ORIGINAL ARTICLE

# EFFECTS OF SIMVASTATIN AND LEVO-CARNITINE ON PLASMINOGEN ACTIVATOR INHIBITOR-1 LEVELS IN OBESE INSULIN RESISTANT RATS

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**Background:** Insulin resistance is considered to be the underlying pathophysiology of the obesity-related cardiovascular risk factors in metabolic. The role of lipid lowering drugs in the treatment of insulin resistant states and metabolic syndrome is significant. Objective of the present study was to determine the effects of Simvastatin and L-carnitine, individually and in combination, on plasminogen activator inhibitor-1 levels as Cardiac Risk Factor in obese insulin-resistant Sprague-Dawley (SD) rats. **Methods:** Eighty healthy SD rats were divided into 5 groups of 16 rats each. Group-1 was given normal pellet diet and served as control while the rest were given high fat diet (HFD) to induce obesity. After two months of HFD, insulin resistance was confirmed and drug administration was carried out for one month in groups 3, 4 and 5 while group-2 served as control for them. At the end of this period, Plasminogen activator inhibitor-1 level was measured. **Results:** Plasminogen activator inhibitor-1 (PAI-1) levels were raised significantly in the group given HFD (11.78±3.58 ηg/ml from 2.66±1.80 ηg/ml in Group 1, $p<0.001$). Compared to HFD group 2, PAI-1 levels were significantly decreased in both simvastatin (5.80±1.65 ηg/ml, $p<0.05$) and combination therapy group (6.315±1.88 ηg/ml, $p<0.05$) but decrease was statistically insignificant in carnitine monotherapy group (10.20±1.8 ηg/ml). **Conclusion:** The combination therapy of simvastatin and levo-carnitine resulted in greater improvement in plasma PAI-1 levels as a cardio-vascular disease risk factor in diet induced obesity than either of the drugs alone.

**Keywords:** obesity, simvastatin, levo-carnitine, insulin resistance, statins, metabolic syndrome

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## INTRODUCTION

The mass ownership of personal transport, processing of food, consumption of carbonated sodas and increasingly sedentary occupation-processes termed 'coca-colonization' by Paul Zimmet, all have contributed towards an unprecedented change in environment over a very short period. Energy dense foods are available to masses at low cost and with minimum effort. The result has been a dramatic increase in obesity occurring in both children and adults in recent years.[1] A 2002 study showed that nearly two thirds of the US adult population is now either overweight or obese.[2] Around the same time, a study conducted in Pakistan showed 25% of Pakistani population to be overweight or obese according to the Asian-specific BMI cut-off value of 23% and 10.3% to be obese according to the BMI cut-off value of 27. Obesity is a major public health problem in Pakistan.[3] Compared with populations in industrialised countries, those in the developing world appear to be at greater risk of the diseases associated with overweight, and cardiovascular disease has become the leading cause of disability and death in many developing countries.[4]

Although increasing body weight, as defined by BMI, is a strong predictor of metabolic disease risk[5], for any given degree of obesity, there is considerable variability in the manifestation of type 2 diabetes and atherosclerosis[6]. One probable explanation for these differences is that individuals with the same BMI may have very different amounts of visceral (also referred to as 'central' or 'abdominal') fat, the adipose tissue known to be associated with the greatest metabolic risk.[7]

Although insulin resistance is now considered to be the underlying pathophysiology of the obesity-related cardiovascular risk factors in metabolic syndrome (MetS)[8], studies suggest that there is more to pathophysiology of the metabolic syndrome than insulin resistance alone[9]. One of the most consistent pathophysiologic features has been evidence of chronic systemic inflammation.[10] Numerous adipocyte-derived pro-inflammatory markers are highly correlated with the degree of obesity and insulin resistance[11]; many of these inflammatory markers are, in turn, highly predictive of vascular disease risk[12]. Hepatic synthesis of C-reactive protein, fibrinogen and Plasminogen activator inhibitor-1 (PAI-1) is induced in response to adipocyte-derived pro-inflammatory cytokines such as TNFα and IL-6 and MetS is associated with impaired fibrinolysis characterised by elevated plasma PAI-1 and fibrinogen levels.[13] Insulin may also increase coagulation factor VII gene expression.[14]

Since high levels of free fatty acids and resultant lipotoxicity are implicated in the pathogenesis of insulin resistance,[15] the role of lipid lowering drugs in the treatment of insulin resistant states and metabolic syndrome is significant. Statins are the most effective

lipid lowering drugs in the market and it has been established that the benefits of statin therapy in cardiovascular disease can be explained not only by the lipid-lowering effects of statins but also by non-lipid-related mechanisms (also-called 'pleiotropic effects').[16] The adverse effects of statins include generalised gastrointestinal disturbances, skin rash or flushing, sensation of tingling and numbness and asymptomatic elevation of liver transaminase levels. More serious adverse events include myotoxicity, peripheral neuropathy and memory loss.[17] Because of the associated side effects of statin group of drugs, it is essential to look at diverse agents for lowering insulin resistance.

