# Chapter 5

## To investigate the possible role of Retinol Binding Protein-4 a novel adipokine, in cardiovascular complications

## 5.1 Introduction

Patients with obesity have an increased risk of developing cardiovascular diseases, extensive artherosclerosis [Rimm et al 1995; Eckel, 1997; Kopelman, 2000; McGill et al., 2002; Lavie et al., 2009]. In the last decade, experimental data have illuminated the role of inflammation in cardiovascular disease while clinical studies have shown that this emerging concept of inflammation in arteriosclerosis applies directly to human patients [Libby et al., 2002]. Over the past few years, accumulating evidence showed that increased cytokines secreted from adipose tissue, also known as adipocytokines, may be responsible for initiating a proinflammatory status that percolates the development of both insulin resistance and endothelial dysfunction (ED; the initial stage in the development of atherosclerosis) [Lau et al., 2005; Berg and Scherer, 2005]. Adipocyte, release inflammatory mediators like cytokines or chemokines, thus orchestrating an ongoing inflammatory response in the vessel wall. In particular, cytokines such as interleukin (IL)-6 or tumor necrosis factor-alpha (TNF alpha), soluble adhesion molecules, and downstream acute phase reactants such as C-reactive protein (CRP), and serum amyloid A (SAA) have been shown to predict the risk of cardiovascular events. Furthermore visfatin and resistin are marker of inflammation and found to be increased in atherosclerotic lesions [Jung et al., 2006; Dahl et al., 2007].

A protein of interest is retinol binding protein-4 (RBP-4), established as an adipokine in the 1990s. [Tsutsumi et al., 1992; Montague et al., 1998] RBP-4 is preferentially expressed in visceral adipose tissues versus subcutaneous13 and appears to be up regulated in obese rodents.[Yang et al., 2005] Elevated serum RBP-4 is associated with insulin resistance, type 2 diabetes, and metabolic abnormalities such as obesity, glucose intolerance, dyslipidemia, and hypertension [Basualdo et al., 1997; Yang et al., 2005; Graham et al., 2005; Takashima et al., 2006; Erikstrup et al., 2006; Cho et al., 2006; Solini et al., 2009]. Von Eynatten et al. stated RBP4 is a valuable marker for the metabolic

syndrome and associated with pro-atherogenic lipoproteins and enzymes of lipoprotein metabolism, suggesting a possible role of RBP-4 in lipid metabolism [von Eynatten et al., 2007]. RBP-4 provides an important link between obesity, insulin resistance and inflammation. In chapter 4, I have reported that, rimonabant, a weight reducing agent, down-regulate the expression of RBP-4 and proinflammatory cytokines in adipose tissues of genetically altered obese (ob/ob) mice [Mohapatra et al., 2009]. There are emerging evidences that RBP-4 is also closely involved in the development of cardio vascular disease [Matsuzawa, 2006; Ingelsson et al., 2009]. Given the increased propensity of obese patients to develop macrovascular events, therapeutic strategies that inflammation in the vessel wall and reduce serum levels of inflammatory surrogate parameters as well as RBP-4 might be a promising tool to influence vascular disease in this high-risk population. The present study was designed to investigate the adipose and aortic expression of RBP-4 and its correlation with inflammatory and lipoprotein mediators of cardiovascular risks and to examine the effect of rimonabant treatment on expression of RBP-4 in aortic tissues and other proinflammatory markers in high cholesterol fed ApoE3 mice a model of hypercholesteremia and atherosclerosis.

**Ratinale for the selection of animal model**: Apoe3 targeted replacement mouse is homozygous for a gene targeted replacement of the endogenous mouse apolipoprotein E gene and expresses the human apolipoprotein E3 isoform. The mice exhibit an increased risk of atherosclerosis and hypercholesterolemia compared with wild type mice on a high fat or high cholesterol diet, but not on a normal diet.

## 5.2 Materials and Methods

### *5.2.1. Animals*

C57BL/6 and ApoE3 leiden mice were housed in individually ventilated cages and given pelleted food (Standard Rodent diet, NIN, Hyderabad, India) and water ad libitum in a temperature (25±3℃) and humidity (50–70%)-controlled environment with a 12-h/12-h dark-light cycle. All animal experimentations were carried out in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines, using (IAEC) Institutional Animal Ethics Committee approved protocols in AAALAC International (association for assessment and accreditation of laboratory animal care international) accredited facility which is comply with the National Institutes of Health guidelines for care and use of animals.

### *5.2.2. Experimental Design*

Female C57BL/6 mice were switched to the high-fat (HF) diet (Research Diet, USA) for 8 weeks, 4 weeks or 0 day (control) before tissue collection. Blood samples were collected for biochemical estimations; white adipose and aortic tissues were harvested and snap frozen for mRNA expression of TNF-alpha, MCP-1, adiponectin and RBP-4 using real time PCR. All mice were 15 to 16 weeks of age at tissue collection, to avoid possible age-related effects.

