SUMMARY

In the search for mechanisms of obesity-mediated vascular pathology, mounting evidence has implicated an important role of adipocytes and adipose tissue in the development of a systemic inflammatory state. Present studies demonstrate that adipocytes derived mediators of inflammation participate in the mechanisms of vascular insult and atheromatous change. Many of these inflammatory proteins such as TNF alpha, IL-6, MCP-1 are secreted from adipocytes and decreased amount of cardioprotective adiponectin from adipose tissues under obese condition. Secretion of inflammatory factors from visceral adipose tissue into the portal system which results in deleterious effects on the liver ultimately lend to systemic inflammation, vasculopathic phenomena and the metabolic syndrome. Studies on effect of age have indicated that in early obesity there was no increase in inflammatory markers however showed various adipocytes markers of adipogenesis in adipocytes decrease. At this stage there was no significant difference between the subcutaneous and visceral fat in the obese db/db mice. However, in late stage of obesity with aging accompanied with an inflammatory state is developed in visceral adipose tissue as evidenced by increased expression of classic inflammatory mediators and decreased expression of the anti-inflammatory nuclear receptor PPAR gamma and adiponectin. There are reports suggesting that visceral adiposity confers more cardiovascular risk than peripheral adiposity. Removal of subcutaneous fat did not significantly alter insulin sensitivity, blood pressure or change the plasma concentrations of glucose, insulin, lipid, CRP, IL-6, tumor necrosis factor-alpha, or adiponectin but reduction in visceral fat in animals and humans is associated with increased in insulin sensitivity, HDL cholesterol, decreased in triglyceride and blood pressure which are the markers of cardiovascular disease. In the present study, the increase in visceral adiposity was found to be associated with different proatherogenic cytokines suggesting that 12 weeks old obese diabetic db/db animals are at cardiovascular risk. One interesting observation was made that visceral obesity is positively correlated with TLR-4 expression in late stage of obese condition. A number of infectious agents have now been associated with

atherosclerotic cardiovascular disorders, including Helicobacter pylori, Human immunodeficiency virus, Herpes simplex virus 1, Herpes simplex virus 2 and hepatitis B and C. More recent models emphasize the relationship of atherosclerosis to total "infectious burden" rather than specific pathogens. Toll like receptors (TLRs) are demonstrated to be key molecules in the induction of inflammation in response to infection and various microbial products and dietary lipids. So TLR-4 may be one of the key mechanisms involved in obesity associated metabolic syndrome and these findings are important in understanding the basis of age-related inflammation and could provide a clue for searching the underlying mechanisms of the age-related increase in insulin resistance and T2D incidence and metabolic syndrome.

It was also observed in this report that inflammatory conditions in adipose tissue of db/db mice are further aggravated by using LPS, a compound of the cell wall of gram-negative bacteria that has been demonstrated to induce inflammatory reactions. In rodents, bacterial infection and LPS also promote atherogenesis. In present study, there are major changes in the expression of key adipokine genes in white adipose tissue following the induction of endotoxaemia in both C57BL/6 and in obese diabetic db/db mice after LPS challange. Endotoxemia increased expression of classic inflammatory mediators such as TNF alpha, IL-6, MCP-1, TLR-4 and COX-2 and decreased expression of the anti-inflammatory nuclear receptor PPAR gamma and adiponectin. The current study demonstrates that adipocytes are the major contributor to the inflammation rather than the macrophage present in adipocytes. LPS further decreased the different adipocytes markers like LPL and aP2 in WAT emphasizing the decrease in adipogenic potential which may be due to the upregulation of inflammatory genes. These cytokines are known to inhibit lipoprotein lipase which leads to hypertriglyceridemia (elevated levels of triglycerides in the bloodstream. Remarkably, higher adipose inflammatory environment and decrease in adipose insulin signal pathways suggest a insulin resistance syndrome observed after acute endotoxemia. Remarkably, decrease in adipogenic marker (aP2, LPL, adiponectin), increase in adipiose inflammation

