insulin production, this will lead to a state of normoglycaemia with hyperinsulinaemia, which is often associated with dyslipidaemia, hypertension and further ectopic fat accumulation. If, however, due worsening of the insulin resistance and, again, to individual genetic and/or lifestyle factors, the secretion capacity of the β-cells is no longer sufficient, hyperglycaemia and hence T2D will develop

Panel B represents the timeline of these events. Genetic and lifestyle factors most likely determine not only the development of (abdominal) obesity (Ow/Ob), low-grade inflammation (LGI) and insulin resistance (InsRes) but also the time scale (years or decades) it takes to progress through the different stages of the development of obesity-associated T2D. The major vascular complications of T2D, i.e. macrovascular and microvascular disease are each presumed to start prior to the development of hyperglycaemia

particular, at the same BMI, more upper body fat (also referred to as abdominal or central obesity), as represented by a higher waist circumference or higher waist-to-hip ratio, has been found to be associated with a higher risk of T2D compared with less upper body fat.[2,3]

Several different fat depots have been identified, each with specific physiological and metabolic functions. Subcutaneous fat is the largest fat depot in the human body and comprises approximately 70-80% of total body fat. The second largest fat depot is visceral fat, which comprises approximately 10-15% of total body fat.[4] The subcutaneous fat depot should probably not be regarded as functionally homogeneous. For example, it may be divided into peripheral versus central subcutaneous fat, which were shown to have specific and sometimes contrasting metabolic functions.[5-7] Another way to identify metabolically distinct parts of the subcutaneous fat depot is to divide it into superficial and deep subcutaneous fat. This distinction appeared to be particularly relevant for abdominal subcutaneous fat, where the deep subcutaneous depot appeared to behave metabolically more similar to visceral than to superficial subcutaneous fat.[8]

Visceral (sometimes referred to as abdominal and/or omental) fat is generally considered the 'bad' fat depot. Adipocytes within the visceral fat depot show substantially higher fatty acid fluxes than superficial subcutaneous

abdominal adipocytes.[9] These non-esterified fatty acids (NEFA), often referred to as free fatty acids, can contribute to insulin resistance and β-cell failure (see below). Visceral fat is characterised by higher secretion of proinflammatory cytokines such as tumour necrosis factor (TNF)-α and interleukin (IL)-6 and lower secretion of adiponectin, the anti-inflammatory adipokine, as compared with abdominal subcutaneous fat.[10,11] Both visceral and deep subcutaneous fat were shown to be associated with insulin resistance.[4,12-15]

In addition to these main fat depots, there are additional fat depots that are usually referred to as 'ectopic' fat. These additional fat depots are considerably less important in volume, but appear highly relevant with respect to regulatory and metabolic functions. Generally, ectopic fat depots are larger in individuals who have a more central fat distribution with a relatively large amount of visceral fat. Ectopic fat includes, for example, perivascular as well as epicardial and pericardial fat depots – which are relatively small but distinct patches of fat around the vasculature and the heart – and intramuscular and intrahepatic fat – which is the accumulation of triglycerides within muscle and liver, respectively.

The adipose tissue depots that are in close proximity to the vasculature and the heart have been implicated in the development of vascular dysfunction,[16] probably via locally produced mediators that can contribute to a local

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inflammatory response[17,18] as well as to local insulin resistance, which may directly affect vascular function[19,20] and might as such contribute to hypertension and CVD (see also below).

Intramuscular fat is mainly derived from the circulation and the amount of fat that is accumulated in muscle was associated with whole body insulin resistance.[21] Indeed, T2D patients were shown to have more visceral and intramuscular fat than non-diabetic controls.[22] Moreover, intramyocellular triglyceride content in the soleus muscle was 40% higher in offspring of T2D parents than in control subjects,[21] suggesting that increased intramuscular fat may precede and contribute to the development of T2D. The main sources of hepatic fat are endogenous fatty acids, which are newly synthesised in hepatocytes, and (diet and adipose tissue derived) exogenous fatty acids. Fat accumulation in the liver (mainly as triglycerides) is currently considered an important risk factor for metabolic and cardiovascular diseases. Uncomplicated hepatic fat accumulation (steatosis) is the first stage of the full spectrum of non-alcoholic fatty liver disease (NAFLD). NAFLD may progress from simple steatosis to steatohepatitis, fibrosis and eventually liver cirrhosis. Obesity and obesity-associated T2D are mostly associated with the earlier NAFLD stages (steatosis, steatohepatitis), although also late stages of NAFLD i.e. liver cirrhosis, were shown to be associated with a high prevalence of T2D.[23] The 'portal theory' is the concept that, with an increasing amount of visceral fat, the liver is exposed to higher concentrations of proinflammatory cytokines and NEFA that are released from the visceral fat depot and directly transported, via the portal vein, to the liver where they contribute to the development of NAFLD.[24] Thus, the occurrence and severity of visceral fat accumulation and NAFLD are highly correlated and inflammatory changes in visceral adiposity and NAFLD are aetiologically intertwined. Hence, it is not easy to dissect their independent contributions to the development of obesity-associated T2D, especially in humans. The general view is that NAFLD adversely affects insulin resistance and the risk of T2D and CVD.[25] The visceral fat depot, in turn, is highly relevant in an aetiological sense, as it precedes and induces the development of NAFLD and other ectopic fat depots. In addition, visceral fat may contribute directly to systemic low-grade inflammation and increased systemic levels of NEFA.