To investigate the effect of single dose rimonabant on RBP-4 expression and lipid profiles, 15-16 weeks-old female C57BL/6 mice, which were on 8 weeks of HF diet, were randomized and kept on overnight fasting. A single dose of rimonabant (10, 20 or 40 mg/kg) or vehicle (0.5% tween 80 in water) was orally administered and after 6 h of administration blood was collected for estimation of biochemical parameters. The animals were then euthanized to open the abdomen and visceral white adipose tissue (WAT) was isolated and flash frozen for mRNA expression of RBP-4 using real time PCR.

In the second study, 18 h fasted female ApoE3 mice which were on high cholesterol (HC) diet containing 1.5% (wt/wt) cholesterol (SD Fine Chemicals, India) and 0.25% (wt/wt) sodium cholate (SD Fine Chemicals, India) for 8 weeks were treated with a single dose of rimonabant (20 or 40mg/kg) or vehicle. Age of

ApoE3 mice were 15-16 weeks old, which were on 8 weeks of HC diet. Blood was collected for estimation of biochemical parameters. For comparison of RBP-4 and MCP-1 expression, animals were euthanized to isolate the aorta and snap frozen for real time PCR. In addition, 10 ApoE3 mice were fed a Standard chow diet as control.

In a third experiment, female ApoE3 mice (7-8 weeks old) were fed with HC diet for 8 weeks. Then the animals were treated with rimonabant 20 mg/kg dose or vehicle (0.5% v/v tween 80 in water) administered orally once daily for 28 days. Total exposure of HC diet in ApoE3 mice was 12 weeks. Twenty four hour of the last dosing, serum was collected and animals were euthanized to isolate the aorta. Aorta from 50% of the animals of each group were snap frozen for real time PCR, while the rest were collected in 10% formal saline for histology (data not shown). In addition, 10 ApoE3 mice were fed a Standard chow diet containing 4% fat as control.

### *5.2.3. RNA analysis & Quantitative Real-Time Polymerase Chain Reaction [qRT-PCR]*

White adipose and aortic tissue samples were homogenized in TRIzol reagent (Invitrogen, Life Technolgies, Carsbad, CA, USA) a Polytron hand- held homogenizer (Kinematica, Switzerland). Total RNA was extracted following the manufacturer's protocol. 1 µg total RNA from each sample was taken for first strand cDNA synthesis using High Capacity cDNA archive kit (Applied Biosystems, USA Part No 4322171). Equal amount of cDNA from each sample was taken for Quantitative Real Time PCR using ABI-prism-7300. FAM labeled Taqman probes viz Adiponectin [Mm00456425_m1], TNF-alpha (Mm00443258_m1) and MCP-1 (Mm00441242_m1) were used for the expression analysis. Taqman Universal Mastermix (Cat. No: 430437) were procured from Applied Biosystems (Foster City, CA, USA) for expression profiling of above mentioned target genes. VIC labeled, housekeeping gene, Mouse beta actin (Part No: 4352339E) probe was purchased from Applied Biosystems as

20X concentrate and was co-amplified, in each sample with every target gene[s], for normalizing the results. Gene expression analysis for RBP4 (F: 5'-ACTGGGGTGTAGCCTCCTTT -3' and R: 5'-GGTGTCGTAGTCCGTGTCG -3') was carried out using SYBR Green quantitative Real Time PCR using QIAGEN QuantiFast SYBR Green kit (Cat. No 204052). Mouse beta actin (F: 5'-TACAGCTTCACCACCACAGC-3' and R: 5'-TCTCCAGGGAGG AAGAGGAT-3') was used as internal control for normalization of the results of SYBR Green PCR.

### *5.2.4. Serum measurement*

Serum triglyceride (Pointe scientific, USA), total cholesterol (Pointe scientific, USA), LDL (Randox Laboratories, UK), HDL (Randox Laboratories, UK), FFA (Randox Laboratories, UK) levels were determined by the colorimetric method using biochemical kit. C reactive protein (CRP) (Randox Laboratories, UK) levels were determined by the immuno turbidometric method using biochemical kit. MCP-1 (R&D System, MinneApolis, USA) levels in the serum were determined by ELISA kits using the methods specified by the manufacturer.