(TLR-4) and modulation of adipose insulin signal pathways (PPAR gamma and SOCS-3) in normal C57BL/6 mice after LPS challenge and their expression pattern are similar to the well established db/db mice an insulin resistant rodent model. Our findings suggest specific targets like SOCS-3 and TLR-4 in humans which warrant further mechanistic focus. Recent studies have now provided in vivo evidence for a direct mechanistic link between TLR4 signaling and innate immune system activation and atherogenesis. The function of TLR4 in atherosclerosis has been investigated in mouse knockout studies and epidemiological studies of human TLR4 polymorphisms. These studies have shown that TLR4 function affects the initiation and progression of atherosclerosis. In the current study, higher expression of TLR-4 in visceral adiposity associated with inflammation may promote atherosclerosis environment and increase its expression in WAT after LPS challenge may further exaggerate the atherogenic condition.

Studies in human subjects have indicated TNF alpha and IL-6 are produced by adipocytes as well as macrophages which signifies a positive correlation between their circulating levels and degree of obesity and thereby indicating the role of circulating proinflammatory cells in obesity. Evidence shows that monocytic cell line cultured with FFA enhances the proinflammatory state which mimics an obese state of inflammation. There is increasing evidence that an cytokine-induced inflammatory response from adipocytes gets communicated to other organs like aorta and macrophage which are major cell types involved in the progression of atherosclerosis. To explore the role of peripheral blood cells in obese state, we challenged LPS in whole blood derived from db/db mice, a model of obesity, insulin resistance, and type 2 diabetes, and compared the responses with normal C57BL/6 mice. This study also helped to determine whether these key cells associated with vascular complications display a preactivated phenotype and altered pathophysiological responses. As the results demonstrate, obesity and diabesity in db/db mice is associated with higher monocyte and lower lymphocytes emphasizing the impairment of immune function. Percentage increase in cytokines per blood cell was enhanced in obese

diabetic mice as compared to the normal C57BL/6 after LPS incubation emphasizing that PBMC are in activated state which is a proatherogenic features.

In case of normal C57BL/6 when fed with high fat diet significantly increase the body weight along with higher pro-atherogenic gene (TNF alpha, IL-6 and MCP-1) expression in visceral adipose tissue, and is positively correlated with RBP-4 mRNA expression and inversely correlated with anti-atherogenic adiponectin suggesting that RBP-4 might play a role in adipose inflammation. An important observation in the current study was that RBP-4 expression was well correlated with LDL, total cholesterol and FFA in HF fed C57BL/6 mice. The observed positive correlation of adipose RBP-4 with pro-inflammatory cytokines and LDL, which are strong predictor of cardiovascular disorders suggest that RBP-4 could be another valuable marker and link between obesity and cardiovascular disease.

In the current study, I have investigated the atherogenic markers and RBP-4 expression in aorta in response to HF diet in C57BL/6 and HC diet in ApoE3 mice. The effect of HC diet in case of ApoE3 mice was much pronounced than that of HF for C57BL/6 mice and there was a dramatic increase in the circulating levels of RBP-4 and its expression in aortic tissues suggesting a pro-atherogenic role of RBP-4. Further support for a pro-atherogenic role of RBP-4 is evidenced by the fact that HC fed ApoE3 mice has a significant elevation in aortic MCP-1 transcripts along with serum CRP levels. MCP-1 and CRP are known to be a marker of inflammation, and their levels reportedly increase in cardiovascular diseases. As RBP-4 is corroborating well with the cardiovascular risk factors like CRP and MCP-1, our new findings add further support to the concept that RBP-4 may be an important effector protein in the interaction between the obesity and cardiovascular disease.

The adipose tissue plays a key role for the insulin resistance and dysregulated state seen in obesity may lead to the Metabolic Syndrome. Adipose cell enlargement in obesity induces a proinflammatory state in the tissue. The increased tissue levels of cytokines (like TNF alpha, IL-6, MCP-1) further

promote a proinflammatory state, impair the normal differentiation of the preadipocytes, alter the pattern of secreted adipokines by the adipose tissue, and induce insulin resistance locally and in other peripheral tissues such as muscle, liver, aorta etc.

