EVALUATION OF THE EFFECT OF ATORVASTATIN ON INSULIN SENSITIVITY

Dissertation submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment of the regulations

for the award of the degree of

M.D. BRANCH - I

GENERAL MEDICINE

GOVT. STANLEY MEDICAL COLLEGE & HOSPITAL
THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI, INDIA

MARCH 2009

CERTIFICATE

This is to certify that the dissertation titled **"EVALUATION OF THE EFFECT OF ATORVASTATIN ON INSULIN SENSITIVITY"** is a original work done by **Dr. A.SANTHI SELVI,** Post-graduate in General Medicine at Govt. Stanley Medical College Hospital, Chennai – 1, which is to be submitted to The Tamilnadu Dr. M.G.R Medical University, Chennai – 32 towards the partial fulfillment of the requirement for the award of **M.D. degree in General Medicine** in March 2009.

Signature of the Unit Chief **Signature of H.O.D. of Medicine**

Dr. J. MOHANASUNDARAM, M.D., Ph.D., DNB

D E A N

Government Stanley Medical College and Hospital

Chennai – 600 001

DECLARATION

I, **Dr. A.SANTHI SELVI**, solemnly declare that the dissertation titled **"EVALUATION OF THE EFFECT OF ATORVASTATIN ON INSULIN SENSITIVITY"** is a bonafide work done by me at Government Stanley Medical College Hospital, during 2006 to 2009 under the guidance and supervision of my Unit Chief **Prof . Dr. P. CHITRAMBALAM, M.D.,**

The dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, towards partial fulfillment of requirement for the award of **M.D. Degree (BRANCH –I) in General Medicine.**

Place: Chennai **(Dr. A. SANTHI SELVI)**

Date:

ACKNOWLEDGEMENT

I sincerely wish to express my wholehearted gratitude to the Dean Stanley Medical College, **Dr. J. MOHANASUNDARAM, M.D., Ph.D., DNB** for permitting me to use this hospital clinical material for the study.

I am grateful to the Professor and Head of the Department of Medicine **Prof. Dr.V.RUCKMANI, M.D.,** and I place on records my deep sense of gratitude to my Unit Chief and Guide **Dr. P. CHITRAMBALAM, M.D.,** for his advice and timely suggestions in preparing this dissertation.

I also thank my Unit Asst. Professors **Dr. NALINI KUMARAVEL M.D.** and **Dr.CHANDRA SEKAR, M.D.,** for their timely suggestions and guidance.

Lastly my gratitude and thanks to patients who were kind and co-operative.

CONTENTS

INTRODUCTION

The 3-Hydroxyl-3 methyl glutaryl Coenzyme A (HMG CoA) reductase inhibitors or statins have been a primary force in the management of hypercholesterolemia for many years and are important in the primary and secondary prevention of heart disease. However, increasingly it is being shown that the statins have clinical benefits that appear to be greater than those one would expect from improvement in the lipid profile alone. These pleotrophic actions include direct effects on vascular tissue, kidney, bone and glucose metabolism.

The hyperinsulinaemic / insulin resistant states is a metabolic condition linked to widespread and heterogeneous clinical syndrome like hypertension, obesity, type-2 diabetes, dyslipidaemia, atherosclerosis and coronary vascular disease. About 25% of the non-diabetic population shows abnormalities of insulin sensitivity and compensatory hyperinsulinaemia.

Diabetes affected 194 million people worldwide in 2003 and is estimated to affect 299 to 333 million by 2025, according to International Diabetes Federation.

The South Asian population is known to be at risk of a atherosclerosis, even though the subject does not have clinical evidence of coronary heart disease.

In India, population is vast, and there is heterogenecity of origin or race, geography and habit, socioeconomic status, dietary habits, methods of cooking and preservation, use of pesticides etc. These factors along with known variables like age, sex etc. influence lipid profile of individuals.

India is facing a diabetic explosion. It has the world's largest diabetic population about 25 million, and the number is predicted to rise to 35 million by 2010 and to 57 million by 2025. The exact nature of the increase in prevalence of type 2 diabetes is unknown, and both genetic and lifestyle factors are being blamed. The urbanization tendency of rural India puts the incidence of diabetes with all its complications and mortality on the rise. Insulin resistance is supposed to play a major role in the development of diabetes. Considering the magnitude and severity of hyperinsulinaemic / insulin resistant state, pharmaceutical measures are initiated early in an Indian.

Clinical trials and animal studies (invivo and invitro) have shown that statins reduce cardiovascular disease risks and events, progression of nephropathy, development of diabetes and fracture rates, these are benefits that go beyond lipid lowering alone. These agents improve insulin sensitivity and

reduce the likelihood of persons progressing from impaired glucose tolerance to type II diabetes.

Various studies have observed the effect of statins on insulin sensitivity in Type 2 Diabetic mellitus. Since statins are commonly used for the treatment of hypercholesterolemia in clinical practice, it is important to know their effect on insulin sensitivity.

AIMS AND OBJECTIVES

- To evaluate the effects of Atorvastatin on insulin sensitivity.

REVIEW OF LITERATURE

STATIN

The statins (or HMG-CoA reductase inhibitors) form a class of hypolipidemic drugs used to lower cholesterol levels in people with or at risk of cardiovascular disease. They lower cholesterol by inhibiting the enzyme HMG-CoA reductase, which is the rate-limiting enzyme of the mevalonate pathway of cholesterol synthesis. Inhibition of this enzyme in the liver stimulates LDL receptors, resulting in an increased clearance of low-density lipoprotein (LDL) from the bloodstream and a decrease in blood cholesterol levels. The first results can be seen after one week of use and the effect is maximal after four to six weeks.

Akira Endo and Masao Kuroda of Tokyo, Japan commenced research into inhibitors of HMG-CoA reductase in 1971 (Endo 1992). This team reasoned that certain microorganisms may produce inhibitors of the enzyme to defend themselves against other organisms, as mevalonate is a precursor of many substances required by organisms for the maintenance of their cell wall (ergosterol) or cytoskeleton (isoprenoids).

The first agent isolated was mevastatin (ML-236B), a molecule produced by *Penicillium citrinum.* The pharmaceutical company Merck & Co. showed an interest in the Japanese research in 1976, and isolated lovastatin (mevinolin, MK803), the first commercially marketed statin, from the mold *Aspergillus terreus*. Dr Endo was awarded the 2006 Japan Prize for his work on the development of statins.

PATHWAYS OF ACTIVITY

Cholesterol is required in maintaining cellular membrane structure and is also a precursor for the synthesis of steroid hormones and bile acid. The mevalonate pathway (FIGURE 1) is the series of biochemical reactions leading to the synthesis of cholesterol. The statins, by inhibiting HMG-CoA reductase, block the rate-limiting step in this pathway, resulting in decreased cholesterol production. Blocking cholesterol synthesis has been believed to be the statins' primary mechanism of action. However, a number of cholesterol-independent or pleiotropic effects of statins relate to their ability to block the synthesis of important intermediate products.

Intermediate products in the mevalonate pathway include isoprenoids such as farnesylpyrophosphate and geranylgeranylpyrophosphate. The biologic mechanism for most of the pleiotropic effects of statins is related to inhibition of isoprenoid metabolism in nonhepatic cells (fig 1).[1] The Ras family of

proteins is necessary for cellular differentiation and proliferation, while the Rho family is important for cytoskeleton formation, superoxide generation, and cell growth progression.[1] Blocking these important isoprenoid intermediates affects mitochondrial respiration, lipid peroxidation, posttranslational modifications of cellular proteins, modifications of certain tRNA, and production of glycoproteins. Therefore, blocking of the mevalonate pathway by the statins may have significant influences on many critical cellular functions.