Levocarnitine besides acting as hypolipidemic agent can also increase the anti-oxidants levels while decreasing the stress markers like C-reactive protein, myeloperoxides and malondialdehyde, and improve the fibrinolytic balance by decreasing plasma PAI-1 levels.[18]

The current study was planned to determine the relative effect of these drugs on impaired fibrinolysis associated with metabolic syndrome as well as to determine the synergistic effect, if any, when given in combination. Simvastatin was chosen as the representative of the statin group as it is one of the most commonly prescribed statin drugs.

## SUBJECTS AND METHODS

It was a randomised controlled trial conducted at Army Medical College Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad from May 2008 to May 2009. Prior to the commencement of the study, permission from Ethical Committee was obtained. Eighty adult Sprague Dawley rats weighing 180±50 grams, and aged 60–90 days were selected from NIH, Islamabad. Rats with deranged lipid profile or who failed to become insulin resistant after HFD were not included. We used Sprague-Dawley rats as they readily gain weight on high-fat diets. They were supplied with diet pellets and water *ad libitum*.

Animal house facility of NIH, Islamabad was used. Room was well ventilated and 12-hour light and 12-hour dark cycle was maintained. Temperature was maintained at 22±3 °C. The normal pellet diet (NPD) or chow diet provided 10% of the calories as fat, 65% as carbohydrate and 25% as protein.

Random allocation of the rats (16 each) to all the 5 groups was done. Control group (Group 1, n=16) rats were fed normal diet *ad libitum*. The rats of this group served as a control for group 2. Blood sampling for insulin resistance after 2 months and for cardiac risk factors after another one month was done for the experimental groups according to sampling schedule.

Group 2 (High Fat Diet, n=16) rats were fed high fat diet for 2 months or 9 weeks, after which 1.5 ml sample of blood from tail vein was drawn to assess the insulin resistance by measuring serum TG/HDL-C ratio which was taken to be indicative of IR if more than 1.8.[19] High fat diet (HFD) was continued for the next 30 days, after which terminal sampling by intra-cardiac puncture was done to assess the cardiac risk factors.

Similar procedure was followed for 2 months for Group 3 (HFD+Simvastatin, n=16) as for Group 2, and then rats were given Simvastatin (12 mg/Kg) mixed with diet for 30 days. HFD was continued till the end. Sampling was done as per schedule.

To Group 4 (HFD+L carnitine, n=16), HFD was given for 2 months, and then L-carnitine was given at dose of 200 mg/Kg for 30 days orally in drinking water, along with continuation of HFD diet. L-Carnitine 500 mg (Sundown, Inc. Boca Raton, FL 33487 USA) was used. Dose per day was calculated and the calculated number of Carnitine tablets was crushed and dissolved in fresh drinking water each morning. Bottles were checked in the evening and refilled with plain water. Sampling schedule was followed.

To Group 5 (HFD+L-carnitine+Simvastatin, n=16) HFD was given for 2 months, and then rats were given L-carnitine 200 mg/Kg and Simvastatin 12 mg/Kg orally in combination with HFD for the next 30 days. Sampling schedule was followed.

On the eve of the sampling, the feed was removed from the cages and intra-cardiac sampling was done in the morning at the laboratory to ensure a fasting sample for plasma PAI-1 concentrations. Within one hour of completion of sampling, samples were shifted from NIH to the Centre for Research in Experimental and Applied Medicine (CREAM) at Army Medical College, Rawalpindi.

## RESULTS

The mean weight of Group 1 rats was 300±50 gm at the age of 5 months, or after 2 months of NPD. The mean PAI-1 level was 2.66±1.80 ηg/ml. This value was taken as standard and was compared with the respective values obtained from experimental Group 2. In Group 2, at the end of first two months, the weight gain was substantial and significant, average weight being 400±30 gm ($p$=0.001). The mean PAI-1 level was 11.77±3.58 ηg/ml. Statistical analysis revealed significant differences in plasma PAI-1 levels ($p$<0.001) (Table-1).

**Table-1: PAI-1 level after two months of HFD**

| Parameter | Group 1 | Group 2 | $p$ |
|---|---|---|---|
| Weight (gm) | 300±50 | 400±30 | 0.001 |
| PAI-1 (ηg/ml) | 2.66±1.80 | 11.78±3.58 | 0.001 |

The mean PAI-1 level in Group 3 was 5.80±1.65 ηg/ml. There were significant differences between plasma PAI-1 levels of Group 2 and Group 3 ($p$<0.05). The mean PAI-1 level in Group 4 was

10.20±1.82 ηg/ml. There were no significant differences in plasma PAI-1 levels between Group 2 and Group 4 ($p$>0.05). In Group 5 the mean PAI-1 level was 6.31±1.88 ηg/ml.

