ORIGINAL ARTICLE

EFFECTS OF LEVO-CARNITINE AND SIMVASTATIN ON FASTING GLUCOSE AND C-REACTIVE PROTEIN LEVELS IN INSULIN RESISTANT RATS

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Background: Obesity is a pro-inflammatory condition in which adipose tissue contributes to increased levels of pro-inflammatory cytokines such as C-reactive protein (CRP), which has been identified as an independent risk factor for cardiovascular disease as well as insulin resistance. 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 plasma glucose and CRP levels on high fat diet fed insulin-resistant obese Sprague-Dawley (SD) rats. **Methods:** Eighty healthy SD rats were randomly divided into five groups of sixteen rats each. Group-1 was given normal pellet diet (NPD) and served as control while the rest were given high fat diet (HFD) to induce obesity. Insulin resistance was confirmed after two months of HFD, and drug administration (simvastatin: 12 mg/Kg and levo-carnitine: 200 mg/Kg orally) carried out for one month in groups 3, 4 and 5. At the end of this period, plasma glucose levels and CRP levels were recorded and statistically analysed. **Results:** The fasting plasma glucose levels were reduced significantly in carnitine group, insignificantly in simvastatin group, and no synergistic effect was seen in the combination therapy group. Significant reduction in CRP levels was induced by both the monotherapy groups; the maximum reduction was seen in combination group. **Conclusion:** The combination therapy of simvastatin and levo-carnitine resulted in greater reduction in CRP levels in diet induced obesity than either of the drugs alone but had no synergistic effect on plasma glucose levels.

Keywords: obesity, insulin resistance, plasma glucose, CRP simvastatin, levo-carnitine
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INTRODUCTION

Obesity is a manifestation of maladaptation of a genetic heritage meant to conserve and store energy, to the overabundance of modern world.[1] According to WHO fact-sheet on obesity in 2014 (updated January 2015), more than 1.9 billion adults, (39% of 18 years and older) were overweight worldwide. Of these over 600 million (13% of all adults) were obese.[2]

Numerous co-morbidities such as metabolic syndrome (MS), type 2 diabetes, cardiovascular diseases (CVD), systemic and pulmonary hypertension, polycystic ovarian diseases, non-alcoholic fatty liver disease (NAFLD) and sleep apnoea are associated with obesity. Indeed, obesity is now considered an independent risk factor for CVD.[3]

There is a differential resistance to insulin in obese individuals.[4] The body compensates for insulin resistance by producing more and more insulin which rises the likelihood of developing hypertension and atherosclerosis.[5] They also have a distinctive dyslipidemia. These deviations increase the danger of CVD. The constellation of clinical conditions increasing the risk of CVD which have a common primary defect in insulin action is labelled as metabolic syndrome or insulin resistance syndrome (IRS).[6]

It has been hypothesised that increased levels of free fatty acids (FFAs) are implicated in insulin resistance. FFAs and several metabolites such as diacylglycerol acyl-CoAs and ceramides activate protein kinases like Protein Kinase C (PKC), the inhibitor of nuclear factor-κB (NF-κB) kinase-β (IKKβ) and Jun kinase (JNK), which increase the serine phosphorylation of insulin receptor substrates (IRS) thus impairing the insulin signalling.[7]

Obesity is a pro-inflammatory condition in which hypertrophied adipose tissue and its resident immune cells (chiefly macrophages and lymphocytes) both contribute to increased circulating levels of pro-inflammatory cytokines.[8] One of these markers, C-reactive protein (CRP) has been established to be an independent risk factor for both CVD and insulin resistance.[9]

Treatment with lipid-lowering drugs is one of the most current therapeutic measures for reducing cardiovascular risk. Statin group of drugs (Hydroxy-3 methylglutaryl-CoA reductase inhibitors) work by inhibiting the de novo cholesterol synthesis in the liver and are the most commonly used lipid lowering drugs.[10] Statins have been associated with reduction in vascular inflammation and oxidative stress as well.[11] However,

the side effects of statins which can include myotoxicity and memory loss can affect the quality of life.[12]

Studies show that supplementation of Levo-carnitine, a natural vitamin-like substance, lowers triglycerides in blood.[13] Carbohydrate consumption is also enhanced as it reduces acetyl co-A/free co-A ratio inside the mitochondria, which stimulates the activity of pyruvate dehydrogenase, increasing the oxidation of pyruvate and reducing lactate formation.[14]

Studies have shown the comparison of the effects of HMG-CoA reductase inhibitor (simvastatin) with *levo*-carnitine on dyslipidemia and pro thrombotic status in MS.[15] In the current study, we combined these drugs to determine their effects on plasma glucose and CRP levels as the cardio-vascular disease risk factors in diet induced obesity in Sprague-Dawley rats.

