ORIGINAL ARTICLE

EFFECTS OF SIMVASTATIN AND LEVO-CARNITINE ON DYSLIPIDEMIA AND INSULIN RESISTANCE IN OBESE INSULIN RESISTANT RATS

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Background: Obesity and its accompanying problem of metabolic syndrome are a worldwide occurrence. To minimise the cardiovascular risks associated with metabolic syndrome, lipid lowering drugs like statins are given. This study was carried out to compare the effects of simvastatin and L-carnitine on insulin resistance and cardiac risk factors in high fat diet fed insulin-resistant obese Sprague-Dawley rats. **Methods**: Sixty-four healthy Sprague-Dawley rats were divided into four groups of sixteen rats each. Group-1 was control and given normal pellet diet while the rest were given high fat diet to induce obesity. After two months, insulin resistance was confirmed by calculating TG/HDL-C ratio. High fat diet was continued and drugs administered (simvastatin 12 mg/Kg, and *levo*-carnitine 200 mg/Kg) for one month in groups 3 and 4 while group 2 served as control. At the end of this period, lipid profile, and plasma glucose levels were recorded and statistically analysed**. Results:** Plasma glucose, serum triglycerides, and total cholesterol were raised significantly in groups given high fat diet ($p<0.000$). Serum triglycerides were significantly decreased by both drugs ($p<0.001$), the reduction was more in carnitine than in simvastatin group ($p>0.047$). Total cholesterol reduction in simvastatin group was highly significant ($p<0.002$) but was insignificant in l- carnitine group ($p<0.069$). The reduction in the fasting plasma glucose levels was highly significant in levo-carnitine group ($p<0.000$), and not significant in simvastatin group ($p<0.290$). Insulin resistance was reduced by both the drug administered groups ($p<0.01$), the difference between the two groups was not significant ($p>0.05$). **Conclusion:** Both simvastatin and *levo*-carnitine succeeded in significantly reducing the risk factors for cardiovascular complications of obesity and metabolic syndrome although their maximal beneficial effect was on different parameters.

Keywords: obesity, insulin resistance, lipid profile, simvastatin, *levo*-carnitine

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INTRODUCTION

The number of obese individuals worldwide has reached up to 2.1 billion.[1] In Pakistan, 25% of total population would be classified as overweight or obese with the use of Indo-Asian-specific BMI cut-off values.[2] It has led to a dramatic increase in obesity-related health problems. Obese individuals develop a differential resistance to the cellular actions of insulin.[3] The insulin resistance is a key etiological factor for development of type 2 diabetes mellitus (DM type 2).[4] The only way the body can compensate for the resistance to insulin action is by secreting the increased amount of insulin. The combination of insulin resistance and compensatory hyperinsulinemia increases the probability of hypertension and atherosclerosis.[5]

These obese insulin resistant individuals also have a dyslipidemia characterised by high plasma triglyceride (TG), low high-density lipoprotein cholesterol (HDL-C) levels and raised low-density lipoprotein cholesterol (LDL-C) particles. These changes increase the risk of cardiovascular disease (CVD), and the cluster of clinical disorders having a common basic defect in insulin action, increasing the risk of CVD is designated as syndrome X or metabolic syndrome (MS) or insulin resistance syndrome (IRS).[6]

Modification of the atherogenic lipid profile by treatment with lipid-lowering drugs is probably one of the most effective methods of reducing cardiovascular risk. Hydroxy-3 methylglutaryl-CoA reductase inhibitors or statin group of drugs are the most effective lipid lowering drugs. Statins work by inhibiting the *de novo* synthesis of cholesterol in the liver. Statins are structural analogues of HMG-CoA reductase which is the key rate-limiting enzyme of the cholesterol biosynthesis and thereby inhibit it competitively.[7] In addition, statins use has been associated with reduction in oxidative stress and vascular inflammation with increased stability of atherosclerotic lesion.[8]

Statins are not free of side effects. The common adverse effects of statins include generalised gastrointestinal discomfort, skin rash or flushing, and asymptomatic elevated transaminase levels. More serious adverse events include myotoxicity, peripheral neuropathy and varying degree of memory loss.[9] These side effects disrupt the quality of life and force many patients to discontinue the drug.[10] There is need to develop better lipid lowering drugs without alarming

side effects and to counter these effects by conventional or non-conventional pharmacological means. One such non-conventional candidate is *levo*-carnitine.