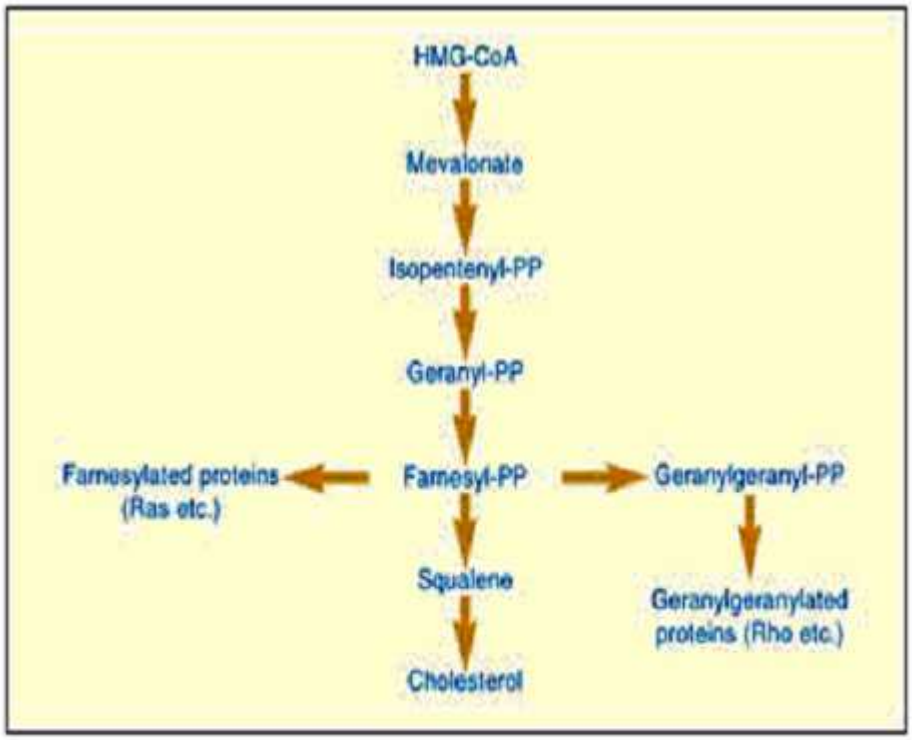


Figure 1. The mevalonate pathway. HMG-CoA, 3-hydroxyl-3-methylglutaryl-coenzyme A; Isopentenyl-PP, isopentenylpyrophosphate; Geranyl-PP, geranylpyrophosphate; Farnesyl-PP, farnesylpyrophosphate; Geranylgeranyl-PP, geranylgeranylpyrophosphate.

ACTIONS OF STATINS

Statin therapy has been found to rapidly improve vasomotor response, enhance coronary blood flow, and reduce the levels of adhesion molecules. This is due in part to the ability of the statins to increase endothelial nitric oxide production secondary to inhibition of Rho and the resulting up-regulation of endothelial nitric oxide synthase (eNOS). Endothelial nitric oxide synthase is the enzyme required for nitric oxide production. Through another unclear mechanism, statins up-regulate the phosphatidylinositol 3'-kinase/Akt pathway (PI3-kinase/Akt pathway). This also activates eNOS (fig 2). The antioxidant effects of this group of drugs may also contribute to their ability to improve endothelial function.[2]

Unstable atherosclerotic plaques are characterized by a lipid-rich core and excess inflammatory cells. The release of matrix metalloproteases by macrophages degrades plaque matrix connective tissue, weakening the fibrous cap. This makes these plaques susceptible to rupture. Statins have been shown to increase plaque stability by decreasing levels of metalloproteases, oxidized-low density lipoprotein, core lipid content, macrophages, and by increasing the

collagen content in plaque matrix. Through the inhibition of Rho, lovastatin has been shown to increase tissue plasminogen activator activity while inhibiting plasminogen activator inhibitor type-1 activity. Thus, statins exert positive effects on the fibrinolytic profile in the vascular endothelium[2].

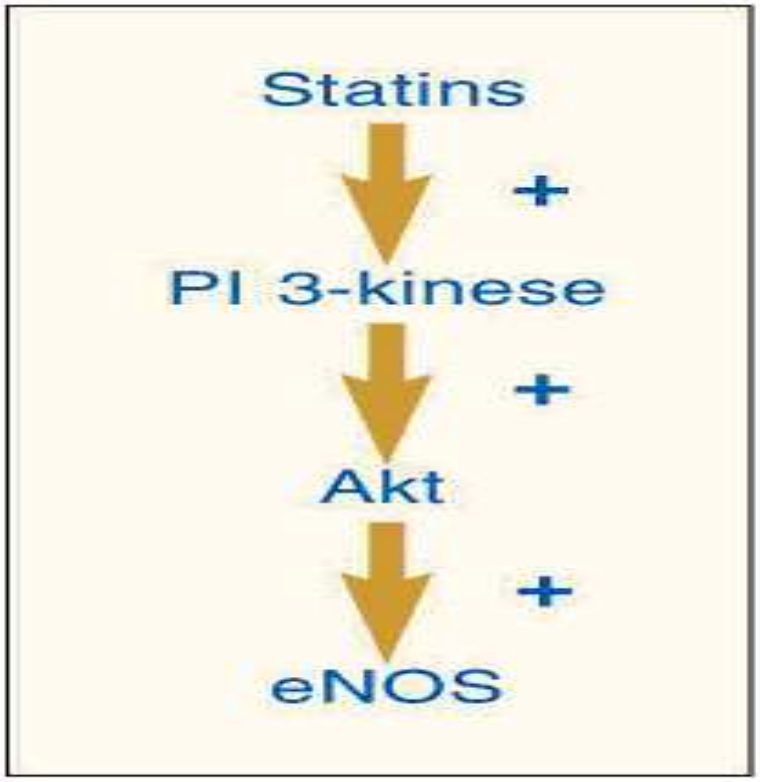


Figure 2 Statins stimulate the PI3 kinase/Akt pathway by an unidentified mechanism. PI3 kinase, phosphatidylinositol 3' kinase; eNos, endothelial nitric oxide synthase.

Statins may exert anti-inflammatory effects by several pathways. The isoprenoids have been shown to activate inflammation via intracellular second messenger systems. Two other pathways include blocking the function of the integrin lymphocyte function-associated antigen-1 (LFA-1) and action on the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. Disruption of these pathways may inhibit lymphocyte recirculation, T-cell activation, and T-cell migration. Other mechanisms yet to be fully elucidated may involve inhibition of adhesion molecules and inhibition of interleukins 6 and 8. High sensitivity C-reactive protein, a clinical marker of inflammation, is lower in hypercholesterolemic patients on statin therapy.

Statins also affect gene expression. Increased gene expression of bone morphogenetic protein-2 (BMP-2) through statin use resulted in increased bone formation in animal studies. This drug group has been demonstrated to inhibit the expression of class II major histocompatibility complex (MHC II) genes. T-cell activation is dependent on interactions involving MHC. These findings indicate that statins may be effective as immunomodulators. In addition, in vitro studies with the HMG-CoA reductase inhibitors have demonstrated the suppression of natural killer cells, inhibition of chemotaxis by monocytes, regulation of DNA in cycling cells, and the inhibition of antibody-dependent cellular cytotoxicity.

Statins modify several processes in the cell cycle. They have been shown to synchronize tumor cells by blocking the transition of G1-S in the cell cycle, thereby exerting antiproliferative effects. This is thought to be secondary to the inhibition of geranylgeranylated proteins. The depletion of geranylgeranylated proteins also appears to mediate statin induced apoptosis. Ras inactivation is considered an important mechanism in the ability of statins to inhibit cell signaling pathways associated with the invasive and metastatic properties of cancer.

PLEIOTROPIC EFFECTS OF STATINS: LIPID REDUCTION AND BEYOND

IMPACT ON CVD

Statins have been shown in primary and secondary prevention[6] trials to significantly reduce fatal and nonfatal CVD events. Cardiovascular benefits of statins have been conventionally attributed to reduction of LDL-cholesterol. However, subanalyses of large clinical trials suggest that statins also have direct cardioprotective effects. For example, in WOSCOPS[3], the time-to-event curves began to diverge within 6 months of initiating therapy, an effect that is earlier than predicted from cholesterol lowering alone. Clinical trials have also

shown larger significant CVD benefits associated with only minimal changes in luminal dimensions on angiography, benefits that cannot be explained by simple plaque regression . Statins also increase myocardial perfusion and reduce recurrent anginal episodes after acute coronary events. Potential mechanisms that may mediate these effects include modulation of endothelial function, plaque stabilization, attenuated atherogenesis, and anti-inflammatory and antithrombotic action[3].

STATINS AND PLAQUE STABILIZATION

Most acute coronary events are due to disruption of unstable atherosclerotic plaques, which result in thrombotic occlusion. These vulnerable lesions occur in moderately stenotic vessels and are characterized by a lipid-rich core and excess activated inflammatory cells . Macrophages release matrix metalloproteases that degrade plaque matrix connective tissue, weaken the fibrous cap, and render them susceptible for rupture . Statins have been shown to decrease the levels of metalloproteases, oxidized-LDL (ox-LDL), core lipid content, and macrophages and to increase collagen content in plaque matrix, actions that increase plaque stability[2].