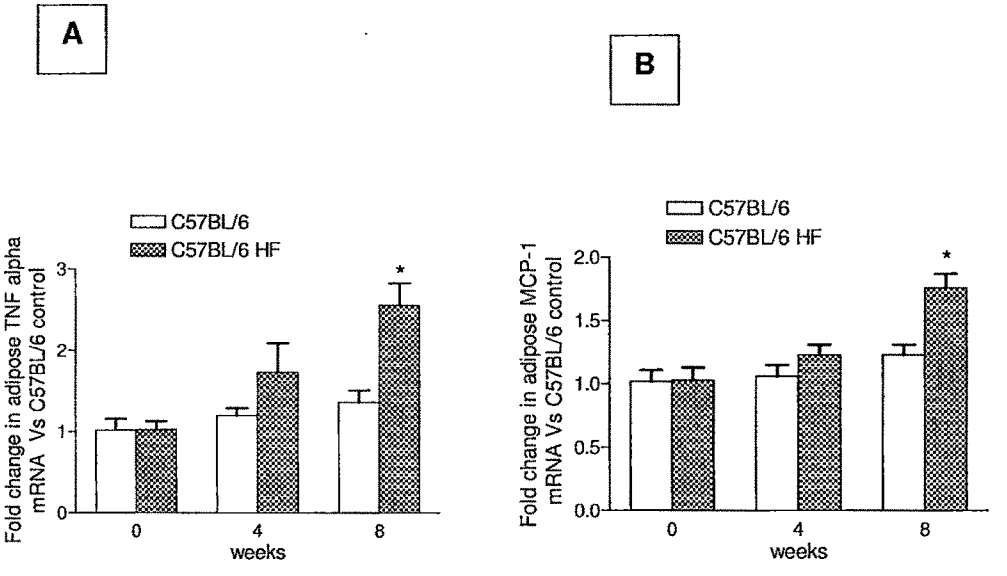
### *5.2.5. Data and Statistical Analysis*

Data are expressed as Mean ± SEM. The statistical analysis was performed using One-way ANOVA followed by Dunnett test used for comparison of all the parameters between treatment and control groups. Differences in expression or biochemical parameters between two groups were compared using Students t-test. For each analysis, *P* values less than 0.05 were considered to be statistically significant. All analyses were performed using graph pad prism software (version 4.0).

## 5.3 Results

### *5.3.1. High-fat diet alters the expression pattern of adipokines in WAT of C57BL/6 mice*

Expressions of TNF-alpha, MCP-1 and adiponectin in WAT were measured at the study initiation and 4 and 8 weeks of HF exposure. As evident from Fig.5.1A & B, both TNF-alpha and MCP-1 mRNAs were significantly increased (1.9 and 1.4 folds respectively, $P< 0.05$) and adiponectin (Fig 5.1C) mRNA was significantly decreased (0.6 fold, $P< 0.05$) in HF fed C57BL/6 mice after 8 weeks of exposure to HF diet. However, slight but non significant alterations in gene expression of TNF alpha, MCP-1 and adiponectin were observed after 4 weeks of HF diet exposure. In case of adipose expression of RBP-4, this was increased early in the course of diet-induced obesity. RBP-4 expression trended up after 2 weeks (data not shown), was significantly increased (1.7 fold, $P<0.05$) at 4 week and was 2.4 fold higher after 8 weeks on the high-fat diet compared with the chow-fed control group (Figure 5.1D).



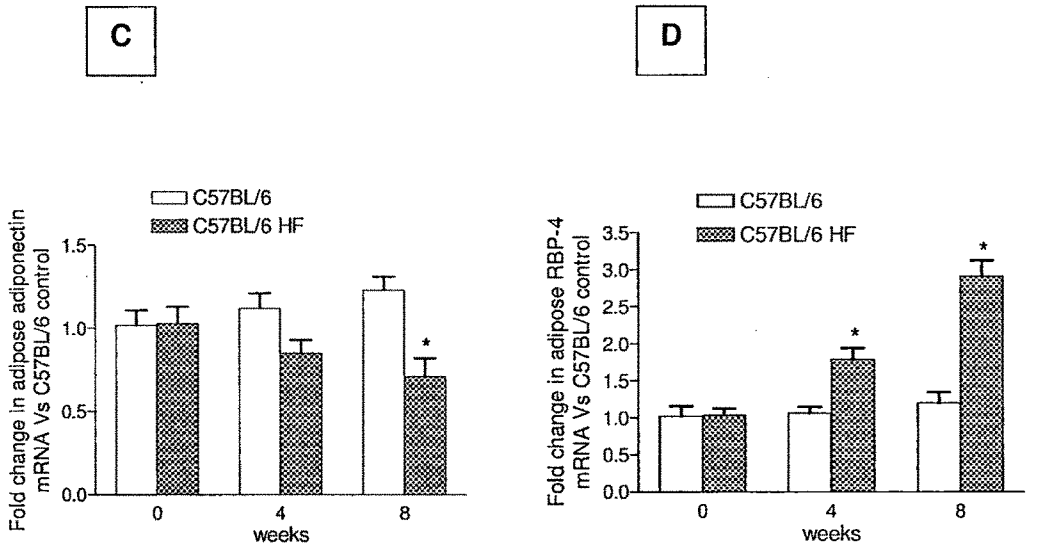



Fig. 5.1: Analysis of the expression of TNF alpha (A), MCP-1 (B), Adiponectin (C) RBP-4 (D) mRNA in WAT of C57BL/6 mice at different weeks of HF diet exposure. Time is shown as weeks on diet. Fold change in HF fed C57BL/6 groups against the C57BL/6 control is represented in the bar diagram. Each value represents mean ± S.E.M. ($n$ = 6 mice/group). * $P < 0.05$ compared with age matched C57BL/6 wild type mice.

