BACKGROUND

Low-grade systemic inflammation

Low-grade systemic inflammation (LGSI) is a chronic condition of sub-clinical inflammation, in which afflicted individuals are subject to a low degree of inflammation for longer periods of time. Inflammation, in the purest sense of the word, is a complex biological response to stimuli, the cardinal signs of which are *dolor* (pain), *calor* (heat), *rubor* (redness), *tumor* (swelling), and the recently added loss of function[13]. The first four of these physiological indicators were first recorded almost 2000 years ago[14]. Today we know that they are the result of a massively coordinated immunological reaction, involving immune cells, inflammatory mediator, and the vascular epithelial cells[15]. None of these cardinal signs, however, are present in the case of LGSI, hence the addendum of "low-grade". Nevertheless, the condition is characterized by slightly but perpetually elevated levels of immunological components, such as circulating cytokines and concentrations of leukocytes. In the following section, I will focus on LGSI in the context of obesity. Furthermore, I will expand upon measures of LGSI.

Obesity and low-grade systemic inflammation

LGSI can be detected concurrently with several inflammatory conditions, including obesity, allergic asthma, inflammatory bowel disease, and rheumatoid arthritis[4], however, the term is most commonly used in connection with obesity. Although observations of associations between obesity and circulating inflammatory components, such as fibrinogen and acute phase proteins, have been found as early as in the 1950's and 1960's[16], the suggestion of a mechanistic link between obesity and inflammation was not actually posed until Hotamisligil *et al.* showed that adipose tissue of rodents and later of obese individuals had increased production of tumour necrosis factor α (TNFα)[17].

The subcutaneous- and visceral adipose tissues represent the two most abundant sites of fat deposition[18], however, we now know that the two adipose tissues sites differ in cell size[19; 20] and produce a distinct combination of adipokines and cytokines[21]. As such, visceral adiposity is the most pathogenic. Adipose tissue quantity alone, however, does not account for the metabolic phenotypes associated with obesity. Rather, cellular composition and function of the adipose tissue is important[5]. Individuals with the same degree of obesity can have

markedly different levels of circulating inflammatory components with concomitant differing risks of type 2 diabetes (T2D) and cardiovascular disease (CVD), dependent upon their level of adipose tissue dysfunction[22]. Nevertheless, since the recognition of obesity as an inflammatory condition, accumulation of evidence in support of actual cytokine production from adipose cells as such has emerged[5; 23]. More recently however, the role of infiltrating macrophages has been highlighted[24].