MATERIAL AND METHODS

Permission from Ethical Committee was obtained before starting the study. The randomised controlled trial was carried out at the Centre for Research in Experimental and Applied Medicine (CREAM) at Army Medical College, Rawalpindi. Animal house facility of National Institute of Health (NIH), Islamabad was used. A total of 80 healthy Sprague-Dawley rats, 60–90 days old, weighing 130–180 gm, were selected and divided into 5 groups of 16 rats each. The normal pellet diet (NPD) or chow provided 65% carbohydrates, 25% proteins and 10% fat calories. The high-fat diet supplied 17% of the calories as carbohydrate, 25% as protein and 58% as fat.[16] Both diets were prepared and supplied by animal house facility of NIH.

Normal pellet diet (NPD) was given to control, Group-1. Its blood parameter values were considered to be baseline. High fat diet (HFD) was given for two months to Group-2[17] after which it was assessed for development of insulin resistance. The criterion for development of insulin resistance was the ratio of plasma triglycerides (TG) to high density-cholesterol (HDL-C) adapted from McLaughlin and colleagues, the cut-off point was 1.8.[18] Group-2 was the control for the drug intervention groups: 3, 4 and 5.

Groups 3, 4 and 5 were given HFD for the first two months to induce insulin resistance, and then drugs were administered for one month with on-going HFD. Simvastatin 12 mg/Kg mixed in diet was given to Group-3.[19] Levo-carnitine 200 mg/Kg body weight mixed with drinking water was given to Group-4.[20] Group 5 was given both drugs simultaneously. At the end of one month, after ensuring proper anaesthesia by ether, terminal intra-cardiac blood sampling was done. Samples were centrifuged and platelet free serum used to assess plasma levels of C-reactive protein, glucose, total triglycerides and high density lipoprotein-cholesterol (HDL-C).

To assess weight gain, rats were weighed both at the beginning and the end of study. Commercial kits were used to assess plasma glucose, total triglycerides, total cholesterol and high- density lipoprotein-cholesterol (HDL-C). Estimation of serum C-reactive protein (CRP) level was done by Rat CRP ELISA Kit (Molecular Innovations Inc. USA).

SPSS-15 was used for statistical analysis. Quantitative variables were expressed as means and standard deviation. ANOVA followed by Tukey's HSD tests was used to evaluate the statistical significance of the various quantitative changes between the experimental and control groups in all the parameters except in CRP group which had high SD values and therefore Kruskal-Wallis test followed by Mann-Whitney U tests were applied for comparison between groups. The difference was regarded statistically significant for p value equal to or less than 0.05.

RESULTS

By the end of two months, the weight gain in Group-2 HFD rats was substantial and significantly more than Group-1 on NPD, (400±30 gm *vs* 300±50 gm). Insulin resistance was confirmed by the TG/HDL-C ratio of 4.50 in the Group-2 after two months of HFD.[17] As compared to the control Group-1, plasma glucose, lipids and CRP levels were significantly increased and HDL-C significantly decreased in Group-2. The p value of all compared parameters between Group-1 and 2 was less than 0.05.

In the simvastatin administered Group-3, simvastatin had negligible effect on rise in plasma glucose induced by HFD (19.34±3.02 mmol/L *vs* 20.48±3.94 mmol/L (p<0.290). The C–reactive protein was decreased from 1098.07±348.16 µg/ml to 327.24±177.60 µg/ml (p<0.001). The HFD-induced increase in TG and TC was statistically significantly modified (p<0.001). The decrease in HDL-C was amended significantly (p<0.002). The TG/HDL-C ratio decreased to 2.80 (p<0.001) indicating waning of IR.

In the levo-carnitine administered Group-4, C-reactive protein, TG, and plasma glucose were reduced significantly (p<0.001) compared to HFD group-2. Change in TC and HDL-C was not statistically significant. The TG/HDL-C ratio decreased to 3.39 (p<0.015). CRP levels and insulin resistance were reduced significantly in both treatment groups with no statistical difference between them (p<0.315).

Statistical analysis of Group-5 (combination therapy) revealed significant decrease in serum glucose and serum CRP levels as compared with the HFD group (Group-2), the simvastatin monotherapy (Group-3) and the levo-carnitine monotherapy (Group-4) (p<0.05). The individual values for intervention groups are shown in Table-1.