### *5.3.2. High-fat diet alters the lipid parameters in C57BL/6 mice*

As shown in Table 5.1, the high fat fed mice were 19% heavier than the chow-fed lean controls (body weight = 31.1 ± 1.6 vs. 25.4 ± 0.4 grams; $P< 0.05$, n =10/group). There was significant increase in LDL, TG, TC, NEFA and decrease in HDL cholesterol 4 weeks after high fat diet. We found a significant time dependent increase in total cholesterol and LDL in the HF fed C57BL/6 mice (Table 5.1).

Table 5.1: Effects of High fat on the various serum biochemical parameters in high fat fed C57BL/6 mice.

| Variables | 0 week | 4 Weeks | 8 weeks |
|---|---|---|---|
| LDL (mg/dl) | 8.60 ± 0.2 | 10.72 ± 0.54* | 11.57 ± 0.65* |
| HDL (mg/dl) | 54.1 ± 2.15 | 74.8 ± 3.0* | 70.8 ± 3.17* |
| NEFA (mMol/L) | 1.15 ± 0.02 | 1.32 ± 0.06* | 1.66 ± 0.09* |
| Triglyceride (mg/dl) | 70.7 ± 8.4 | 101.5 ± 8.37* | 107.2 ± 8.37* |
| Total Cholesterol (mg/dl) | 96.0 ± 3.7 | 131.4 ± 5.2* | 171.9 ± 5.2* |
| Body weight (g) | 25.4 ± 0.4 | 28.0 ± 1.4* | 31.1 ± 1.6* |
| RBP-4 (µg/ml) | 22.3 ± 0.92 | 26.5 ± 1.6* | 29.3 ± 1.5* |

Values are described as mean ± SEM. * $P < 0.05$ as compared to day 0.

### *5.3.3 Effect of single dose rimonabant on adipose RBP-4 expression and serum LDL levels in 8 weeks HF fed C57BL/6 mice*

Treatment with rimonabant reduced the circulating LDL in a dose dependent manner (10 mg/kg: 5.4 %, 20 mg/kg: 16.9 %, 40 mg/kg: 29 %; Figure 5.2) without altering serum HDL cholesterol (data not shown). Parallel to its effect on LDL, rimonabant dose-dependently reduced expression of RBP-4 in WAT (10 mg/kg: 16.2%, 20 mg/kg: 24.8%, 40 mg/kg: 36.2%; Figure 5.3). A significant reduction in LDL and RBP-4 was observed at 20 and 40 mg/kg dose of rimonabant treated animals.

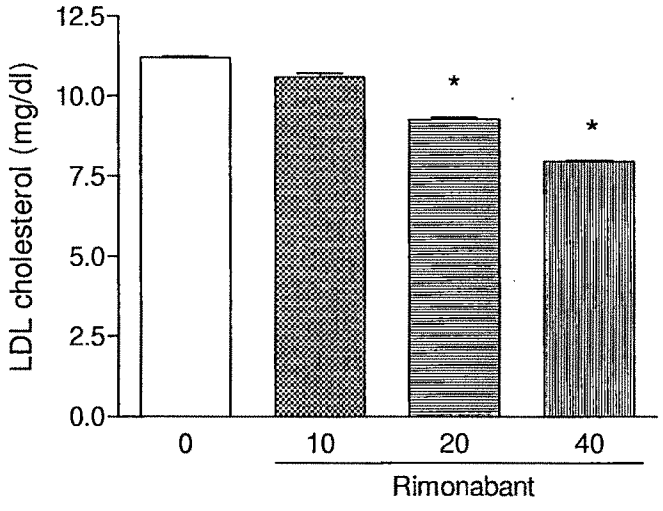



Fig. 5.2 Acute dose response effect of rimonabant treatment on the LDL in HF fed C57BL/6 mice. Change in LDL in HF fed C57BL/6 group against the treatment group is represented in the bar diagram. Each value represents mean ± S.E.M. (*n* = 8 mice/group). * P < 0.05 compared with HF fed C57BL/6 mice.

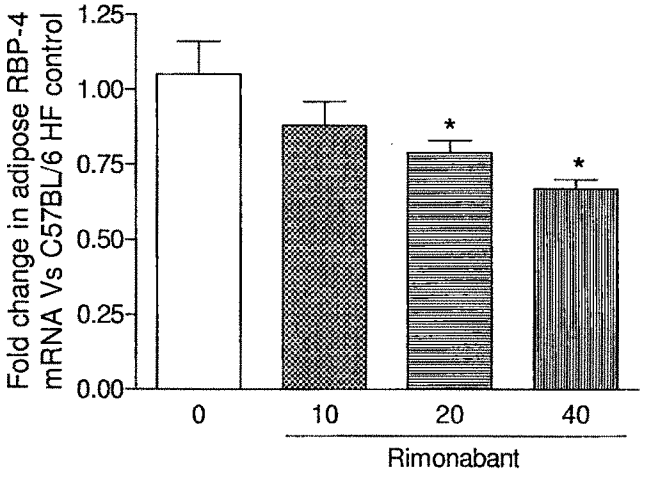



Fig 5.3 Expression of RBP-4 mRNA in white adipose tissue of HF fed C57BL/6 mice, determined by quantitative real-time PCR. The bars represent the fold change in the treatment groups compared with the vehicle control group, mean ± SEM (n = 6). *P < 0.05 vs untreated group.