STATINS AND ENDOTHELIAL FUNCTION

Statins have beneficial effects on vascular endothelium and many of these effects are mediated by the inhibition of small molecular weight G-proteins of the Ras superfamily (Ras and Rho). These small molecular weight G-proteins are involved in cell proliferation, differentiation, apoptosis, migration, contraction, and regulation of gene transcription. Activated Ras/Rho proteins are key components in signal-transducing kinase cascades involved in NO production and glucose metabolism. Thus, inhibition of these proteins can critically affect various cellular processes. The anchoring of these small G-proteins to cell membranes requires prenylation; Ras proteins are farnesylated, whereas Rho proteins are geranylgeranylated. Small G-proteins exist in an inactive GDP-bound cytosolic form, and upon cellular activation they exchange GTP and translocate to the active-membrane form (Fig.1). Lack of protein isoprenylation leads to cytosolic sequestration and loss of biological activity. Statins, in addition to lowering cholesterol by inhibiting HMG-CoA reductase enzyme, also reduce cellular isoprenoid intermediates such as dolichol, ubiquinone, farnesol, and geranylgeraniol (Fig.2). Statins, by inhibiting isoprenylation, effectively lower membrane levels and activity of Ras/Rho proteins and thus improve vascular function[2].

Studies in humans and animals have demonstrated a positive effect of statins on endothelial function. Basal and stimulated endothelium-dependent forearm blood flow responses in hypercholesterolemic subjects are improved in 4 wk of treatment with statins.. Simvastatin has been reported to increase endothelial NO production and improve NO-dependent vasorelaxation in different vascular beds . Chronic administration of simvastatin or mevastatin to rodents up-regulates endothelial NO synthase (eNOS) expression ,augments blood flow in cerebral vessels, and reduces infarct size . These studies suggest a direct action of the statins on NO production in the endothelium. Nevertheless, a major mechanism of action of statins in improving endothelial-derived vasorelaxation is through LDL-cholesterol lowering. Indeed, acute lowering of LDL by apheresis has been shown to improve endothelium-dependent vasodilatation in persons with hypercholesterolemia .