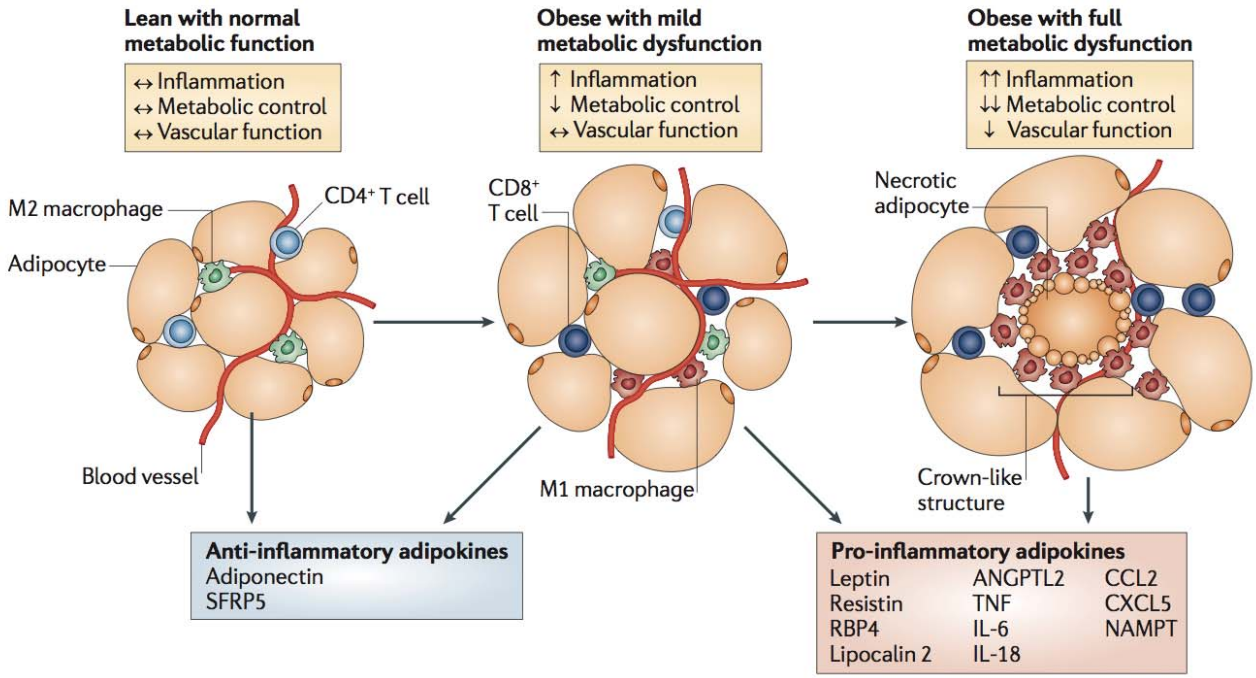

Figure 1: Progression from normal metabolic function to metabolic dysfunction as a result of obesity. During normal conditions the adipose tissue is infiltrated by macrophages of the anti-inflammatory M2-phenotype, while progression towards obesity and metabolic dysfunction results in adipose cell hypertrophy and increasing infiltration of macrophages of the M1-phenotype. Concomitantly the production of inflammatory components is skewed towards an overweight of pro-inflammatory adipokines and cytokines, such as interleukin (IL)6 and tumour necrosis factor (TNF)α. Figure was taken from Ouchi *et al.*[5].

In obese individuals, and in animal models of obesity, adipose tissue is infiltrated by a large number of macrophages, proportional to the degree of obesity[25; 26]. Furthermore, this accumulation of infiltrating macrophages has been associated with severity of LGSI[25; 26]. Similarly, the abundance of infiltrating macrophages is also increased in visceral adipose tissue, compared to subcutaneous adipose tissue[27]. Evidence from mice studies suggests that obesity-associated inflammation of adipose tissue is correlated with differing subsets of infiltrating macrophages. Although this may differ humans, it would appear that adipose tissue macrophages (ATMs) associated with obesity are of the subset classified as "classically activated" or M1 phenotypic macrophages, whereas ATMs present in lean mice appear to be M2 phenotypic

or "alternatively activated"[28]. In line with this, M1-ATMs produce and secrete the pro-inflammatory cytokines IL6 and TNFα, among others, while the M2-ATMs produce anti-inflammatory components, such as the cytokine IL10 or the IL1 receptor antagonist (IL1Ra)[29] (**Figure 1**). It is, however, not known to which degree the adipocytes themselves or differing subsets of macrophages are responsible for production of the pro-inflammatory components that can be detected in circulation.

Measures of low-grade systemic inflammation

Traditionally, circulating markers of inflammation have been used to form the idea of LGSI[16]. Pentraxin 3 (PTX3) is an acute phase protein, similarly to C-reactive protein (CRP), and is part of a group of soluble pattern recognition molecules such as collectins, ficolins, and pentraxins, which represent the functional evolutionary ancestors of antibodies[30]. Fully functional PTX3 can be released rapidly from neutrophil vesicles in response to microbial components and Toll-like receptor (TLR) agonists[31], however, *in vitro* studies have also indicated that PTX3 can be produced and released from many cell types including endothelial cells, fibroblasts, dendritic cells, monocytes, and macrophages through stimulation with inflammatory signals including TNFα and IL1β[30; 32; 33; 34; 35]. PTX3 has been shown to harbour immune-modulatory capabilities partly through its ability to recognize and opsonize a broad spectrum of microorganisms and microbial components and partly through its ability to recognize self and modified self-ligands[30]. The latter seems to be of particular importance in association with CVD where PTX3 is released rapidly and in high concentrations, presumably from neutrophils vesicles, immediately preceding acute myocardial infarction. Mice studies have shown PTX3 to be cardio-protective in such a scenario [36], and this may be related to its ability to dampen complement mediated inflammation. PTX3 can interact with C1q, the main activator if the classical pathway in the complement system, and promote complement activation, if this occurs e.g. on a bacterial surface, however, in the fluid phase, this interaction leads to complement inhibition[37]. In addition to this, PTX3 can interact with factor H, the main regulator or the alternative complement pathway, favouring its deposition on PTX3-coated surfaces (e.g. apoptotic cells) and thus limiting exaggerated activation of the alternative complement pathway[38]. PTX3 can also interact with the complement component C4BP by promoting its recruitment to late apoptotic cells, which dampens excessive complement activation and thus exaggerated inflammation and tissue damage[39]. As expression and release of PTX3 can be induced