### *5.3.4. Effect of single dose rimonabant on serum Lipid parameters in 8 weeks HC fed ApoE3 mice*

To further explore the LDL lowering effect, single dose of rimonabant was administered to ApoE3 mice, which were on 8 weeks of HC diet. Rimonabant treatment caused dose dependent reduction of LDL (20 mg/kg: 19.2% 40 mg/kg: 35.7%; Figure 5.4A) and total cholesterol (20 mg/kg: 9.6% 40 mg/kg: 16.3%; Figure 5.4B) in the serum of ApoE3 mice.

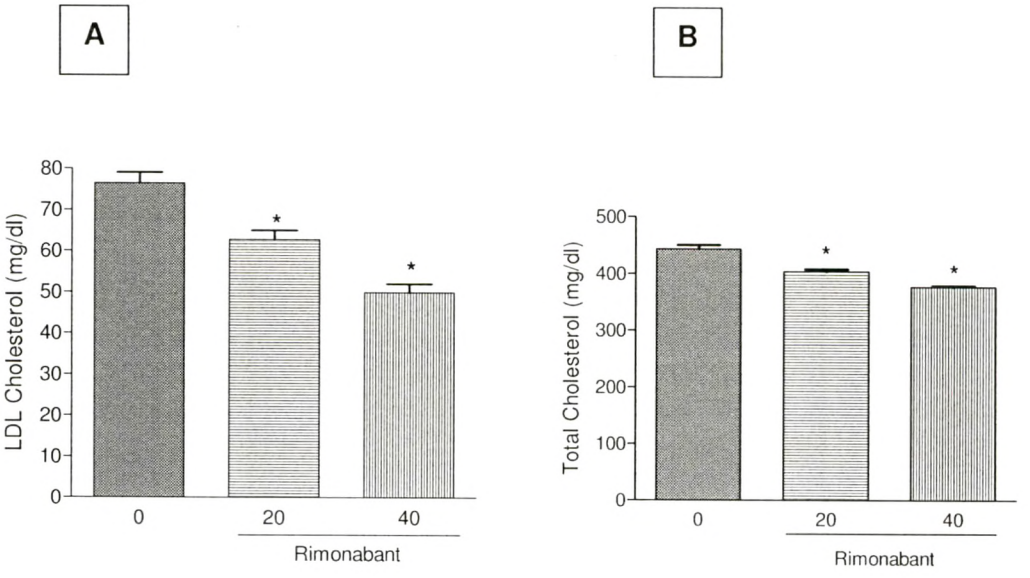



Fig 5.4: Acute effect of rimonabant treatment on the LDL (A) and total cholesterol (B) in HC fed ApoE3 mice. Change in LDL and total cholesterol in HC fed ApoE3 group against the treatment group is represented in the bar diagram. Each value represents mean ± S.E.M. ($n$ = 6 mice/group). * $P < 0.05$ compared with HC fed ApoE3 mice.

### *5.3.5. Effect of 8 weeks exposure to HF diet in C57BL/6 and HC diet in ApoE3 mice on serum RBP-4 and its expression in aortic tissues*

Interestingly, RBP-4 expression was found to be significantly higher (1.6 fold, P< 0.05, Figure # 5.5) in aortic tissues of HF fed C57BL/6 than chow fed animals.

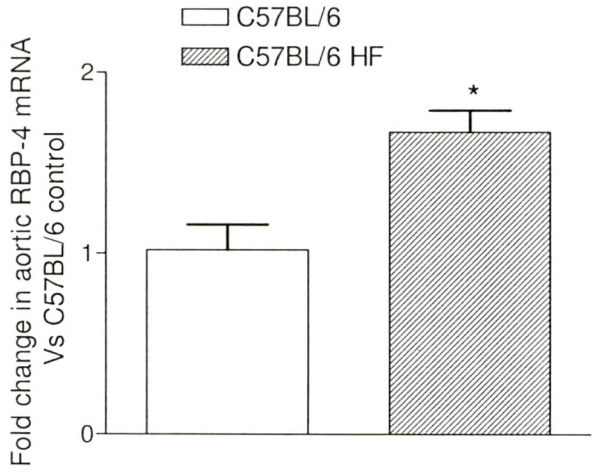



Fig. 5.5 Expression of RBP-4 mRNA in aortic tissue of HF fed C57BL/6 mice, determined by quantitative real-time PCR. The bars represent the fold change in the HF fed C57BL/6 groups compared with the C57BL/6 control group, mean ± SEM (n = 6). *P < 0.05 vs C57BL/6 mice.