But it appears that not all fat is bad. It has consistently been shown that approximately 25-30% of obese individuals do not develop insulin resistance; these are the so-called healthy obese. There is also accumulating evidence that expansion of the fat depot(s) will not by definition lead to an inflammatory response and insulin resistance. Efficient expandability of the superficial subcutaneous fat depot, through e.g. intrinsic genetic properties and/or an attenuated inflammatory response, is likely to improve flexibility to process excess caloric intake with limited triglyceride overflow into the visceral and ectopic fat depots. A large capacity for storage of triglycerides in the superficial subcutaneous, metabolically less active fat depot, may thus result in less 'overflow' of triglycerides into the deep subcutaneous and visceral fat depots.[26,27] Very recently it was indeed shown that upon feeding healthy men a high-fat diet, accumulation of fat in the visceral fat depot was highest in those subjects who had the lowest expression of lipid storage-related genes in their subcutaneous fat.[28] The possibility that subcutaneous adipose tissue function and/or inflammation may contribute to redistribution of fat towards the visceral depot is also corroborated by recent data that infiltration of macrophages into human abdominal superficial subcutaneous adipose tissue was associated with larger visceral fat depots.[29] Accordingly, the expression of inflammation-related genes was significantly upregulated in abdominal subcutaneous adipocytes of obese, as compared with non-obese individuals.[30,31] In line with these data, we recently showed that preadipocytes isolated from subcutaneous adipose tissue of T2D patients had a gene expression profile that was consistent with a decreased differentiation capacity.[32] In animal models subcutaneous fat expansion could, for example, be achieved by fat-specific overexpression of adiponectin in genetically obese mice, which resulted in increased peripheral obesity but less accumulation of ectopic fat (visceral, liver, muscle) with significant improvement in insulin resistance. Adiponectin-overexpressing mice showed an increased expression of peroxisome proliferator-activated receptor (PPAR)-γ target genes and, despite massive obesity, had few macrophages in their fat depots, concomitant with lower plasma IL-6 and TNF-α levels.[33] Notably, recent data show that adiponectin can exert part of its anti-inflammatory effects on adipose tissue via regulation of microRNAs that can suppress intracellular proinflammatory pathways, such as toll-like receptor (TLR)-4 signalling (see below).[34] MicroRNAs comprise a promising new field of potential novel treatment targets for insulin resistance and T2D, because they appear to have a vast functional and regulatory capacity, also in other pathways that may contribute to insulin resistance and T2D.[35] PPAR-γ activation by rosiglitazone in mice was also associated with higher body weight and adipose tissue expansion, but with less accumulation of fat in the liver. In these mice a higher macrophage infiltration into adipose tissue was seen, but these were primarily alternatively activated (M2) macrophages that are considered to have anti-inflammatory capacities (see below), and their presence was associated with ameliorated insulin resistance.[36]

Together, current data suggest that visceral/omental, abdominal deep subcutaneous, as well as ectopic fat depots appear to be the culprit fat depots with respect to the generation of an inflammatory response and insulin resistance. There may, however, very well be underlying

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metabolic characteristics of the (superficial) subcutaneous fat depots that contribute to the size of these visceral/omental depots. Prevention of adipose tissue dysfunction, of (visceral) fat inflammation, and of ectopic fat deposition may therefore all help to maintain a metabolically healthy obese phenotype.[37]

Obesity – fat distribution | Key points:
- Obesity is strongly associated with T2D
- Visceral, abdominal deep subcutaneous, and ectopic fat were all shown to be associated with an adverse metabolic phenotype
- Subcutaneous fat, especially of the lower body, may have metabolically beneficial functions
- Better capacity for triglyceride storage in adipocytes of superficial subcutaneous fat may prevent overflow of triglycerides into the metabolically unfavourable fat depots

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HOW DOES OBESITY CAUSE CHRONIC INFLAMMATION?

It is currently well-accepted that that obesity promotes a state of chronic low-grade inflammation,[38-40] which is reflected not only by an increased production of cytokines and proinflammatory adipokines by adipose tissue, but also by a cellular component. Adipose tissue is heterogeneous in composition and contains, besides mature adipocytes, also immature adipocytes (preadipocytes), endothelial cells, fibroblasts, macrophages and other immune cells. Adipose tissue macrophages are largely bone marrow derived and their number is increased in obesity.[41] Thus, local production of chemoattractants that enhance the homing of monocytes to adipose tissue depots can contribute to adipose tissue inflammation. Macrophages in adipose tissue are overrepresented around dead or dying adipocytes, thereby forming so-called crown-like structures.[42,43] This suggests that adipocyte necrosis may underlie the proinflammatory response and macrophage attraction, but at present their concomitant presence represents an association and a direct causal relation remains to be established.[44]

Accumulation of abdominal fat can induce inflammation via several mechanisms. For example, caloric intake in excess of energy expenditure will lead to expansion of adipose tissue and adipocyte hypertrophy, which may be associated with local hypoxia and adipocyte apoptosis, which in turn generate signals to recruit macrophages.[45] Hypertrophic adipocytes begin to secrete low levels of TNF-α, which stimulate preadipocytes and endothelial cells to produce monocyte chemotactic protein (MCP)-1 (also known as CCL2).[46] Indeed, in a study of monozygotic twins it was shown that *acquired* obesity is characterised by adipocyte hypertrophy and increased expression of the macrophage marker CD68 and TNF-α in subcutaneous abdominal adipose tissue.[47] These proinflammatory changes in acquired obesity were associated with an increase in insulin resistance.[47] In addition to proinflammatory effects induced by local hypoxia, the high rate of protein synthesis during adipose tissue expansion may lead to accumulation of unfolded or misfolded proteins and hence to endoplasmic reticulum (ER) stress,[48-50] which may then also contribute to the production of inflammatory and chemotactic signals.