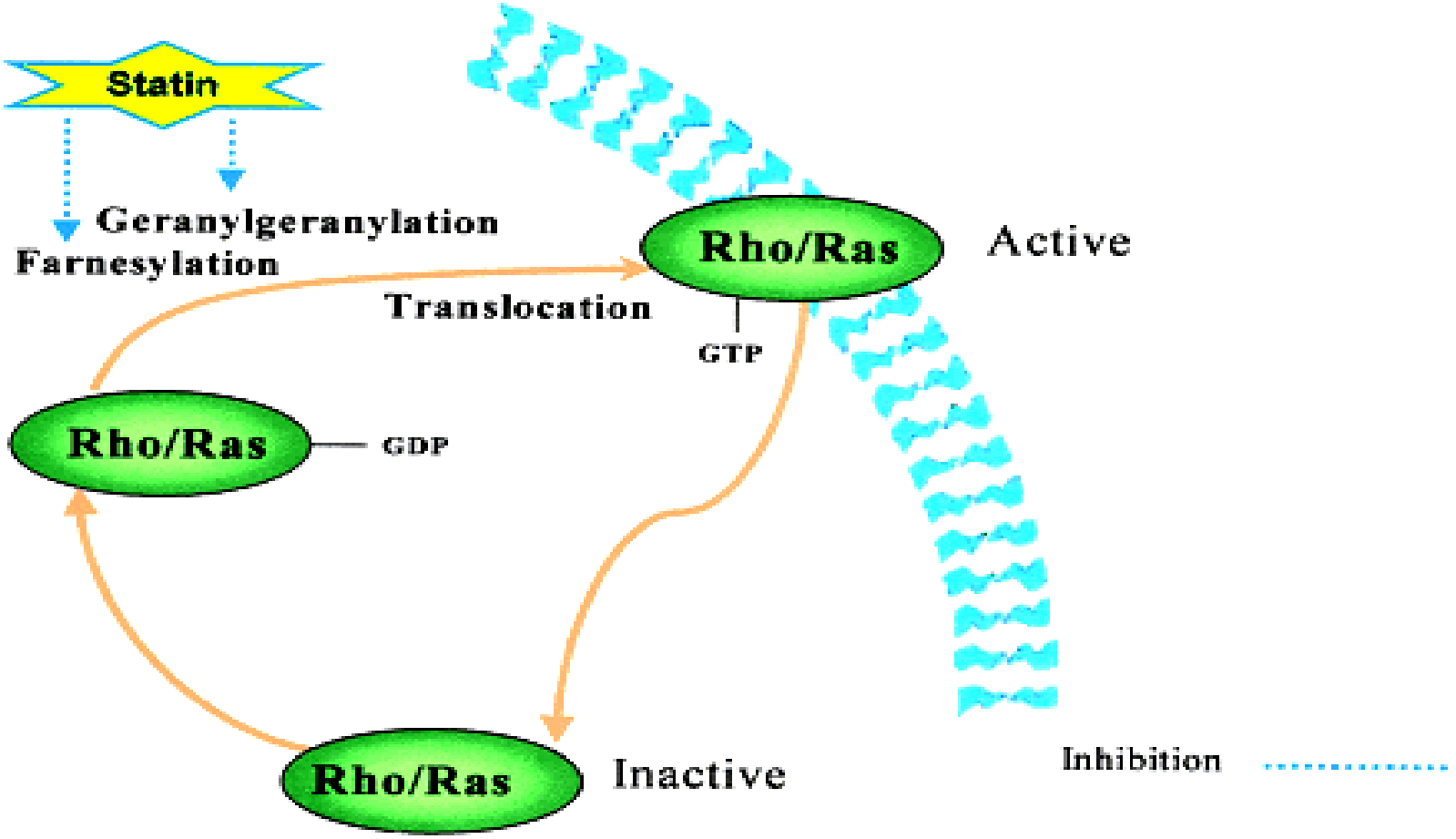


Figure 1. Effects of statins on small G-proteins

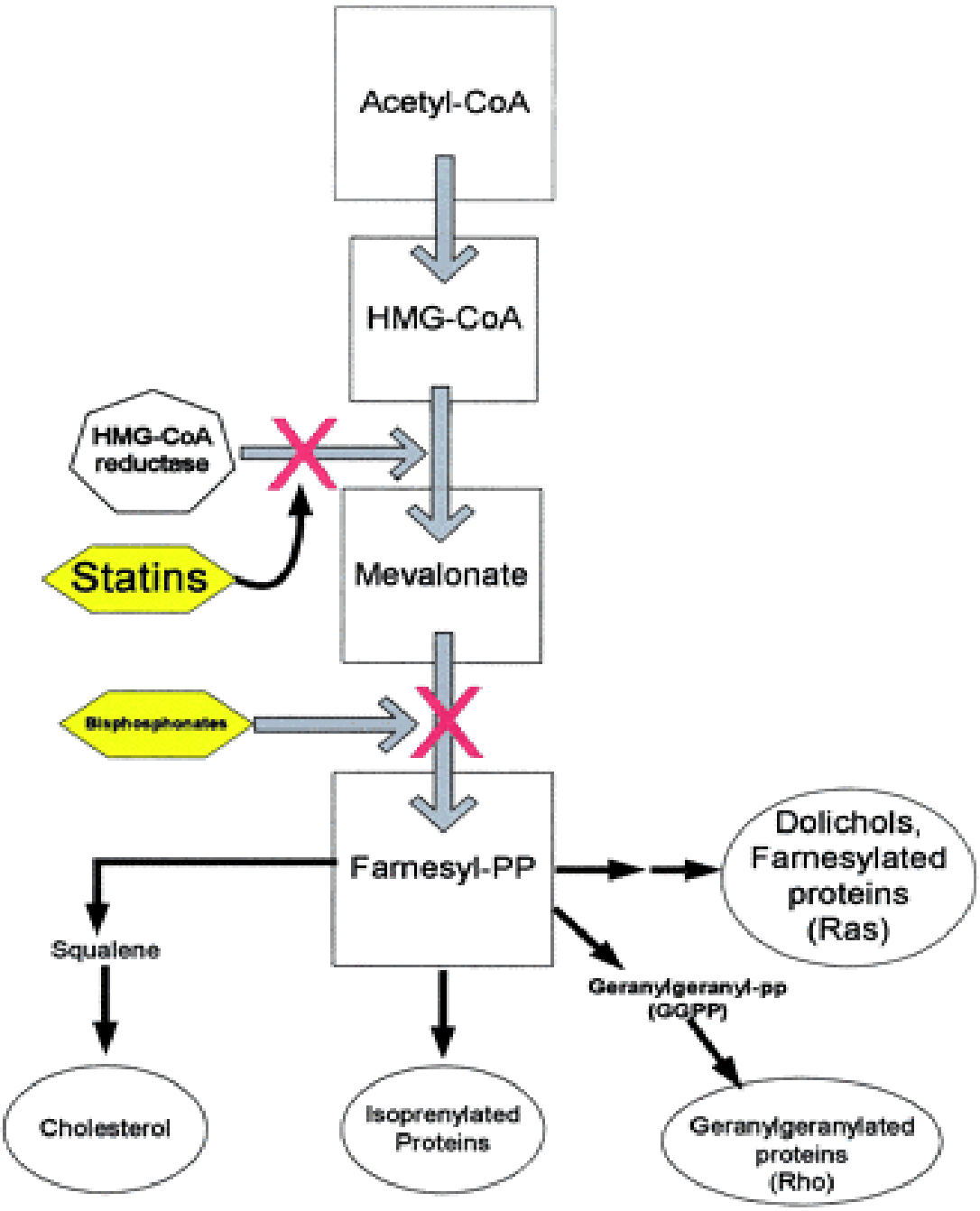


Figure 2.

Cholesterol biosynthesis and mevalonate pathway. Bisphosphonates exert inhibitory effect a step distal to that of statins.

eNOS resides in the caveolae and produces small amounts of NO on demand in a transient fashion that is both calcium- and calmodulin-dependent. In the caveolae, eNOS is bound to the caveolar protein, caveolin that inhibits its activity. Elevations in cytoplasmic calcium promote binding of calmodulin to eNOS that subsequently displaces caveolin, thus activating eNOS (Fig.3). In addition to undergoing regulatory posttranslational modifications, eNOS is regulated by a serine-threonine kinase, Akt. Akt is activated by insulin/IGF-I binding to endothelial and vascular smooth muscle cells (VSMCs). Phosphorylation by Akt increases the affinity of eNOS to calmodulin and enhances the activity of eNOS. Statins activate Akt and thus increase NO production. Statins also decrease cellular caveolin levels and attenuate the inhibition of eNOS by caveolin, resulting in increased NO production. In addition to affecting posttranslational regulatory mechanisms, statins increase eNOS transcription, stability, and protein level. These class effects of statins contribute to improved NO-mediated vascular relaxation.

Endothelial dysfunction is a hallmark of diabetes and insulin-resistant states and is characterized by reduced effective vascular NO action . Statins ameliorate the abnormal vascular relaxation and partially restore NO production in the aorta of diabetic mice. Hyperglycemic states both *in vivo* and *in vitro* stimulate Rho activity, which in turn activates Rho-kinase resulting

in increased vascular tone. The protective effect of statins on diabetic vascular disease may be due to the suppression of Rho kinase cascades, resulting in increased NO production and decreased vascular tone. Statins not only increase endothelial cell NO production but also up-regulate the inducible form of NOS (iNOS) in Vascular Smooth Muscle Cells. iNOS is expressed after vascular injury, and induction of iNOS in these states may be beneficial in preventing restenosis.

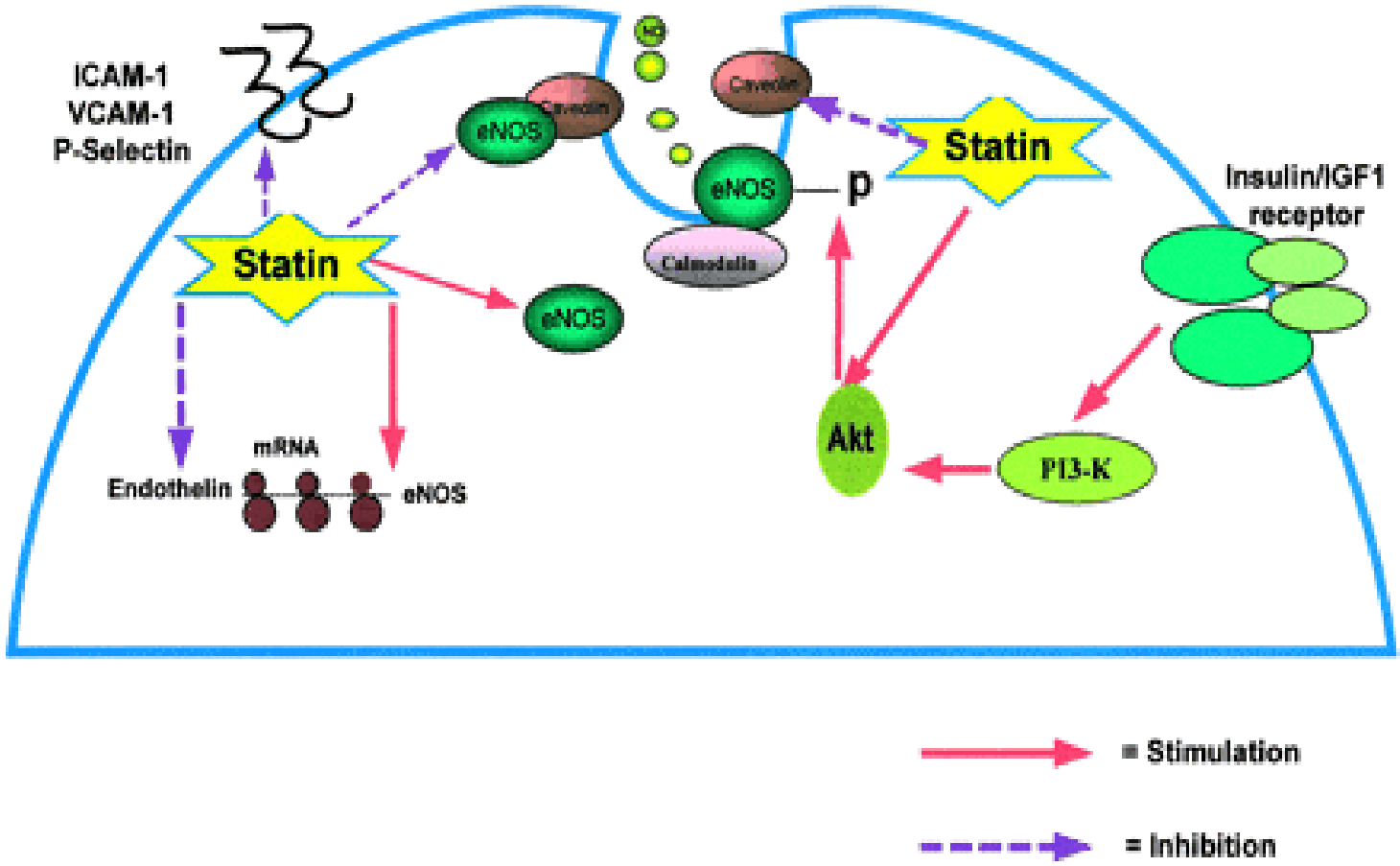


Figure 3: Effects of statin on the endothelium.

Statins also modulate the release and action of vasoconstrictors (*e.g.* endothelin and angiotensin II). Clinical studies show that hypercholesterolemic men have exaggerated hypertensive responses to infused angiotensin II, and this response is reversed by statins . In a study using double transgenic rat model harboring the human renin and angiotensin genes, cervistatin improved survival, decreased blood pressure, and reduced cardiac hypertrophy . Statins also have a direct effect on endothelin-1 (ET-1) production (Fig.3). These agents reduce, in a dose- and time-dependent fashion, the expression of ET-1 in endothelial cells. This reduction is maintained even in the presence of ox-LDL. Because ET-1 is a powerful vasoconstrictor, decreasing ET-1 levels potentially reduces vascular resistance and improves blood flow in coronary and systemic vascular beds.

THE ANTI-INFLAMMATORY ACTIONS OF STATINS

The vascular inflammatory response is a complex process that leads to thrombus formation, angiogenesis, neointimal thickening, and atherosclerosis (4). Markers of inflammation such as C-reactive protein, IL-6, TNF-, and monocyte-chemotactic protein-1 (MCP-1) have, in varying degrees, been proposed as CVD risk factors. Recent evidence indicates that statins decrease C-reactive protein levels in just 6 wk of treatment, independent of LDL

cholesterol reduction, and suggests that statins possess anti-inflammatory actions.