### *5.3.6. Effect of 8 weeks exposure to HC diet in ApoE3 mice on serum RBP-4 and CRP and the expression (MCP-1 and RBP-4) in aortic tissues*

However, dramatic increase in aortic RBP-4 mRNA expression was observed in HC fed ApoE3 mice and was 3.1 fold as compared to ApoE3 wild type mice (Fig 5.6A). Interestingly, basal serum RBP-4 levels were significantly higher in ApoE3 mice when compared with lean C57BL/6 and there was further significant increase in ApoE3 mice after high cholesterol diet when compared to NIN fed ApoE3 mice (Fig 5.6D). As shown in Fig-5.6B we also investigated aortic MCP-1 expression, there was significant increase in MCP-1 expression in high cholesterol fed ApoE3 mice compared with wild type. Further we investigated the CRP levels in ApoE3 mice as they are an established inflammatory marker and

correlates with cardiovascular disease risk. As shown in Fig 5.6C, HC fed ApoE3 mice led significantly higher CRP levels (6 fold) compared to their control animals. emphasizing the animals are in risk to cardiovascular complications.

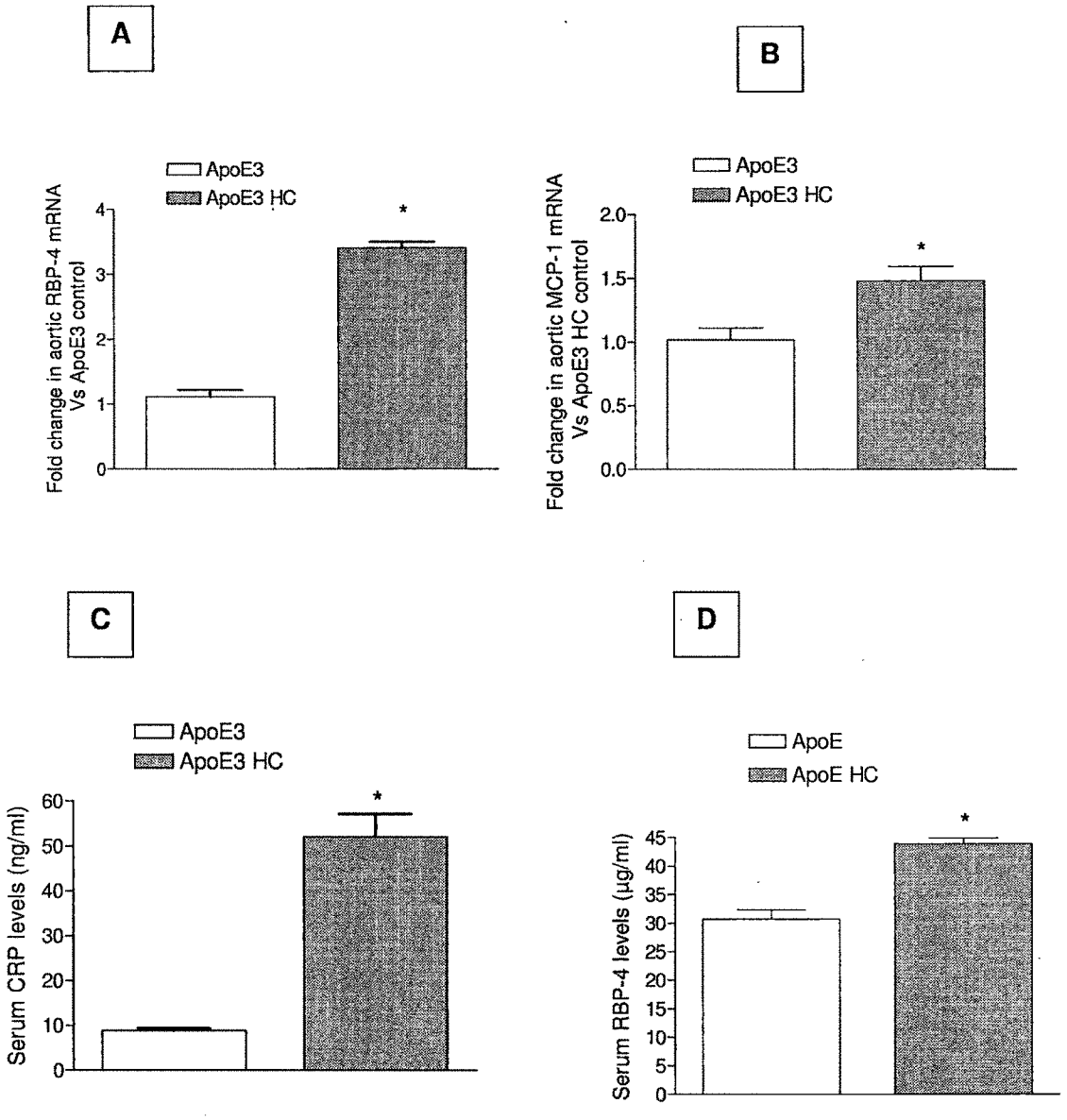



Fig 5.6 Expression of RBP-4 (A) and MCP-1 (B) mRNA in aortic tissue of HC fed ApoE3 mice, determined by quantitative real-time PCR. Effect of HC diet on the serum CRP (C) and RBP-4 (D) levels in ApoE3 mice. The bars represent the change in the HC fed ApoE3 groups compared with

their age matched ApoE3 control. Values are Mean ± SEM ($n$ = 6 mice/group). * P < 0.05, as compared to ApoE3 mice.