The exact signals from adipose tissue that initiate macrophage infiltration have not yet been identified. In obesity, TNF-α production is increased in both the adipocyte and the macrophage fraction of adipose tissue and an increase in MCP-1/chemokine (C-C motif) ligand (CCL2) that may be induced by TNF-α has been proposed as primary macrophage attractant[51,52] although these data are not fully consistent.[53] Recently it was shown that TNF-α also induces the production of CXCL5, which is a strong chemoattractant for macrophages. Moreover, mice that were knock-out for the receptor for CXCL5 (i.e. CXCR2) or treated with anti-CXCL5 were less insulin resistant.[54]

Macrophages that are located within the adipose tissue may be pro- or anti-inflammatory, depending on their activation status.[55] Classically activated macrophages (referred to as M1 macrophages) are considered proinflammatory, and the M1 status is induced by, among others, TNF-α and lipopolysaccharides (LPS). Alternatively activated macrophages (referred to as M2 macrophages), on the other hand, primarily function to resolve or dampen the M1-induced inflammatory response and are therefore considered anti-inflammatory. The M2 status is induced by, among others, IL4 and IL10. Although the M1 versus M2 status is a gradient rather than a black-and-white phenomenon, macrophages present in adipose tissue in obesity appear to be predominantly polarised towards the M1 phenotype.[56]

Obesity – chronic low-grade inflammation | Key points:
- Adipocyte hypertrophy, hypoxia and stress may all be involved in adipose tissue inflammation via induction of pro-inflammatory cytokines, as well as of chemokines that attract macrophages
- Adipose tissue macrophages may have a pro-inflammatory (M1), an anti-inflammatory (M2), or an intermediate phenotype, depending on the activating cytokines that are present
- In obesity, macrophages in adipose tissue were shown to be mainly M1

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HOW DOES INFLAMMATION CAUSE INSULIN RESISTANCE?

Insulin resistance is a state in which the sensitivity of target cells to insulin, especially with regard to its metabolic actions, is reduced. Inflammatory cytokines, with TNF-α and IL-6 as most extensively studied examples, can directly induce insulin resistance and the level of insulin signal transduction, by using a physiological negative feedback mechanism of normal insulin signalling.[57] Binding of insulin to its functional receptor induces autophosphorylation of tyrosine residues on the intracellular part of the receptor. In the so-called metabolic pathway of insulin signalling, the insulin receptor substrate (IRS), docks the insulin receptor and is trans-phosphorylated in its tyrosine residues via the kinase activity of the phosphorylated insulin receptor. Subsequently, more members of the insulin signal transduction pathway, including phosphatidylinositol-3-kinase (PI3K) and Akt/protein kinase B (PKB), are recruited and activated in order to induce downstream effects.[58] Insulin signal transduction via PI3 kinase mainly affects metabolic pathways such as GLUT-4 translocation and inhibition of hormone-sensitive lipase. The other main pathway of insulin signal transduction involves signal transduction via the renin-angiotensin system/ mitogen-activated protein (Ras/MAP) kinase pathway and primarily stimulates mitogenic rather than metabolic processes.[58]

Several processes interrupt signalling via the insulin receptor in order to maintain a physiological insulin response. Firstly, protein phosphatases can dephosphorylate the insulin receptor and the IRS proteins; secondly, there may be ligand-induced downregulation of the insulin receptor; and thirdly, insulin receptor signalling induces pathways that inhibit signalling via the insulin receptor. The *physiological* negative feedback mechanism is induced when insulin activates mTOR and PKCζ. These intracellular serine (ser)/threonine (thr) kinases can then either directly, or indirectly (e.g. via IkappaB kinase beta (IKKβ)), phosphorylate ser/thr residues in IRS. Ser/thr phosphorylation of IRS, which occurs at multiple residues in the IRS protein, hampers its tyrosine phosphorylation via insulin receptor and thus interrupts, or at least reduces, insulin signal transduction via the IRS proteins.[59] In addition, ser/thr phosphorylation can induce dissociation of IRS proteins from the insulin receptor,[60,61] induce degradation of IRS proteins,[62,63] remove IRS proteins from complexes that keep them in close proximity to the insulin receptor,[64,65] and turn IRS proteins into inhibitors of insulin receptor kinases.[66]

There are various other intracellular and extracellular substances that can also induce ser/thr phosphorylation of the IRS proteins and thereby hamper insulin signalling, but with *pathophysiological* consequences.[57] These include,

for example, the proinflammatory cytokines TNF-α,[67-69] IL-6[70] and IL-1α,[71] and saturated NEFA,[72] which are all involved in obesity-associated low-grade inflammation. These factors employ various intracellular ser/thr kinases such as Jun NH₂-terminal kinase (JNK), protein kinase C (PKC), IKKβ and mTOR,[57,72] which can be activated via multiple mechanisms. IKK-β is particularly interesting in this respect since it is a central effector protein in the inflammatory responses that are activated upon stimulation of the intracellular protein transcription factor NF-κB. Notably, the factors described here mainly affect signal transduction via IRS, and it has indeed been shown that it was the PI3 kinase pathway that was impaired in obesity and in T2D, while insulin signalling via MAP kinase was largely unaffected.[73] Moreover, (saturated) fatty acids, TNF-α and IL-6 have all been demonstrated to induce insulin resistance in healthy humans,[74,75] suggesting that the above-described induction of insulin resistance is indeed relevant in humans, even though a large body of information was obtained in cell and animal studies.

