Obesity and Cardiovascular Health Award 2010

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Cardiovascular disease (CVD) and its sequelae are the leading cause of premature death, leading not only to significantly increased mortality rates but also to high levels of morbidity. Whilst the causes of CVD are complex there is increasing evidence suggesting an integral role for inflammation in CVD pathogenesis, with recent research examining therapeutics targeting this aspect of the disease. However, the cause of inflammation and its link with CVD is still poorly understood, particularly in humans, due to the difficulty in studying the relevant human tissues.

Studies have further established that adipose tissue distribution has significant impact on disease risk with central abdominal fat increasing both CVD and T2DM risk compared with gluteo-femoral fat. Such differences in risk may be attributable to the depot specific differences in the expression and secretion of adipocytokines. However, whilst many investigations have elucidated the relative pathogenic risk of abdominal and gluteo-femoral adipose tissue, to date, few studies have investigated the adipocytokine profile of epicardial adipose tissue. This depot, situated predominantly on the right-ventricular free wall and the left-ventricular apex, has been shown to have a high capacity for non-esterified fatty acid (NEFA) release and is proposed as a source of this preferred metabolite for the myocardium. In addition, the lack of any fascia between the adipocytes and the myocardial layer does suggest that factors secreted by the adipocytes would readily interact with the adjacent cardiomyocytes. Clinical studies have noted a strong correlation between the fat mass of epicardial adipose tissue, central abdominal fat and the associated risk of T2DM and CVD. Studies by Mazurek and co-workers comparing expression of pathogenic factors between epicardial and subcutaneous fat from the leg in patients with CAD undergoing coronary artery bypass grafting (CABG), also highlight the potential importance of the macrophage and inflammatory response of epicardial adipose tissue.

In addressing the mechanisms of inflammation, our previous studies have demonstrated that human isolated abdominal sc adipocytes possess many of the key components of the nuclear factor-kinase B (NFkB) inflammatory pathway. Furthermore, our previous findings indicate increased activation of the innate immune pathway in response to bacterial fragments known as lipolysaccharide (LPS)

or endotoxin) in vitro, via the toll like receptors (TLRs). However it is apparent that this inflammatory response may also occur in vivo, through stimulation of the TLRs by gut-derived LPS. Such in vivo observations have previously been reported in CVD and T2DM subjects as well as subjects with impaired glucose tolerance, in which soluble CD-14 (sCD-14), a monocytic marker for LPS activity, is up-regulated.

With this prior work and background literature we addressed the function of epicardial fat in CAD and non-CAD patients and the signaling pathways that may lead to an inflammatory response, we sought therefore to: 1) investigate systemic LPS levels in pre-operative CAD patients compared with age and body mass index (BMI)-matched controls, 2) examine the protein expression of key inflammatory signaling molecules such as NF-kinase B and c-Jun N-terminal kinase (JNK) in epicardial AT compared with paired sc thigh AT, and 3) investigate the gene expression of TLR-2 and -4 and determine any correlations with markers of inflammation, such as TNF-alpha, and markers of macrophages. For such a study serums and AT biopsies (epicardial and thigh) were obtained from CAD (n = 16) and non-CAD (n = 18) patients. The intracellular signalling pathways of inflammation were assessed in tissue and serum samples through Western blot, real-time PCR, ELISAs, and activity studies.

Our primary end results highlighted that epicardial AT had significantly higher NF-kinase B, inhibitory-B kinase (IKK)-, IKKβ, and JNK-1 and -2 compared with thigh AT. Furthermore that human Epicardial AT mRNA data showed strong correlations between CD-68 (macrophage marker) and toll-like receptor-2, toll-like receptor-4, and TNF-alpha. Circulating endotoxin was elevated in patients with CAD compared with matched controls [CAD: 6.80 ± 0.28 endotoxin unit (EU)/ml vs. controls: 5.52 ± 0.57 EU/ml; $P<0.05$]. As a result of these studies we noted that human Epicardial AT from patients with CAD showed an increased in NF kinase B, IKKβ, and JNK expression compared with CAD thigh AT and non-CAD epicardial AT, suggesting a depot-specific as well as a disease-linked response to inflammation. These studies implicate both NFB and JNK pathways in the inflammatory profile of epicardial AT and highlight the role of the macrophage in the inflammation within this tissue. This also highlights that in spite of medication to reduce inflammation, the response to inflammation persists which may arise due to the continual presence and increase in endotoxin in patients with CAD. As such new therapeutic insights are required to continue to mitigate the potential effects gut derived endotoxin may have in patients with CAD.