Augmented expression of adhesion molecules on leukocytes (*e.g.* CD11b) and endothelial cells (*e.g.* P-selectin, intracellular adhesion molecule, ICAM-1) is necessary and critical in the early vascular response to injury. Cytokines, in addition to enhancing cellular adhesion, promote chemotaxis and stimulate vascular proliferation. Statins affect many of these events in the inflammatory cascade by inhibiting receptor-dependent activation of signal-transducing cascades. In a rat model of coronary inflammation, pravastatin reduces MCP-1 expression, monocyte infiltration, and proliferation. Simvastatin reduces leukocyte rolling, adherence, and transmigration in a rodent model of NO deficiency and attenuates endothelial adhesion molecule and monocyte CD11b expression in the absence of lipid lowering (Fig.4). Statin therapy reduced the levels of soluble P-selectin in patients with acute coronary syndromes. In another rat model associated with elevated serum levels of TNF- and IL-1ß, cerivastatin has been shown to reduce serum levels of these markers and improve survival rate. Statins also mediate the suppression of cytokine and adhesion molecule expression by reducing NF_kB activity in inflammatory and vascular cells. These observations underlie the

importance of statins in attenuating the inflammatory process and the consequent impact on CVD risk reduction[8].

STATINS AND OXIDATIVE STRESS

Oxidative stress is a result of altered balance in the relative concentrations of oxidants and antioxidants. Ox-LDL is deleterious to endothelial and Vascular Smooth Muscle Cells. It activates macrophages, induces release of various cytokines, and increases endothelial adhesiveness resulting in vascular injury and inflammation. Statins as potent antioxidants and antiatherosclerotic agents are attractive therapeutic options for preserving normal vascular function and blood flow. In several human and animal studies, various statins have been shown to: 1) inhibit the uptake and generation of ox-LDL[4], 2) attenuate vascular and endothelial superoxide anion formation by inhibition of NADH oxidases via Rho-dependent mechanisms[4]; and 3) preserve the relative levels of vitamin E, vitamin C, and endogenous antioxidants such as ubiquinone and glutatione in LDL particles[4]. Thus, statins not only decrease oxidants but also restore antioxidants, thereby possibly reducing the level of oxidative stress in the vascular milieu, which may explain some of the observed clinical beneficial effects.

STATINS AND THROMBOSIS

Different statins have varying effects on prothrombotic factors, such as tissue factor, tissue factor pathway inhibitor, platelet aggregation, blood and plasma viscosity, fibrinogen, plasminogen activator inhibitor 1 (PAI-1), and lipoprotein (a)[4]. Cellular expression of tissue factor in human macrophages is suppressed by lipophilic statins. Statins normalize thrombin generation in hypercholesterolemic patients and reduce platelet aggregation. Furthermore, decreases in platelet aggregation after statin therapy may be partially related to relative reductions in the cholesterol to phospholipid content in the platelet membrane.

STATINS AND VASCULOGENESIS

Statins, in addition to modulating endothelial and vascular function, may mediate neovascularization (vasculogenesis) and collectively contribute to the reduction in recurrent CVD events. Increased vasculogenesis has been demonstrated in rabbits treated with simvastatin via the activation of vascular Akt[4]. Statins mobilize endothelial progenitor cells (EPCs) from the bone marrow that play a role in maintenance vasculogenesis. Increased EPCs are seen immediately after a coronary event and line the endothelium of myocardial vessels. Indeed, statin therapy is associated with enhanced EPCs in patients with coronary artery disease[4].

STATINS AND KIDNEYS

Statins have been shown to attenuate renal injury in both *in vivo* and *in vitro* studies. Renal injury initiates inflammatory cascades that involve similar cellular events as seen in vascular tissue. Statins inhibit key events in this process that alter the progression of renal injury. In hyperglycemic insulin-deficient diabetic rats, pravastatin ameliorates the structural and functional changes of diabetic nephropathy[13]. Statins have been demonstrated to decrease TGF-ß production and suppress the enhanced Ras-dependent activation of MAPK cascade (Fig.4). In another model of renal injury due to overexpression of Ang II, cerivastatin decreased systolic blood pressure, albuminuria, and cortical necrosis. These changes were associated with reduced infiltration of inflammatory cells, diminished expression of adhesion molecules, and lower levels of transcription factor (NF_kB) activity (Fig.4). In rats with glomerulonephritis, simvastatin decreased mesangial cell proliferation and monocyte/macrophage infiltration.

Statins have been shown to inhibit the proliferative actions of platelet-derived growth factor and TGF-ß. Cytokines released during renal injury activate NF-B and growth-regulating pathways in mesangial and tubular cells. Statins both decrease the levels of cytokines and inhibit the NF-B-dependent gene activation, such as MCP-1 and IL-6. In humans, statins also decrease

urinary albumin excretion in patients with nephrotic syndrome and in patients with type II diabetes[4]. Thus, statins modulate glomerular mesangial and interstitial inflammatory process independent of lipid reduction.

STATINS AND GLUCOSE METABOLISM

A retrospective analysis of the WOSCOPS examining the development of new diabetes mellitus revealed that pravastatin therapy reduced the risk of developing diabetes by 30%. This prevention in the onset of diabetes was associated with significant reduction in triglyceride levels, but upon further analyses the reduction in triglycerides did not account for the effect of statins on the development of diabetes[14].

Statins may affect substrate delivery to insulin-sensitive tissues or modulate insulin-activated signaling cascades that mediate glucose uptake. Insulin increases skeletal muscle perfusion and substrate delivery by enhancing eNOS activity. As described previously, statins also increase eNOS expression, which may result in increased capillary recruitment and glucose disposal . Insulin activates a series of kinase cascades that involve PI3K and Akt, resulting in the translocation of glucose transporters to cell membrane and enhanced glucose uptake. This cascade is inhibited by circulating cytokines (TNF- and IL-6).

Statins, like insulin, activate PI3K and Akt, which may play a role in glucose uptake. Statins, in addition to decreasing cytokine levels, also inhibit the cellular cascades such as Rho-kinase that inactivate the insulin receptor and signaling. NO is a potential intermediary, because it has been shown to stimulate skeletal muscle glucose uptake .

There is also evidence that statin mediated effects on fatty acid metabolism influence glucose metabolism. The peroxisome proliferator activator protein receptors are known to known to be major regulators of intra and extracellular fatty acid metabolism, especially PPAR alpha (5). PPARs belong to the superfamily of nuclear receptors that are ligand activated transcription factors.

There is preliminary evidence that PPAR alpha activation would also result in improved insulin sensitivity. A possible mechanism for this PPAR alpha activation is the statin induced inhibition of Rho, which results in PPAR alpha activation. This raises the possibility that atorvastatin positively affects insulin sensitivity and help to prevent transition from impaired glucose tolerance to manifest type 2 diabetes.

Inflammatory markers linked with insulin resistance is associated with the development with type 2 diabetes in adults. The mechanism by which inflammation leads to glucose intolerance and diabetes is not known, but

proinflammatory cytokines may affect insulin receptor or impair insulin action and secretion(11).

In the WOSCOPS study ,pravastatin was found to produce 30% reduction in the risk of diabetes.The possible roles of pravastatin in the development of diabetes are

1. The triglyceride lowering effect of pravastatin could reduce the risk of developing insulin resistance. But other lipid lowering agents do not appear to improve insulin resistance.[3].
2. Pravastatin has been shown to reduce levels of interleukin 6 and TNF –a through its anti - inflammatory effects. These cytokines are known to inhibit lipoprotein lipase activity and to stimulate lipolysis in adipose tissue . Pravastatin may therefore interrupt the progression from central obesity to insulin resistance mediated by adipose tissue – derived cytokines.
3. Impaired endothelial function has been shown to correlate with insulin resistance. Pravastatin, by restoring endothelial function, may beneficially affect glucose and insulin transport.