Table-1: Effects of monotherapy and combination therapy of simvastatin and l-carnitine on fasting plasma glucose, lipid profile, insulin resistance and C-reactive protein levels (Mean±SD)

Parameters	Group-3 Sim+HFD	Group-4 L-Car+HFD	Group-5 Sim+L-Car+HFD
Fasting Plasma Glucose (mmol/L)	19.12±3.47	13.04±1.41	13.56±3.63
Total triglycerides (mmol/L)	2.00±0.20	1.77±0.24	1.57±0.12
Total cholesterol (mmol/L)	3.20±0.52	3.86±0.48	2.97±0.25
HDL-C (mmol/L)	0.71±0.10	0.53±0.08	0.725±0.09
C-Reactive protein (µg/ml)	466.00±294.41	446.31±232.95	297.65±98.33
TG/HDL-C ratio	2.81	3.33	2.16

Table-1: Differences in CRP between Groups (*p*-value)

Groups Compared	*P*
Group 2 vs Group 3	<0.001
Group 2 vs Group 4	<0.001
Group 2 vs Group 5	<0.001
Group 3 vs Group 4	0.315
Group 5 vs Group 3	0.005
Group 5 vs Group 4	0.089

DISCUSSION

The high fat diet model for the development and progression of metabolic syndrome in rodents was successfully validated. Inbred Sprague-Dawley rats were used. After 2 months of high fat diet all the rats gained weight and exhibited fasting hyperglycaemia, along with a rise in plasma C-reactive protein level indicating a pro-inflammatory response, presumably induced by obesity.

Many other studies have also reported development of hyperglycaemia with HFD. In another study HFD was given for 4 weeks and fasting glucose levels increased from 5.98±0.09 mmol/L to 6.18±0.26 mmol/L.[21] Smaller duration and a different diet may explain the relatively less marked increase as compared to our study.

Scientists examined the effects of high-fat or high-sucrose diets on insulin resistance in male C57BL/6J mice.[22] The plasma glucose concentrations of mice fed on the HF diet was 20.61% greater than in those fed by HS diets, (8.25±1.25 mmol/L *vs* 5.50±0.46 mmol/L). The greater increase in plasma glucose concentrations in our study may be attributed to difference in diet as well as species.

C-reactive protein (CRP) has been established to be an independent risk factor for both CVD and insulin resistance[9]. It has been positively associated with body mass index.[23] In our study, CRP levels increased 55.25% in response to high fat diet and these results are in agreement with the concentration of CRP seen in obesity as reported in most of the previous studies.

In a population-based cross-sectional study conducted in 2004 to examine the association of CRP with various features of the metabolic syndrome, authors concluded that obese subjects more frequently had CRP levels in the range indicative of a source of infection or inflammation (>10 mg/L) than non-obese subjects.[24]

Moreover, disruption of vasodilatory effects of Nitric oxide on coronary vasculature in obesity was assessed in 2008 in an animal study. Male Wistar rats were fed the high-fat diet for 10 weeks. The body weight, plasma glucose, total cholesterol, and serum insulin levels became significantly more compared with NPT fed rats, however both groups had similar CRP levels.[25] However, no reason was offered for this discordant result as compared to prevalent view.

On the other hand, a study published in 2009 in which Male Zucker Fa/fa rats were fed a high-fat (HF) diet, a high-fat diet with added antioxidants as procyanidins from grape seed or a low-fat (LF) diet for 19 weeks, confirmed the rise in CRP levels after HFD ingestion.[26] Raised CRP levels resulting from diet-induced obesity in our study were modified by drug intervention. Both Simvastatin and l-carnitine lowered the CRP levels and the combination therapy had by far the most positive effect.

In 2000, a prospective trial was conducted to directly evaluate the anti-inflammatory effect of statins as indicated by decrease in plasma levels of C-reactive protein. It was a community-based, double-blind, randomised, prospective trial. In the primary prevention trial, pravastatin reduced median CRP levels by 16.9% ($p<0.001$) as compared with placebo at 24 weeks, in the secondary prevention cohort treatment with pravastatin reduced CRP levels by 14.3% at 12 weeks and 13.1% at 24 weeks ($p<0.005$ for both).[27]

Effectiveness of l-carnitine in lowering inflammatory markers is also indicated by some studies. In one study evaluating patients undergoing kidney dialysis with resultant increased CRP levels, treatment with L-carnitine (20 mg/Kg, given intravenously at the end of each dialysis session for 6 months), resulted in a reduction of CRP levels.[28] Another study in which L-carnitine was used intravenously three times a week at a 20 mg/Kg dose after each haemodialysis session in chronic haemodialysis patients, revealed that supplemental L-carnitine not only lowered CRP levels, it also resulted in improved body mass index and reduction in insulin resistance.[29]

The combination therapy reduced the raised fasting plasma glucose and plasma CRP seen after two months of high fat diet more effectively than the monotherapies.

CONCLUSION

Simvastatin and *levo*-carnitine given in combination have a greater beneficial effect on the predisposing

factors for cardiovascular problems in obesity and MS than monotherapy of either of the drugs. It becomes apparent from the results that *levo*-carnitine may have a place in the line-up of hypoglycaemic drugs in diabetes type 2, as well as a component of combination therapy in metabolic syndrome with pro-inflammatory state.

LIMITATIONS

Experimentations with sub-optimal doses could not be performed.

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