Weight loss correlates with decreased inflammation and the cardioprotective effects of many of the most popular drug regimens. It has been demonstrated that the improvements in systemic inflammation may be attributable to decreased inflammatory signaling within adipocytes or adipose tissue macrophages. These observations have provided great insight into the central role of adipose tissue toward the metabolic syndrome. Studies on antiobesity and anti-inflammatory therapeutic interventions corroborate these casual relationships.

Obesity or diabesity associated pro-inflammatory state can be reversed by different classes of drugs through their actions on the adipokines which will ameliorate the insulin resistance and thus the metabolic syndrome. In the present study, treatment with therapeutic relevant dose of rimonabant a CB-1 receptor antagonist improved insulin sensitivity. However, low dose of rimonabant was found to reduce proinflammatory adipokines such as visfatin and TNF alpha in WAT whereas no such insulin sensitizing effect was observed. Rimonabant is known to enhance lipolysis, decrease fat mass and reduced body weight. Global CB1 blockade enhances insulin sensitivity or glucose utilization; however CNS CB1 blockade did not improve the insulin sensitivity indicating involvement of peripheral CB1 receptors. The improvement in insulin resistance by rimonabant due to its direct effect on adipocytes causing modulation of adipokines may be speculated. These findings may give a new insight to the development of peripheral CB1 antagonists as target for cardiometabolic disease including atherosclerosis.

We investigated the effect of rimonabant on cardiovascular complications and features of associated metabolic syndrome (inflammation, dyslipidemia) in HC fed ApoE3 mice and the possible role of RBP-4. Our results demonstrate that rimonabant ameliorates dyslipidemia, a major biochemical disorder associated

with cardiovascular diseases. Moreover, rimonabant treatment reduces the elevated levels of CRP, and MCP-1 (tissue and circulatory levels) suggesting its anti-inflammatory role. Furthermore, elevated levels of aortic RBP-4 and circulating levels in ApoE3 mice may relate to cardiovascular diseases and its normalization due to rimonabant may represent one of the possible mechanism by which rimonabant ameliorates the cardiovascular complications in HC fed ApoE3 mice.

In this report, low dose of rimonabant was found to reduced proinflammatory adipokines such as visfatin and TNF alpha in WAT the emphasizing its anti-inflammatory effect. Similarly, low dose of pioglitazone exerted an anti-inflammatory by reducing the expression of TNF alpha, IL-6 and resistin mRNA in WAT, whereas no PPAR gamma related metabolic effect was observed.

Anti-atherosclerotic effect of systemic TNF alpha inhibition is well established. IL-6 is also known to promote the release of endothelial adhesion molecules and chemokines. Adding to the possible link between adipokines and cardiovascular risk, it was recently shown that resistin is secreted primarily by the macrophage in the human rather than the adipocyte as in rodents. Further, potential role of resistin in atherosclerosis has been documented. The anti-inflammatory effects of low-dose pioglitazone or rimonabant thus include several mediators of inflammation, which might indicate a reduced cardiovascular disease (CVD) risk. It is therefore possible that, pioglitazone and other TZDs may be used as anti-inflammatory and anti-atherogenic drugs in future because atherosclerosis is a chronic inflammation of the arterial wall. However, at clinical relevant dose, PPAR gamma activator pioglitazone increases insulin sensitivity and modulates various PPAR gamma related biomarkers. Pioglitazone treatment, which results in adipogenesis and facilitates the expansion of fat mass, body weight gain on the other hand rimonabant which is known to enhance lipolysis and reduce fat mass, interestingly both exert similar effect. Furthermore, inactivation of PPAR gamma or PPAR gamma KO mice also limits fat mass expansion but enhances insulin sensitivity. In animal models, a number