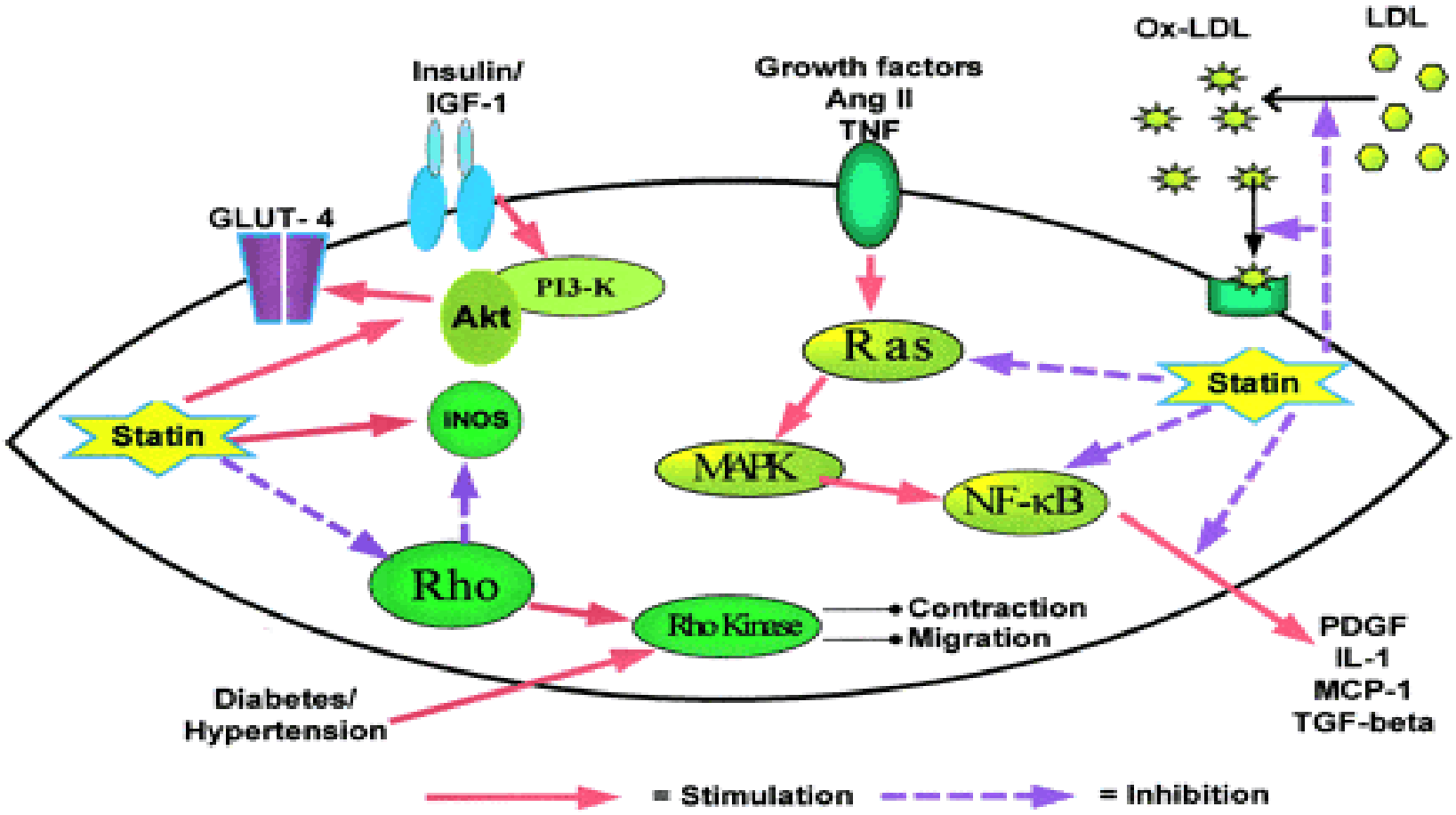


Figure 4. Effects of statins on inflammation and glucose metabolism

STATINS AND CHOLESTEROL

Insulin resistance and type 2 diabetes are associated with high triglyceride and low HDL-cholesterol levels. Increased synthesis of VLDL particles in the liver has been proposed the main cause of increased concentrations of triglyceride-rich lipoproteins. This overproduction of VLDL and triglycerides in the liver has been proposed to be driven by high levels of serum free fatty acids in patients with insulin resistance.

Insulin resistance could explain the increase in cholesterol synthesis in patients with obesity and type 2 diabetes[6] .This hypotheses is supported by the finding that in subjects with normal glucose tolerance, high glucose is linked to increased cholesterol synthesis. Increased cholesterol synthesis is always accompanied by low rates of cholesterol absorption. Therefore insulin resistance is associated with high cholesterol synthesis and cholesterol absorption. Because fasting insulin correlated with cholesterol synthesis independent of the rates of BMI and whole body glucose uptake, it is possible that regulation of cholesterol synthesis by hyperinsulinemia may be a link between insulin resistance and cholesterol metabolism[7].

Various studies have shown that insulin sensitizing effect of statins may be linked to their triglyceride lowering effect.

STATINS AND BONE REMODELING

Statins were also shown to stimulate bone formation in several studies. In vitro, statins increase the number of osteoblasts and the amount of new bone formation in mouse skull bones. Similar effects were also seen in vivo when simvastatin or lovastatin was injected subcutaneously over the skull bone of mice. Furthermore, oral administration of simvastatin to rats increased trabecular bone volume and the rate of new bone formation. These findings were confirmed by further studies; for example, transdermal lovastatin and cerivastatin were shown to increase bone mass in rodents at doses similar to the dose used in humapins in the treatment of hypercholesterolemia .All of these findings illustrate positive effects of statins on bone remodeling in the form of inhibition of bone resorption and stimulation of bone formation.

MATERIAL AND METHODS

SELECTION OF VOLUNTEERS

The study deals with the effect of Atorvastatin on insulin sensitivity conducted at Hypertension outpatient department of Government Stanley Medical College Hospital, Chennai. Ethical approval for the study was obtained from the institutional ethical review board. Ninety patients are screened for the study from a random population of 110 hypertensive patients receiving atenolol as anti-hypertensive drug, by a random selection process, from which 68 patients are considered based on patient compliance, intelligence to understand dietary prescriptions and directions and whether free from any other disease on initial medical testing. Written consent for the study as per protocol is obtained.

The patients were randomized into two groups, of 34 patients each, by a random selection process. The experimental group consisting of 34 dyslipidaemic and hypertensive patients receiving atorvastatin 10mg/day and atenolol 50 mg/day at the Hypertension OPD at Govt Stanley Medical College Hospital are chosen as volunteers and are compared with another group of 34 hypertensive patients receiving atenolol 50 mg/day only. Uniform diet pattern is prescribed to all of them.

INCLUSION CRITERIA

Dyslipidaemia, Hypertension, Age 40 to 50 years, not receiving any drugs other than mentioned above, not suffering from any other diseases.

EXCLUSION CRITERIA

Those patients not satisfying the inclusion criteria are excluded.

CLINICAL CHARACTERS OF VOLUNTEERS

Clinically, both the groups show no abnormality, other than hypertension in both groups, along with dyslipidaemia in the experimental group.

DATA COLLECTION

Height, Weight, BMI and blood pressure measurements were done and other information collected with the help of predesigned questionnaire.

BIOCHEMICAL PARAMETERS

Fasting blood sugar, fasting insulin, fasting lipid profile (cholesterol, triglyceride, HDL, LDL) were done by the standard methods.

Other parameters like liver function tests (LFT), total leukocyte count (TLC), differential leukocyte count (DLC), haemoglobin (Hb), urea, creatinine, total proteins, serum electrolytes, urine tests, electro-cardiograph (ECG), X-ray of chest, etc. are almost identical and within normal range in both the groups.

COLLECTION OF BLOOD SAMPLES

Twelve hours fasting values are taken initially and at monthly intervals for 1 year.

ASSESSMENT OF INSULIN SENSITIVITY

Insulin sensitivity was assessed by calculating Homeostasis model assessment (HOMA –IR) as follows :

$$\frac{\textbf{Fasting Insulin µ/ml x Fasting Glucose mg/dl}}{\textbf{22.5}}$$

STATISTICAL METHODS

The statistical analysis is done based on paired t-test, and p-value is calculated using paired t-statistic.

RESULTS AND OBSERVATIONS

Table – 1 shows the anthropometric and biochemical characteristics of the subject in the control and Atorvastin treated groups at the start of the study. The proportion of the male & female in both the groups was similar. Proportion of subjects having positive family history of diabetes and blood pressure in both the groups were also similar.