Levo-carnitine is a natural vitamin-like substance which plays an integral part in β-oxidation of fatty acids favouring energy production and blocking esterification of fatty acids to triglycerides. Thus supplementation of L-carnitine lowers triglycerides in blood.[11] It also enhances carbohydrate utilisation as it reduces acetyl co-A/free co-A ratio inside the mitochondrion stimulating the activity of pyruvate dehydrogenase and increasing the oxidation of pyruvate and reducing lactate formation.[12] Pyruvate dehydrogenase activity is depressed in the insulin resistant status.[13]

This study was planned to assess and compare the effects of administration of Simvastatin and *levo*-carnitine, on cardiac risk factors: plasma glucose, Serum triglycerides, total cholesterol in Sprague-Dawley rats which were high fat diet (HFD) fed, insulin- resistant and obese.

MATERIAL AND METHODS

This laboratory based randomised controlled trials were carried out at the Centre for Research and Applied Medicine (CREAM) at Army Medical College, Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad. Animal house facility of NIH was used. Sixty-four healthy Sprague-Dawley rats, 60–90 days old, weighing 130–180 g, were selected and divided into 4 groups of 16 rats each. The normal pellet diet or chow provided 10% of the calories as fat, 65% as carbohydrate and 25% as protein. The high-fat diet supplied 58% of the calories as fat, 25% as protein and 17% of the calories as carbohydrate comprised of sucrose.[14] Both diets were prepared and supplied by animal house facility of NIH.

Group-1 was given normal pellet diet (NPT) and considered as control group for the receiving high fat diet and its blood parameter values were taken to be normal.

Group-2 was given High Fat Diet (HFD) for two months[15] and then assessed for development of insulin resistance by measuring the increase in weight and determining the lipid profile and plasma glucose levels. The criterion for development of insulin resistance was adapted from McLaughlin and colleagues as the ratio of plasma triglycerides (TG) to high density-cholesterol (HDL-C), the cut-off point being 1.8.[16] This group acted as the control for the drug intervention groups.

Group-3 and 4 were the drug intervention groups. For the first two months rats were given HFD to induce insulin resistance, and then drugs were given for one month along with HFD. Group-3 was given simvastatin 12 mg/Kg mixed in diet.[17] Group-4 was given levo-carnitine 200 mg/Kg/body weight mixed with drinking water.[18] At the end of one month, rats were weighed, and terminal intracardiac blood sampling was done after ensuring proper anaesthesia by ether. Platelet free serum was obtained and used to assess plasma glucose, plasma total triglycerides, total cholesterol, high density lipoprotein-cholesterol (HDL-C) and low density-lipoprotein-cholesterol (LDL-C).

Rats were weighed at the beginning of study and again at the end of two months of HFD to compare groups on NPT and on HFD. Plasma glucose, total triglycerides, total cholesterol and high- density lipoprotein-cholesterol (HDL-C) were assessed by commercially available kits. LDL-cholesterol was calculated as: LDL-C=total cholesterol-(HDL-C+VLDL-C); VLDL-cholesterol was calculated as 0.456×total triglyceride concentration expressed in mmol/L (Friedewald formula).[19]

Statistical analysis was done using SPSS-15. Quantitative variables were expressed as means and standard deviation. To see the significance of differences among the groups, ANOVA and Tukey's HSD were calculated and $p<0.5$ was taken as significant.

RESULTS

By the end of two months, the rats on HFD were significantly heavier than their counterparts on NPD. The weight gain in group-2 was significant, average weight being 400±30 g, 26.66% more than the group-1 on NPD (average weight 300±50 g, $p=0.00$). The TG/HDL-C ratio of the high fat diet group after two months of HFD was 4.502, which confirmed insulin resistance. Total cholesterol, total triglycerides, LDL-C and plasma glucose levels were significantly increased and HDL-C significantly decreased in group-2 as compared to the control group-1 (Table-1).

In the group-3, the increase in the TG, TC and LDL-C levels induced by HFD was lessened to a statistically significant extent ($p<0.001$). The HDL-C was significantly increased ($p<0.002$), but not normalised. Simvastatin had negligible effect on HFD-raised plasma glucose (19.34±3.02 mmol/L *vs* 20.48±3.94 mmol/L, $p<0.290$). The metabolic marker for insulin resistance, the TG/HDL-C ratio decreased from 4.50 in HFD group-2 to 2.80 ($p<0.001$) in group-3 indicating lessening of IR.

In the *levo*-carnitine group-4, plasma glucose and TG were reduced significantly ($p<0.000$) compared to HFD group-2. Reduction in TC, LDL-C and HDL-C was not statistically significant. The marker for insulin resistance, the TG/ HDL-C ratio decreased significantly to 3.39 from 4.50 in HFD group-2 ($p<0.015$). Reduction in insulin resistance was significant in both treatment groups with no statistical difference between them. (Table-3, 4).