Obese, hypertrophic and/or insulin resistant adipocytes were shown to have an increased release of fatty acids. Specifically, the saturated fatty acids that are released can, in a paracrine fashion, activate the TLR-4/NF-κB pathways on macrophages in adipose tissue, which then release TNF-α, which in turn binds to TNF receptors on the adipocytes, further stimulating fatty acid release and thus inducing a vicious cycle of worsening inflammation and insulin resistance.[76,77] JNK is activated upon exposure not only to cytokines and NEFA, but also to internal cues such as ER stress.[49] Given the relevance of NAFLD in insulin resistance and T2D, it is also of interest that experimental activation of JNK in the liver appeared to be sufficient to induce systemic insulin resistance.[78] The proinflammatory effects of fatty acids appeared to be mainly restricted to the saturated fatty acids while unsaturated and in particularly ω-3 fatty acids, in contrast, appeared to exert anti-inflammatory effects.[79,80]

Inflammation – insulin signalling | Key points:
- In obesity and T2DM it is insulin signalling via IRS and PI3 kinase – i.e. the metabolic pathway – that is primarily affected
- Inflammatory cytokines, e.g. TNF-α and IL-6, as well as saturated fatty acids can all hamper insulin signalling via the IRS and PI3 kinase pathway via activation of intracellular ser/thr kinases
- IKK-β and JNK are important intracellular mediators in metabolic insulin resistance

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HOW DOES INFLAMMATION CAUSE β-CELL DYSFUNCTION?

The healthy β-cell has a large capacity to maintain normoglycaemia via an increase in β-cell mass and subsequent hyperinsulinaemia.[81] However, once the demand for insulin exceeds its production, hyperglycaemia will develop (*figure 1*). β-cell failure can result on the one hand from an intrinsic insulin secretion defect in existing β-cells and on the other hand from reduced β-cells mass.[82] β-cell failure may be partly due to genetic and partly to acquired factors. It is probable that genetic disposition may render some individuals more sensitive to those acquired factors than others. Prolonged exposure of pancreatic β-cells to high levels of glucose and lipids, also known as glucotoxicity and lipotoxicity, may contribute to oxidative stress – potentially via effects on mitochondrial function – and to high rates of β-cell apoptosis in T2D.[83-86] Moreover, impaired insulin signalling may add to β-cell dysfunction.[87,88] In addition, inflammatory cytokines may also contribute to β-cell dysfunction and, as such, to enhanced development of T2D.[89]

Hyperglycaemia can induce the production of IL-1β by β-cells,[82,90] and this proinflammatory cytokine was shown to be involved in β-cell deterioration in both T1D and T2D.[91,92] IL-1β may, via induction of specific signal transduction pathways that include Fas (CD95), initially induce β-cell proliferation, but with prolonged hyperglycaemia switch to increased β-cell apoptosis.[93] Notably, leptin, which circulates in considerably increased concentrations in obesity, was shown to increase the release of IL-1β by β-cells.[94] In addition to its effects on β-cells, IL-1β may also induce insulin resistance via direct effects in insulin signalling. For example, IL-1β can down-regulate IRS mRNA expression in adipocytes.[95] The relevance of IL-1β in human T2D, and in particular β-cell function, was recently shown in a placebo-controlled proof-of-concept study with an IL-1 receptor antagonist.[96,97] Clearly, the effects of IL-1β are not the only way through which β-cell mass and function are affected in the development of T2D, but the IL-1β pathway is a relevant representative of the many (inflammatory) pathways that are involved in the generation of β-cell failure in response to obesity-associated low-grade inflammation and the concomitant increased insulin demand.

Pancreatic lipotoxicity partly results from dyslipidaemia (high small dense LDL cholesterol, low HDL cholesterol, high NEFA) and partly from accumulation of fat (triglycerides) in the pancreas as an ectopic fat depot. Increased concentrations of NEFA, particularly saturated fatty acids, were shown to be harmful for β-cells, in among other ways via the induction of IL-1β,[98] and induced an inflammatory response in pancreatic islets.[99] NEFA also induced the local production of other IL-1-dependent proinflammatory cytokines such as IL-6 and IL-8.[99] It was also recently shown that insulin gene transcription was decreased when JNK was activated by palmitic acid in pancreatic β-cells.[100] In addition, reduction of pancreatic triglyceride content was shown to improve insulin secretion capacity.[101]