Plasma PAI-1 levels were significantly decreased when compared with Group 2 and Group 4 but difference was insignificant when compared with Group 3. The PAI-1 activity was increased in HFD group, which was significantly attenuated in simvastatin monotherapy group. The decrease in PAI-1 activity was statistically significant in L-carnitine monotherapy group, whereas the differences in PAI-1 values of group 1, 3 and 5 were insignificant. (Table-2)

**Table-2: Differences in PAI-1 between Groups ($p$-value)**

| Groups Compared | $p$ |
|---|---|
| Group 2 vs Group 3 | 0.001 |
| Group 2 vs Group 4 | 0.001 |
| Group 3 vs Group 4 | 0.001 |
| Group 5 vs Group 1 | 0.799 |
| Group 5 vs Group 2 | 0.001 |
| Group 5 vs Group 3 | 0.378 |
| Group 5 vs Group 4 | 0.001 |

## DISCUSSION

We were able to demonstrate the success of high fat diet model for the development and progression of metabolic syndrome in rodents. After 2 months of high fat diet (fat source being beef tallow) all the rats gained weight and exhibited a rise in PAI-1 levels indicating a pro-inflammatory response and a disturbances of fibrinolytic system presumably induced by obesity. From the results of our study, however, reduction in fibrinolytic homeostasis marker PAI-1 was not significantly more in combination group as compared to simvastatin monotherapy group.

An elevated plasma PAI-1 concentration is predictive for myocardial infarction in humans.[20] However, the predictive value of circulating PAI-1 levels is highly dependent on the insulin resistance syndrome.[21] As a matter of fact, PAI-1 is stated to be the true benchmark for metabolic syndrome.[22]

In 2002, a study was conducted by Festa *et al* investigating the relation of PAI-1, C-reactive protein (CRP) and fibrinogen to incident type 2 diabetes during a 5-year period in the Insulin Resistance Atherosclerosis Study (IRAS).[23] A significant association of the three variables of interest, i.e., CRP, fibrinogen, and PAI-1 with BMI, waist circumference, fasting insulin, fasting glucose, and SI was found.[23] In our study, the levels of PAI-1 raised from 2.66±1.80 µg/ml in control group to 11.78±3.58 µg/ml in high fat diet fed, obese group, which is in accord with the earlier studies.

In previous studies, variable results are reported with different statin drugs. In one study, Simvastatin increased PAI-1 by 18% in 111 patients treated for 2 years.[24]

Wiesbauer *et al*[25] compared the effect of 6 different statins (atorvastatin, cerivastatin, fluvastatin, lovastatin, pravastatin, simvastatin) on components of the fibrinolytic system expressed by human vascular endothelial cells and smooth muscle cells and by the human hepatoma cell line HepG2. In their study, all statins used except pravastatin significantly decreased PAI-1 production in human endothelial and smooth muscle cells and increased t-PA production in human smooth muscle cells.[25]

Extending the investigation in the same direction, a study[26] published in 2008 investigated the effects of simvastatin on pro-atherosclerosis markers (PAI-1, sP-selectin, and sCD40 ligand) in metabolic syndrome. Compared to baseline, simvastatin significantly reduced the circulating PAI-1 activity ($p$<0.05).

Although, there are not many recent studies documenting the effect of levo-carnitine on fibrinolytic parameters, in 1992, Pola *et al*[27] attempted to restore the coagulative fibrinolytic homeostasis that is compromised in peripheral vascular disease. Patients with peripheral vascular disease were given oral propionyl-L-carnitine 1 g thrice a day for a period of 20 days. The authors observed a significant increase of t-PA synthesis and a significant decrease in PAI-1 activity on the 20$^{th}$ day of therapy.[27]

Our study confirms the efficacy of the simvastatin therapy on fibrinolytic marker, PAI-1. The negligible effect of carnitine therapy on PAI-1 in our study may be attributed to smaller dose used (less than half the human dose) as well as the pharmacological compound used (propionyl vs L-carnitine, L-tartrate).

## CONCLUSION & RECOMMENDATIONS

The positive effect of simvastatin on the fibrinolytic system marker PAI-1 in insulin resistant state was confirmed in our study. However, the levo-carnitine therapy had no effect on raised PAI-1 levels. Further studies should be designed to elucidate the cellular mechanism of action of carnitine for the reduction of pro-inflammatory cytokines like CRP and pro-fibrinolytic marker PAI-1.

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