of interventions would appear to improve insulin sensitivity without an increase in fat mass. So based on my work, I propose an expandability hypotheses that, adipocyte can continue to safely store fat, which will remain metabolically healthy even if they become obese. The beneficial effects of other antidiabetic and antihypertensive drugs on the subclinical inflammation have been reported. Similarly, the hypolipidemic statins have also demonstrated anti-inflammatory properties. Inhibitors of 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) have been shown to decrease CRP levels in subjects with high cardiovascular risks and they accelerated the decline of CRP and SAA3 levels after myocardial infarction. It is likely that statins mediate their anti-inflammatory actions by ameliorating the proinflammatory effects of atherogenic lipoproteins ACE inhibitor, ATII antagonist and HMG- CoA reductase inhibitors may improve cardiovascular disease partly by inhibiting the inflammatory cytokines. In the context of obesity and adipose tissue derived inflammation, it was discovered recently that the statin class of drugs may have direct anti-inflammatory actions on adipocytes themselves. Treatment of cultured adipocytes with cerivastatin decreased expression and secretion of IL-6. So it is speculated that the quality of fat (decrease in adipocytes inflammation) is more important which was observed in both the treatments of rimonabant and pioglitazone. Present study imply that the inhibition of proinflammatory cytokines observed at low dose of pioglitazone and rimonabant treatment may first correct the proinflammatory state of adipose tissue and prime these tissues for the therapeutic outcome at the clinical relevant dose.

Visceral fat may be a potent stimulus for promoting vascular complications and may represent a link between obesity and vascular disease. Therapies targeting fat inflammation may be particularly effective in subjects with visceral obesity, who are at risk for cardio vascular disease.

FUTURE PERSPECTIVE

This thesis provides evidence that inflammation related to adipose tissues may provide potential therapeutic approaches to target metabolic and cardiovascular disorders. This research highlights the benefits of subtherapeutic/low doses of pioglitazone (Paper-1) and rimonabant (paper-II). At low dose ranges these agents may exert cardiovascular benefits through anti-inflammatory mechanisms. Further long term studies of low dose of pioglitazone and rimonabant may be needed in different animal models of atherosclerosis such as ApoE or LDLR knockout mice in order to confirm their anti-atherosclerotic effect.

This research has further shown that SOCS-3 and TLR-4 are important regulators that are up regulated in visceral adipose tissue and positively correlated with classic markers of inflammation in an animal model of obese diabetic db/db mice. Future studies may be carried out to explore molecular mechanisms for increased SOCS-3 and TLR4 expression. The role of other isoforms of SOCS and TLR family and their contribution to the proinflammatory state can be investigated using diabetic TLR knockout mice or TLR antagonist.

We have also made in interesting observation that acute treatment with rimonabant has a pronounced effect on LDL reduction in C57BL/6 mice. This LDL reduction was positively correlated with reduction in Retinol Binding Protein-4 (Chapter-5). Future studies may be needed to find out how rimonabant's acute effect on LDL reduction is mediated by RBP-4, or if there is any additional mechanism involved in this effect.

It is well known that insulin has a potent anti-inflammatory effect on vascular endothelium. It is also evident that insulin resistance is associated with accelerated atherosclerosis; a major question is whether or not insulin resistance occurs in cardiovascular tissues and whether such a process is main driving force behind accelerated cardiovascular diseases. Since, pioglitazone and

rimonabant, both have insulin sensitizing properties, it is possible that these agents may be used as anti-inflammatory and anti-atherogenic drugs because atherosclerosis is a chronic inflammation of the arterial wall.

In conclusion, an attempt has been made in this thesis to understand the association of adipocyte derived factors with inflammation and their potential implication in cardiometabolic disorders including atherosclerosis. Since, insulin resistance is the primary mechanism of various conditions associated with metabolic syndrome, a greater understanding of the insulin resistance in vessel wall will provide new insight to alleviate the huge burden of cardiovascular disease that is confronting the world at present.