The effects of lipotoxicity may be enhanced in case of hyperglycaemia.[102] Thus, both glucotoxicity and lipotoxicity induce local production of cytokines and inflammation in pancreatic islets, but it remains to be established to what extent circulating cytokines can also directly affect β-cell survival at their systemic concentrations, although they do appear to affect the secretory function of β-cells, *in vitro*.[103] Other mechanisms that were proposed to explain β-cell failure in obesity-associated T2D include ER stress, oxidative stress and amyloid deposition. Most of these mechanisms have also been implicated in inflammation, either because they induce a (local) inflammatory response or because they result from inflammation.[104] The detrimental effects of inflammation on β-cell function may be particularly relevant in situations of a sustained inflammatory response, as is probably the case in obesity and associated glucose and lipid overload.[104] Increased numbers of macrophages have been shown in pancreatic islets of T2D patients,[99,105] most likely in response to increased islet expression of IL-1β and chemokines.[99,106]

Inflammation and β-cell failure | Key points:
- Glucotoxicity and lipotoxicity may both contribute to β-cell failure, in among other ways via induction of local production of cytokines, e.g. IL-1β, and hence of inflammation in the pancreatic islets
- ER stress, oxidative stress and amyloid deposition may also induce inflammation and β-cell failure in obesity-associated T2D

HOW DOES INFLAMMATION CAUSE MACROVASCULAR DISEASE IN T2D?

CVD comprises the major long-term complication of diabetes. Various aspects of (obesity-associated) inflammation and macrovascular disease have been extensively reviewed elsewhere.[16,107-109] In short, atherosclerosis, the main process underlying macrovascular disease, starts with activation of the endothelial cells that line the intima. Endothelial cell activation, which may be induced by e.g. lipids (including NEFA and cholesterol) or inflammatory cytokines,[16,107-109] can lead to expression of leucocyte adhesion molecules and binding of leucocytes, which migrate through

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the endothelium to the intima where they can attract monocytes which ultimately transform into lipid-laden foam cells. These processes may be enhanced in T2D. Further progression of the atheroma and generation of rupture-prone atherosclerotic plaques involves a complex interplay of immune cells and inflammatory mediators. Inflammatory pathways are also involved in thrombosis, the late complication of atherosclerosis which is responsible for most of the complications of macrovascular disease.[107] Macrovascular disease is thus perceived to be a major consequence of obesity-induced inflammation and T2D.

HOW DOES INFLAMMATION CAUSE MICROVASCULAR DYSFUNCTION?

Microvascular dysfunction may not only be a resultant, but also a cause of T2D and hypertension. We recently showed that microvascular dysfunction was associated with a higher incidence of T2D[110] and other studies showed that diet-induced insulin resistance in the microvasculature develops before the development of skeletal muscle insulin resistance.[111,112] How can microvascular dysfunction affect the development of insulin resistance, T2D, and hypertension?

Obese insulin-resistant humans and rats are characterised by impaired capillary recruitment, which has been shown to be necessary for normal insulin-mediated glucose uptake by skeletal muscle.[113] Such microvascular dysfunction may result from increased systemic concentrations of NEFA and inflammatory cytokines, and decreased concentrations of adiponectin, which can induce endothelial insulin resistance, reduce local NO production, lower insulin-mediated glucose uptake in muscle by as much as 40% and, as such, contribute to whole body insulin resistance. Microvascular dysfunction may be further aggravated in the expanding adipose tissue since adipose tissue produces all factors of the RAS necessary to produce angiotensin II, and RAS activity is enhanced in obesity.[113,114] Perivascular fat around resistance arterioles of muscle may directly affect the function of these vessels and indeed it appeared that in lean mice perivascular fat had a beneficial effect to stimulate insulin-induced vasodilation due to local adiponectin production, which was hampered in obese mice.[115] Moreover, this impairment in obese mice was ameliorated by inhibition of JNK.[115]

Microvascular dysfunction may also contribute to the vicious cycle of adipose tissue dysfunction and inflammation. Functional capillaries in the expanding adipose tissue are necessary to provide optimal blood flow and delivery of nutrients and oxygen to adipocytes. Thus, insufficient adipose tissue angiogenesis and capillarisation may lead to hypoxia and induction of an inflammatory response.[116] A relative reduction in the density of the capillary network combined with microvascular dysfunction may therefore aggravate the hypoxic and inflammatory processes in adipose tissue depots and thus lead to deterioration of insulin resistance and metabolic homeostasis.[117]

Microvascular dysfunction may additionally contribute to the development of T2D via effects on β-cell function. For example, transient periods of (mild) hyperglycaemia that coincide with insulin resistance as well as low-grade inflammation – possibly in combination with increased NEFA and dysregulation of adipokines – may lead to reduced islet perfusion and (mild) islet ischaemia,[118] and control the recruitment of inflammatory cells to the islets.[119]

Interestingly, microvascular dysfunction is also thought to contribute to the development of hypertension (reviewed elsewhere[120]), and may thus provide an explanation, at least in part, for the typical co-occurrence of insulin resistance and hypertension in obesity.