It is found that in the group receiving atenolol and atorvastatin (experimental group), TC is reduced from initial values of 280 +/- 20 mg/dl to 202 +/- 12 mg/dl ($p = 0.05$). HDLC is increased from 45 +/-10 mg/dl to 52 +/- 12 mg/dl ($p = 0.04$). LDLC reduced from 180 +/-20 mg/dl to 148 +/-12 mg/dl ($p = 0.05$). VLDLC, Triglycerides values remain almost same, and changes are statistically insignificant. FBS values changed from initial 106 +/- 6 mg/dl to 88 +/-4 mg/dl ($p = 0.04$).

In the control group receiving atenolol only, it is observed that TC is from initial values of 140 +/-20 mg/dl to 112+/-8 mg/dl. HDLC changes from 40 +/-10 mg/dl to 44 +/-9 mg/dl. LDLC changes from 110 +/-30 mg/dl to 95 +/-13 mg/dl. VLDLC, TG and FBS values remain almost same, and all the value changes are statistically insignificant. Table 4 shows serum insulin and homeostasis model assessment of insulin resistance (HOMA 2-IR) values of

different groups, in order to determine insulin sensitivity. It was observed that in the experimental group, serum insulin value initially is 20 +/-5 microU/ml and finally is 18 +/-3 microU/ml ($p = 0.03$), and in the control group, serum insulin value initially is 18 +/-5 microU/ml and finally is 31 +/-2 microU/ml. HOMA 2 values (insulin resistance or IR) of the two groups of patients show that in the experimental group it was 4.3 +/-0.5 microU/ml initially and 4.3 +/-0.3 microU/ml finally, showing increase in insulin sensitivity by atorvastatin.

Table 1: Anthropometrical, Clinical and Biochemical characters of volunteers

	Experimental Group (34)	Control Group (34)
Age	**45 ± 4**	**43 ± 3**
Males	**21**	**21**
Females	**13**	**13**
BMI	**27.3 ± 12**	**27.5 ± 2.1**
SBP	**154 ± 16**	**146 ± 24**
DBP	**100 ± 12**	**94 ± 8**
T. Cholesterol	**280 ± 20**	**140 ± 20**
LDL	**180 ± 20**	**110 ± 20**
HDL	**45 ± 10**	**40 ± 10**
VLDL	**40 ± 5**	**35 ± 15**
TGL	**220 ± 20**	**120 ± 20**
FBS	**106 ± 6**	**96 ± 6**
Fasting Insulin	**20 ± 5**	**18 ± 5**

Table 2: Values of Blood parameters of Experimental Group

	0 mon	1 mon	2 mon	3 mon	4 mon	5 mon	6 mon	7 mon	8 mon	9 mon	10 mon	11mon	12 mon
T. cholesterol	2.80 ± 20	276 ± 18	270 ± 16	260 ± 15	256 ± 16	248 ± 18	240 ± 16	234 ± 12	226 ± 11	218 ± 13	212 ± 12	208 ± 10	202 ± 12
HDL	45 ± 10	45 ± 12	46 ± 12	46 ± 13	47 ± 12	48 ± 16	48 ± 8	49 ± 11	50 ± 14	50 ± 10	51 ± 13	51 ± 11	52 ± 12
LDL	180 ± 20	178 ± 18	17 ± 12	172 ± 11	172 ± 12	168 ± 18	166 ± 10	164 ± 8	160 ± 12	160 ± 8	158 ± 10	152 ± 8	148 ± 12
VLDL	40 ± 5	40 ± 3	40 ± 2	39 ± 6	39 ± 5	39 ± 6	39 ± 5	39 ± 4	39 ± 2	39 ± 4	39 ± 3	39 ± 4	39 ± 3
TGL	220 ± 20	218 ± 18	218 ± 16	214 ± 10	214 ± 8	216 ± 8	212 ± 10	210 ± 11	210 ± 12	206 ± 6	214 ± 8	208 ± 8	212 ± 6
FBS	106 ± 6	106 ± 5	105 ± 3	103 ± 4	103 ± 3	101 ± 4	98 ± 5	98 ± 6	96 ± 5	93 ± 4	92 ± 5	90 ± 3	88 ± 4

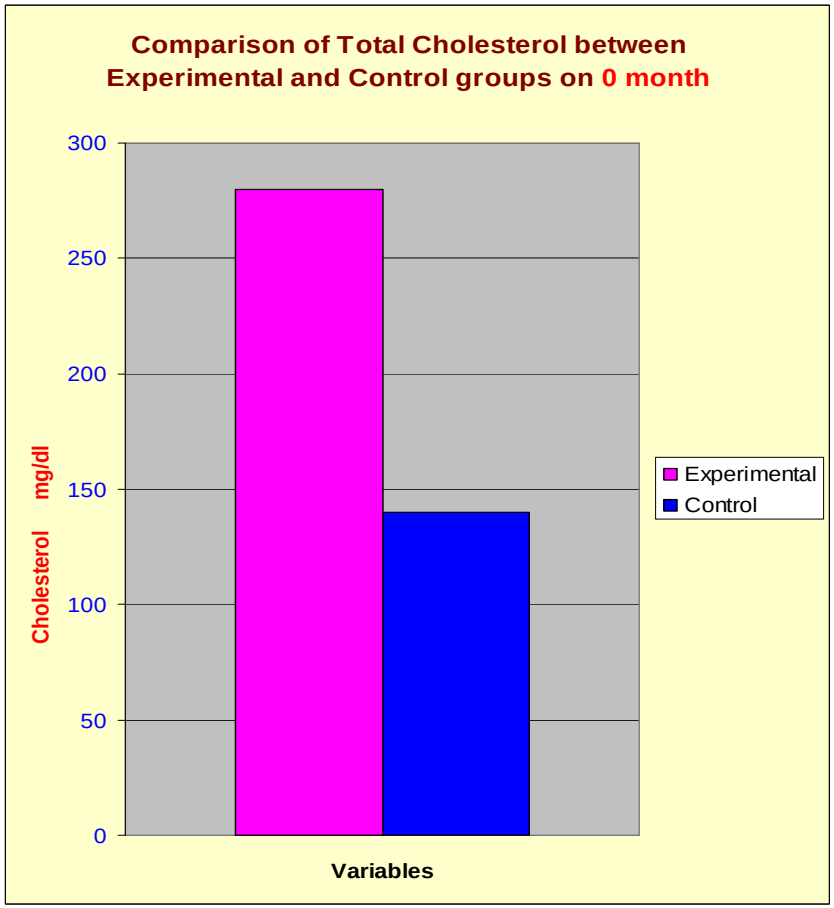
Table 3: Values of Blood parameters of Control Group