### *5.3.6. Effect of chronic treatment of rimonabant on the lipid concentration, RBP-4 and MCP-1 in 12 weeks HC fed ApoE3 mice*

As shown in Fig. 5.7, rimonabant treatment decreased the expression of RBP-4 in aortic tissues by 35% and MCP-1 by 29% Vs control. However, treatment with rimonabant slightly reduced the serum RBP-4 level which was not significant when compared to control group (Table. 5.2). Four weeks treatment with 20mg/kg dose of rimonabant significantly decreased serum CRP levels in ApoE3 mice when compared with vehicle treated animals (Table. 5.2). In parallel with reduction in CRP, serum MCP-1 levels were significantly reduced in rimonabant 20mg/kg dose group in comparison to control animals (Table. 5.2). Rimonabant was also found to reduce the triglyceride, total cholesterol, LDL, FFA levels significantly in HC fed ApoE3 mice (Table. 5.2)

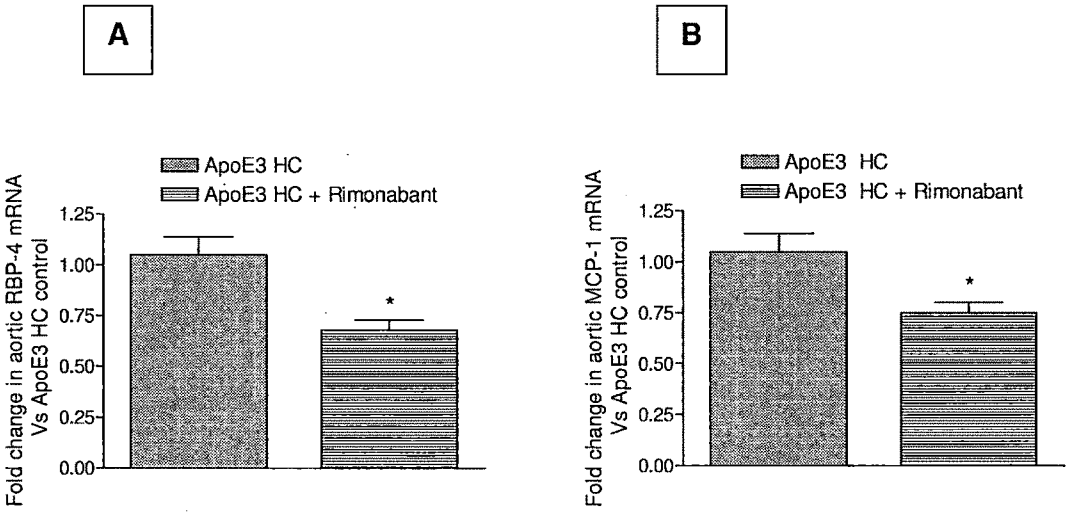



Fig. 5.7: Effect of rimonabant treatment on the expression of RBP-4 (A) and MCP-1 (B) mRNA in aortic tissue of HC fed ApoE3 mice, determined by quantitative real-time PCR. The bars represent the fold change in the treatment groups compared with the vehicle control group, mean ± SEM (n = 6). *P < 0.05 vs vehicle treated group.

**Table 5.2** Effects of rimonabant on serum biochemical parameters in HC fed ApoE3 mice after 28 days treatment.

| Metabolic Parameters | Vehicle | Rimonabant (20mg/kg) |
|---|---|---|
| LDL (mg/dl) | 92.6 ± 8.1 | 62.31 ± 4.2 * |
| HDL (mg/dl) | 71.05 ± 2.15 | 75.14 ± 3.01 * |
| Triglyceride (mg/dl) | 232.05 ± 5.23 | 141.4 ± 7.09 * |
| Total Cholesterol (mg/dl) | 576.3 ± 22.5 | 389.5 ± 15.8 * |
| NEFA (mMol/L) | 1.89 ± 0.16 | 1.25 ± 0.07 * |
| C reactive protein (ng/ml) | 62.3 ± 7.1 | 48.3 ± 3.6 * |
| MCP-1 (pg/ml) | 842.3 ± 42.7 | 645.6 ± 56.2 * |
| RBP-4 (μg/ml) | 48.3 ± 3.7 | 43.6 ± 2.2 |

Values are described as mean ± SEM. * $P < 0.05$ as compared to vehicle control.