Inflammation – vascular disease | Key points:

- Endothelial dysfunction: a shared factor underlying both micro- and macrovascular dysfunction
- Macrovascular disease is a major consequence of obesity-induced inflammation and T2D
- Microvascular dysfunction may be both cause and consequence of obesity-induced inflammation and T2D
- Microvascular endothelial insulin resistance may lead to reduced capillary recruitment in muscle and, as such, contribute to whole body insulin resistance
- Microvascular dysfunction may also contribute to adipose tissue hypoxia and dysfunction

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INITIATION OF INFLAMMATION IN OBESITY: RECENT INSIGHTS

Although the concept of low-grade inflammation as an important causal factor in obesity-associated insulin resistance is currently well accepted, less is known about the processes that induce the inflammatory response in adipose tissue. Several processes have been proposed, including the above-described adipocyte hypertrophy, apoptosis and macrophage infiltration, which most likely act simultaneously. Recently, inflammasomes have been proposed as central regulators of early adipose tissue inflammation. Inflammasomes, of which NOD-like receptor family pyrin domain containing 3 (NLRP3) is

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the best characterised member, are pattern-recognition receptors (PRRs) that assemble into high-molecular-weight platforms that control maturation and secretion of proinflammatory interleukins such as IL-1β.[121] NLRP3 releases bioactive caspase-1 which can cleave procytokines into their mature active forms.[122] The expression of inflammasome NLRP3 components is increased in obesity, while whole-body knockout of components of this complex resulted in protection from obesity (due to higher energy expenditure), and from inflammation and insulin resistance in mice.[56] Several endogenous stress signals, including glucose, palmitate, cholesterol crystals, islet amyloid peptides and reactive oxygen species, have been suggested as potential *in vivo* inflammasome inducers, but their relevance in the aetiology of human obesity and insulin resistance remains to be elucidated.

A growing body of evidence suggests that cellular components of not only the innate but also the adaptive immune system contribute to adipose tissue dysfunction. The stromal vascular fraction of adipose tissue consists of various types of immune cells, in addition to the macrophage populations discussed earlier. For example, the role of proinflammatory T-cells in obesity-induced T2D has gained significant interest in recent years. Human adipocytes and preadipocytes appear to possess the full machinery to prime inflammation and attract T-cells independently of macrophages.[123] Moreover, subcutaneous adipose tissue of T2D patients has increased presence of not only macrophages, but also of proinflammatory T-cells,[124] infiltration of which preceded the infiltration of macrophages in mice fed a high fat-diet.[124,125] T-cells derived from adipose tissue of obese mice produced more interferon-gamma (IFN)-γ than those from control mice,[126] and hampered preadipocyte-to-adipocyte differentiation.[127] T-cells that are infiltrated in adipose tissue may not only attract macrophages, but also skew their differentiation towards the M1 phenotype. In contrast, induction of T-regulatory cells was beneficial and reduced adipose tissue inflammation and insulin resistance.[128,129] Notably, the anti-inflammatory master switch in adipocyte differentiation, PPAR-γ, was recently identified as major driver of visceral adipose-tissue-resident regulatory T-cells.[130]

Another emerging factor that may underlie, at least part of, the inflammatory response that is seen in insulin resistance and T2D is the gut microbiome. Obese humans and rodents were shown to have higher concentrations of gut-derived endotoxins than non-obese, and these can potentially trigger TLRs in e.g. adipose tissue or on pancreatic β-cells, thus contributing to both insulin resistance and β-cell failure.[131,132] Experimental endotoxaemia can induce adipose tissue inflammation and insulin resistance in lean human subjects.[133] Moreover, portal endotoxaemia may contribute to inflammation

in hepatic steatosis and be a relevant risk factor for nonalcoholic steatohepatitis (NASH).[134]

Initiation of obesity-induced inflammation – novel insight | Key points:

- Inflammasomes, of which NLRP3 is the best characterised member, were recently proposed as central regulators of early adipose tissue inflammation
- Pro-inflammatory T-cells may comprise an early inflammatory cellular infiltrate and contribute to cytokine release and attraction of additional inflammatory cells
- Composition of the gut microbiome may contribute to, among other things, endotoxaemia which may induce adipose tissue inflammation

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Below we will discuss in more detail two additional emerging early activators of adipose tissue inflammation in obesity: the complement system and advanced glycation end products (AGEs). We will also discuss their potential roles in the development of diabetes.

THE COMPLEMENT SYSTEM IN INFLAMMATION AND T2D

The complement system is a complex protein network that was initially identified as part of the innate immune system. Historically, the liver was regarded the major source of complement, but in recent years, various non-hepatic sources of complement, including adipose tissue and endothelial cells, have been identified. Complement can be activated via several pathways – the classical, the lectin and the alternative – which all converge on complement C3, the central component of the complement system. The alternative pathway also functions as an 'amplification loop' and thereby enhances complement activation once it is initiated by activation of any of the three pathways. All three pathways result in the activation of the terminal complement pathway (*figure 2*). The complement system is increasingly recognised as an essential regulator of cell and tissue homeostasis, in addition to its well-known role in immunity.[135,136] Higher systemic C3 concentrations have been associated with several diabetes risk factors, including obesity, insulin resistance and NAFLD,[137,138] and were shown to be independently associated with incidence of T2D, at least in men.[139]

Various lines of evidence suggest a biologically relevant, functional role for complement activation in adipose tissue homeostasis and insulin resistance. First, adipose tissue expresses a large variety of complement

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components and regulators, with contributions from both the stromal vascular and the adipocyte fractions.[140-145] It has been known for over two decades that the alternative complement pathway is activated in adipocytes,[146] and more recently expression of proximal components of the classical pathway has been shown to be altered in subcutaneous adipose cells of insulin resistant individuals.[147] There may also be local effects of complement activation in specific ectopic fat depots. As an example, it was recently reported that the induction of complement C3 from perivascular fat may adversely affect adventitial fibroblast function[148] and complement activation was shown in human NALFD.[149] In addition, C1q, which is an early component of the classical complement pathway, can suppress macrophage inflammation *in vitro*,[150] while, on the other hand, the anaphylatoxins (C3a and C5a) that are generated during complement activation can efficiently attract inflammatory cells to the site of complement activation[151] and, as such, also induce inflammation and insulin resistance in adipose tissue and the liver. C3a-receptor knock-out mice are protected from high-fat-diet-induced insulin resistance and have less macrophage infiltration in adipose tissue[152] and, very recently, similar data were obtained in C5a-receptor knock-out mice.[153] In particular the observations on C3a and C5a and their receptors suggest that complement activation in obesity, and in particular in hypertrophic, metabolically stressed adipocytes, may contribute to recruitment of immune cells from the circulation to the adipose tissue. This may enhance the cellular immune response in adipose tissue and thereby contribute to adipose tissue dysfunction.