through cytokine signalling, and as it harbours immune-modulatory properties it may serve as an interesting new marker for MS-associated LGSI.

The most commonly measured marker of LGSI, however, is CRP[1]. CRP plays a central role in part of the innate immune system, namely the complement system, and is released from hepatocytes upon binding of circulating cytokines, such as IL6, TNFα, or IL1β[15]. As such, the serum CRP level reflects the level of circulating cytokines, including IL6, TNFα, and IL1β, and while these may be more direct, as measures of immune status, their use does present some problems. Short bouts of intense physical exercise for example, such as running up a flight of stairs, can produce significant bursts of IL6, which would then confound subsequent measurements[40].

Ex vivo production of cytokines from whole blood or isolated immune cell sub-populations, stimulated with bacteria or bacterial components, such as lipopolysaccharide (LPS), has been used in several contexts to assess immune capacity[3; 41]. The clinical significance of *ex vivo* cytokine production, however, has not been thoroughly established in the literature. Relative to obesity, only a few studies have investigated the production of cytokines from stimulated peripheral blood mononuclear cells (PBMCs) or whole blood, however, this distinction seems to be important (see Table 1). One study included a comparison of cytokine levels, including IL6 and TNFα, generated by stimulating PBMCs from 20 obese and 20 normal weight healthy individuals with the plant mitogen concanavalin A[42]. A second study similarly compared production of IL6 and TNFα between 14 obese and 14 normal weight healthy individuals, but here LPS was used to stimulate PBMCs[43]. Finally, a third study isolated and stimulated PBMCs with LPS from 20 obese and 20 normal weight schizophrenic hospitalized patients and measured the *ex vivo* production of IL6, TNFα, and IL1β[44]. The first two studies found increased production levels of both TNFα and IL6 in obese, compared to normal-weight individuals. The third study found similar increases for TNFα and IL1β, but not for IL6. In contrast, a fourth study, featuring 17 morbidly obese and 19 non-obese pre-menopausal women, found reduced production of both IL6 and TNFα in the obese, compared to the non-obese women[45], however, here whole blood and not PBMCs was *ex vivo* stimulated with LPS. Partially in support of this, another study showed higher *ex vivo* production of IL1 in LPS-stimulated whole blood after 6 weeks of weight loss in morbidly obese individuals, compared to baseline[46], while there was only a tendency towards increased production of IL6, TNFα,

and sTNFR2 in the obese, compared to the lean group. From these studies it would appear that when evaluating immune status via *ex vivo* cytokine production in overweight or obese individuals, PBMCs behave differently than immune cells of whole blood. This is probably not the case in lean men, as it has been shown in such a study population that *ex vivo* production of TNFα and IL6 from LPS-stimulated whole blood is positively associated with production from both PBMCs and isolated monocytes[47]. Due to the above-mentioned problems with circulating cytokine levels, *ex vivo* cytokine production might constitute an interesting alternative method for assessing immune status in the context of LGSI. However, as can be seen from the studies mentioned above, more research into the mechanisms underlying cytokine production in different settings is necessary. In the following section I will focus on LGSI in the context of the metabolic syndrome (MS) and development of T2D.