## 5.4 Discussion

In the previous study, I have demonstrated that rimonabant reduces the expressions of RBP-4 and the proinflammatory cytokines in adipose tissues of genetically altered obese (ob/ob) mice [Mohapatra et al., 2009]. To explore the potential association of RBP-4 with varying degrees of obesity, I measured the expression of RBP-4 and proinflammatory cytokine in WAT of diet induced obese mice at different time interval. The data demonstrate that adipose RBP-4 expression and circulating levels steadily increased with increase in adiposity due to high fat diet exposure. In this study, the proinflammatory cytokine MCP-1 and TNF alpha were measured which have been reported to increase with obese condition [Xu et al., 2003]. TNF alpha is a well established marker for

inflammation and MCP-1 is a chemoattractant molecule whose increased production in obesity aids in the infiltration of macrophages into adipose tissue contributing to the inflammatory state. Significantly higher TNF-alpha and MCP-1 mRNA expression were observed in WAT of 8 weeks of high fat fed mice. Previously Yao-Borengasser et al have made that, there was a significant positive relationship between adipose tissue MCP-1 and RBP4 expression in humans [Yao-Borengasser et al., 2007]. The positive association in gene expression pattern of RBP-4 with MCP-1 and TNF-alpha and inverse correlation with adiponectin in WAT of high fat fed mice, suggest 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. We examined the effects of anti-obesity agent rimonabant, a selective CB1 antagonist, on LDL cholesterol and RBP-4 expression in WAT of high fat fed mice. Surprisingly, a single dose of rimonabant reduced the LDL cholesterol as well as adipose RBP-4 expression in obese mice. This is the first report to indicating the acute effect of rimonabant on LDL and RBP-4 expression in HF fed mice. Erikstrup et al. found a significant correlation of serum RBP4 levels with TG, LDL and HDL levels in patients with type 2 diabetes and proposed a strategy that lowering LDL levels might lower RBP4 levels [Erikstrup et al., 2006]. Acute rimonabant mediated suppression of adipose RBP-4 expression confirm previous observation and further establish direct or indirect observation of LDL cholesterol and RBP-4 levels. There are several reports describing association of LDL level with circulating RBP4 concentrations in patients with type 2 diabetes [Erikstrup et al., 2006; von Eynatten et al., 2007; Usui et al., 2009]. Furthermore, RBP4 homozygous knockout (RBP4-/-) mice demonstrate lower free fatty acid levels indicating the role of RBP4 in lipid metabolism [Quadro et al., 2003].

To further explore the LDL lowering effect, hypercholesteremic ApoE3 mice were treated with single dose rimonabant. Rimonabant treatment caused dose dependent reduction in LDL in ApoE3 mice. The observed positive

correlation of adipose RBP-4 with pro-inflammatory cytokines and LDL, which are strong predictor of cardiovascular disorders, led us to draw the hypothesis that RBP-4 may involve in cardiovascular complications. To explore the hypothesis, we have investigated the aortic RBP-4 expression and its correlation with established cardio-vascular markers in response to HF diet in C57BL/6 and HC diet in ApoE3 mice.

The effect of diet, HF in case of C57BL/6 mice and HC for ApoE3 mice, was much pronounced in the latter 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. CRP levels have been reported to increase in vascular inflammation [Dutta et al., 2009], endothelial dysfunction [Stokes et al., 2009] and atherosclerosis [Tuomainen et al., 2008; Zhang et al., 2008] in mice emphasizing the role of CRP in cardiovascular diseases. MCP-1, like CRP, is known to be a marker of inflammation, and its levels reportedly increase in cardiovascular diseases [Niu et al., 2009]. Balagopal et al recently described a study in obese children, in which a correlation was found between RBP-4 levels and plasma levels of CRP and IL-6 [Balagopal et al., 2007]. Our new findings add further support for the concept that RBP-4 may be an important effector protein in the interaction between the metabolic syndrome and cardiovascular disease.

Rimonabant has been reported to have anti-atherosclerotic effects [Dol-Gleizes et al., 2009]. On a molecular level, CB1 antagonist increases adiponectin [Bensaid et al., 2003] which is known to have anti-inflammatory as well as anti-atherosclerotic effect [Han et al., 2009]. Our results in HC fed ApoE3 mice demonstrate that rimonabant ameliorates dyslipidemia, a major biochemical disorder associated with cardiovascular diseases. Rimonabant treatment of ApoE3 mice reduced serum levels of cholesterol, free fatty acids, and triglycerides and, importantly, decreases the LDL. Moreover, our results show

that rimonabant treatment reduces the elevated levels of CRP, and MCP-1 (tissue and circulatory levels) suggesting its anti-inflammatory role. These finding are in agreement with in vivo data from other laboratories [Schafer et al., 2008; Nissen et al., 2008]. 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.

The data of the present study showing increased expression of RBP-4 and plasma levels in association with inflammatory markers and LDL levels in obese and atherogenic condition reinforces the possible involvement of RBP-4 in cardio-metabolic complications. However, studies are needed to validate these associations and to evaluate the clinical implications of our findings. Further, our results suggest that reduction of RBP-4 along with pro-inflammatory cytokine may be a possible mechanism through which rimonabant improves the cardiovascular complications.