In addition to the proposed effects of complement activation on adipose tissue (dys)function, there may also be a role for complement activation in the progression of β-cell dysfunction in T2D, as absence of complement C3 in mice prevents diabetes development in response in mice treated with low-dose streptozotocin,[154,155] which causes β-cell deterioration in a way similar to what is observed in T2D.[82] There is also a substantial body of data, including from our own research group,[156] that implicates complement activation in the development of macrovascular and microvascular disease[156-158] (*figure 2*) as reviewed elsewhere.[135,137,159] In short, complement system activation leads to endothelial dysfunction, especially when protection of the endothelial cells is compromised due to decreased expression of complement inhibitors.[160] Since the microvasculature comprises approximately 98% of the total vascular surface area,[161] complement activation will inevitably affect microvascular function. With respect to atherosclerosis, complement activation by the classical and perhaps also the lectin pathway may aid in removal of apoptotic cell and cell debris and hence have protective effects in the atherosclerotic plaque, while complement activation beyond C3, which is associated with the release

of anaphylatoxins and assembly of the (soluble) terminal complex, may be proatherogenic.[159] Moreover, complement activation may be instrumental in the development of atherothrombosis since proteases of the coagulation and fibrinolysis systems may activate the complement system, and vice versa.[162]
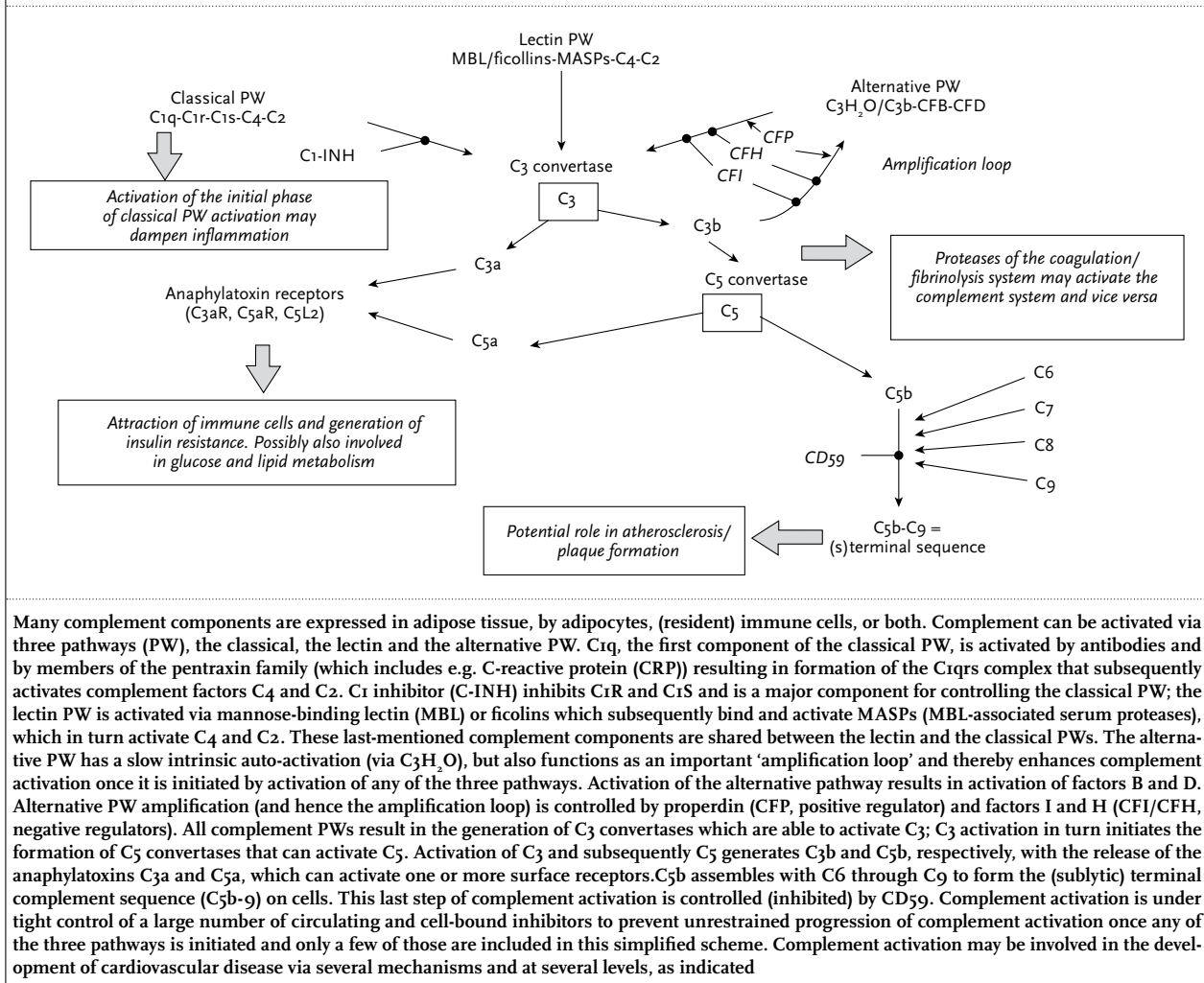