Table-1: Effect of high fat diet on weight, plasma glucose levels and lipid profile

Parameters	Group-1	Group-2	*p*	% Change
Weight (gm)	300±50	400±30	0.000	26.66
Fasting Plasma Glucose (mmol/L)	5.80±2.30	20.48±3.94	0.000	69.15
Total triglycerides (mmol/L)	1.10±0.33	2.46±0.35	0.000	53.26
Total cholesterol (mmol/L)	1.80±0.33	4.34±0.55	0.000	57.31
HDL-C (mmol/L)	0.74±0.10	0.56±0.09	0.000	24
LDL-C (mmol/L)	0.83±0.25	3.29±0.49	0.000	73.62
TG/HDL-C ratio	1.50	4.50	0.001	189.74

Table-2: Plasma glucose, lipid profile and insulin resistance in interventional and control groups

Parameters	HFD	Sim+HFD	L-car+HFD
FPG mmol/l	20.48±3.94	19.34±3.02	13.04±1.4
TG mmol/l	2.45±0.35	1.98±0.20	1.77±0.24
TC mmol/l	4.34±0.55	3.19±0.56	3.86±0.48
HDL-C mmol/l	0.55±0.09	0.72±0.09	0.53±0.08
LDL-C mmol/l	3.20±0.49	2.07±0.61	2.97±0.43
TG/HDL-C ratio	4.50±0.92	2.80±0.62	3.39±0.61

Table-3: Differences between interventional and control groups (*p*-values)

Variable	HFD vs Sim+HDF	HFD vs L-car+HDF	Sim+HDFvs L-car+HDF
FPG mmol/l	0.885	0.000	0.000
TG mmol/l	0.001	0.000	0.327
TC mmol/l	0.001	0.000	0.005
HDL-C mmol/l	0.001	0.969	0.001
LDL-C mmol/l	0.000	0.425	0.000
TG/HDL-C ratio	0.000	0.000	0.181

DISCUSSION

Modification of the atherogenic lipid profile by treatment with lipid-lowering drugs is practical and one of the most effective methods of reducing cardiovascular risk in obesity. This study was conducted to compare the relative effectiveness of two lipid-lowering agents, one a conventional statin and the second a non- conventional drug, L-carnitine in obesity-induced insulin resistant states.

In this study, we successfully demonstrated that high fat diet regimen as advocated by Srinivasan *et al*[15] is a successful model to induce obesity and insulin resistance; *levo*-carnitine may have a role as a hypoglycaemic drug in metabolic syndrome with cardiac risk factors like dyslipidemia and insulin resistance as well as in diabetes type 2.

Previous studies experimenting with high fat diet model demonstrated the disturbances in the lipid profile and the raise in TG/HDL-C ratio indicating insulin resistance as seen in our study.[20] However the magnitude varied, which may be attributed to difference in dietary plan and route of feeding as well the rodent species.

In a 2004 study to see the effects of statins and niacin in dyslipidemia, simvastatin reduced LDL-C level by 42.79% ($p<0.05$) at 12 weeks, TC levels decreased by 32.97% ($p<0.05$), while HDL-C,TG and Lp(a) levels did not change significantly.[21]

In a retrospective cohort analysis in 2006 comparing the relative effectiveness of the different members of statin group, it was found that simvastatin-treated patients had a 24.9% decrease in LDL-C from baseline and that attainment of NCEP ATP III LDL-C goal among patients was 72.8% among simvastatin users.[22] The results from our study show that all lipid parameters benefited from simvastatin therapy, thus improving the insulin resistance but the effect was less on triglycerides while having little effect on plasma glucose.

A review of carnitine supplementation studies on effects of either carnitine supplementation or carnitine deficiency on parameters of glucose homeostasis and insulin sensitivity in DM concluded that it has a beneficial effect on glucose tolerance during insulin-resistant states.[23] In a study to evaluate the effects of L-carnitine on insulin-mediated glucose uptake and oxidation in type II diabetic patients, it was concluded that L-carnitine improves insulin sensitivity in insulin resistant diabetic patients with significant effect on whole body insulin-mediated glucose uptake.[13]

A study investigating the effect of L-carnitine treatment on very low density lipoprotein kinetics in the hyperlipidemic rabbit found that plasma VLDL-triglycerides (VLDL-TG) and VLDL-apoprotein B (VLDL-apoB) were significantly decreased after four weeks of treatment.[11]

In a study in 2007, Power *et al* concluded that carnitine supplementation relieves lipid overload and glucose intolerance in obese rodents.[24]

In our study, only plasma glucose and TGs wee decreased while no effect was seen in TC, LDL-C and HDL-C. The difference in reduction parameters may be explained by the less duration of carnitine administration.

CONCLUSION

Both simvastatin and *levo*-carnitine have a beneficial effect on the risk factors for cardiovascular complications of obesity and metabolic syndrome although their maximal beneficial effect was on different parameters. This study can be further expanded to investigate the anti-inflammatory and anti-oxidant effects of carnitine to counter the production of reactive oxygen species in metabolic syndrome and DM.

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