Complement – inflammation – T2D | Key points:
- Many complement components are produced by human adipose tissue (by both adipocytes and stromal vascular cells), and are increased in obesity, insulin resistance and low-grade inflammation
- Complement activation in adipose tissue, liver or pancreatic islets may contribute to inflammation and attraction of immune cells
- Complement activation may lead to endothelial dysfunction, and has been implicated in macro- and microvascular disease

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Advanced glycation, inflammation and T2D

AGEs form a heterogeneous family of unavoidable by-products that are formed by reactive metabolic intermediates derived from glucose and lipid oxidation.[163] In addition to the overwhelming amount of data, including ours,[164-167] demonstrating a role of AGEs in the development of vascular disease in diabetes (reviewed elsewhere[168,169], AGEs are implicated in the development of obesity and diabetes[170] and have been found to be associated with insulin resistance.[171] In obesity, the combined effects of enhanced food consumption, low energy expenditure, hyperglycaemia, hyperlipidaemia and increased oxidative stress may augment the formation of specific AGEs such as N[Carboxymethyl]lysine (CML). Peroxidation of lipids may also lead to the formation of the reactive dicarbonyl compound methylglyoxal (MGO), which is believed to be the most potent glycation product. Accelerated endogenous formation of both CML and MGO in obesity has been described in a few studies. We recently demonstrated the accumulation of a major AGE, CML, in adipose tissue and fatty liver and provided evidence that this is a core mechanism leading to the dysregulation of cytokines production.[172,173] CML is a major ligand for the receptor for AGE (RAGE) and we demonstrated that RAGE$^{-/-}$ obese$^{db/db}$ mice have reduced inflammation and improved insulin sensitivity, indicating a role for the CML-RAGE axis in inducing insulin resistance.[173] In addition to the effects in insulin resistance, AGEs have also been shown to induce β-cell dysfunction and apoptosis, at least partly via the AGE-RAGE axis[174-177] and via effect of MGO.[178,179]

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Figure 2. *Complement activation and the development of vascular disease*

Many complement components are expressed in adipose tissue, by adipocytes, (resident) immune cells, or both. Complement can be activated via three pathways (PW), the classical, the lectin and the alternative PW. C1q, the first component of the classical PW, is activated by antibodies and by members of the pentraxin family (which includes e.g. C-reactive protein (CRP)) resulting in formation of the C1qrs complex that subsequently activates complement factors C4 and C2. C1 inhibitor (C-INH) inhibits C1R and C1S and is a major component for controlling the classical PW; the lectin PW is activated via mannose-binding lectin (MBL) or ficolins which subsequently bind and activate MASPs (MBL-associated serum proteases), which in turn activate C4 and C2. These last-mentioned complement components are shared between the lectin and the classical PWs. The alternative PW has a slow intrinsic auto-activation (via $C3H_2O$), but also functions as an important 'amplification loop' and thereby enhances complement activation once it is initiated by activation of any of the three pathways. Activation of the alternative pathway results in activation of factors B and D. Alternative PW amplification (and hence the amplification loop) is controlled by properdin (CFP, positive regulator) and factors I and H (CFI/CFH, negative regulators). All complement PWs result in the generation of C3 convertases which are able to activate C3; C3 activation in turn initiates the formation of C5 convertases that can activate C5. Activation of C3 and subsequently C5 generates C3b and C5b, respectively, with the release of the anaphylatoxins C3a and C5a, which can activate one or more surface receptors.C5b assembles with C6 through C9 to form the (sublytic) terminal complement sequence (C5b-9) on cells. This last step of complement activation is controlled (inhibited) by CD59. Complement activation is under tight control of a large number of circulating and cell-bound inhibitors to prevent unrestrained progression of complement activation once any of the three pathways is initiated and only a few of those are included in this simplified scheme. Complement activation may be involved in the development of cardiovascular disease via several mechanisms and at several levels, as indicated

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Advanced glycosylation end products – inflammation – T2D | Key points:
- Obesity is characterised by increased formation of advanced glycation end products
- AGEs in obesity may have important biological effects on the dysregulation of adipokine secretion and the induction of insulin resistance
- AGEs have been shown to induce β-cell dysfunction

CONCLUDING REMARKS

Taken together, the larger picture on how obesity, inflammation and T2D are interrelated is becoming increasingly clear. We have provided an overview of the different fat depots and their potential contribution to obesity-associated inflammation, on how inflammatory cytokines can affect insulin signalling at the molecular level and on how similar molecular events may also affect β-cell function. We have additionally discussed novel insights into the processes that may initiate the obesity-associated inflammatory response, including complement activation and advanced glycation end products. However, the details on exactly where and how inflammation is induced, the temporal order of the events that contribute to insulin resistance and the development of β-cell function, and the role of vascular dysfunction therein remain to be further elucidated. More detailed knowledge of these events will help to pin-point optimal targets for prevention of, and intervention in, T2D.

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