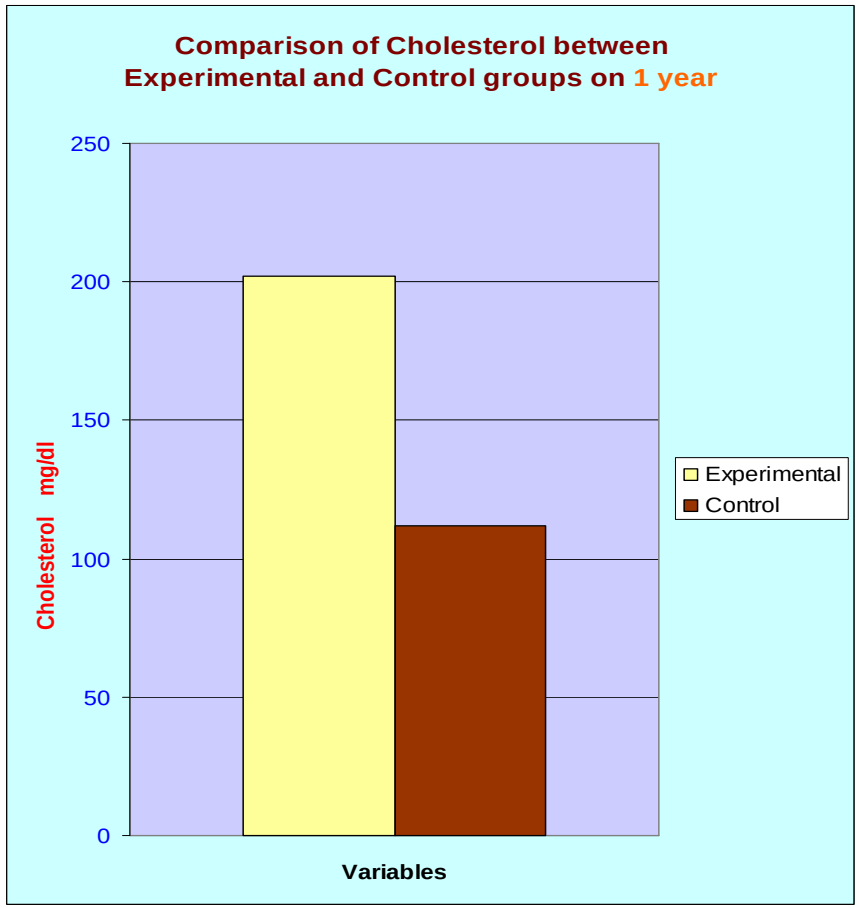
	0 mon	1 mon	2 mon	3 mon	4 mon	5 mon	6 mon	7 mon	8 mon	9 mon	10 mon	11 mon	12 mon
T. cholesterol	140 ± 20	138 ± 8	136 ± 16	134 ± 15	132 ± 15	130 ± 16	128 ± 8	125 ± 10	123 ± 8	120 ± 12	117 ± 6	115 ± 6	112 ± 18
HDL	40 ± 10	40 ± 8	41 ± 9	41 ± 7	41 ± 5	42 ± 6	42 ± 7	42 ± 7	42 ± 6	43 ± 8	43 ± 9	43 ± 8	44 ± 9
LDL	110 ± 30	110 ± 12	108 ± 18	108 ± 16	108 ± 14	106 ± 12	106 ± 13	106 ± 13	102 ± 11	100 ± 18	98 ± 16	97 ± 10	95 ± 13
VLDL	35 ± 15	35 ± 9	35 ± 8	35 ± 7	35 ± 8	35 ± 10	35 ± 10	35 ± 8	35 ± 12	35 ± 14	35 ± 11	35 ± 6	35 ± 8
TGL	120 ± 20	120 ± 11	120 ± 8	119 ± 12	120 ± 14	118 ± 12	119 ± 8	116 ± 15	118 ± 8	118 ± 11	110 ± 12	116 ± 12	116 ± 13
FBS	96 ± 10	96 ± 8	94 ± 6	94 ± 5	92 ± 8	94 ± 8	94 ± 5	92 ± 6	92 ± 9	92 ± 8	96 ± 7	94 ± 8	94 ± 6

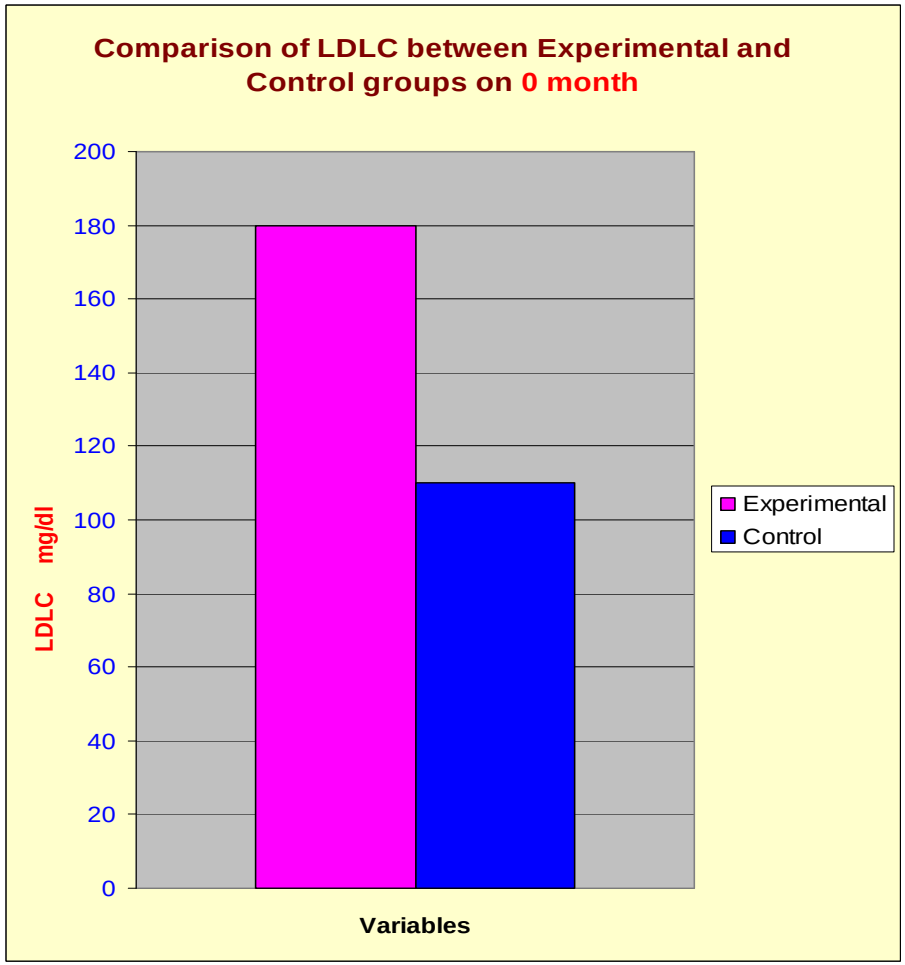
Table 4: Showing serum insulin and HOMA-IR 2 values of Different group

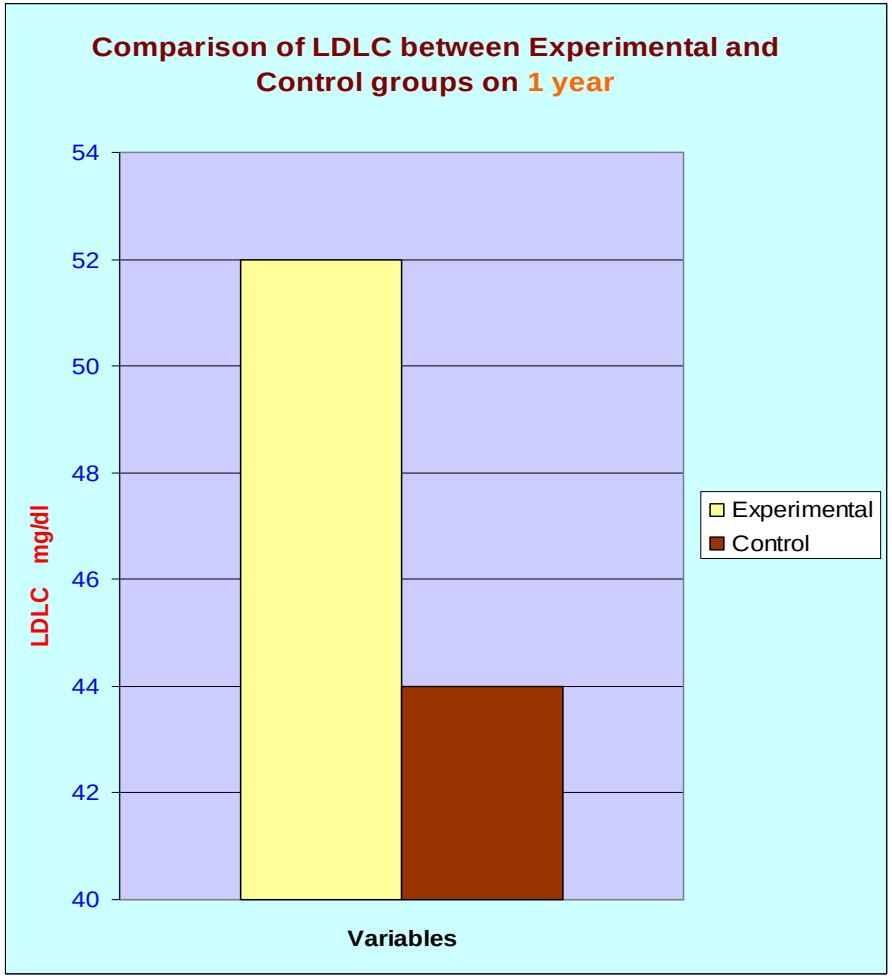
	S. insulin initial value	S. insulin end value	Homeostasis model assessment of insulin resistance 2-IR (initial value)	HOMA - 2 IR end value
Exp Group	**20 ± 5**	**18 ± 3**	**4.3 ± 0.5**	**3.7 ± 0.4**
Control Group	**18 ± 5**	**18 ± 5**	**4.3 ± 0.3**	**4.3 ± 0.2**

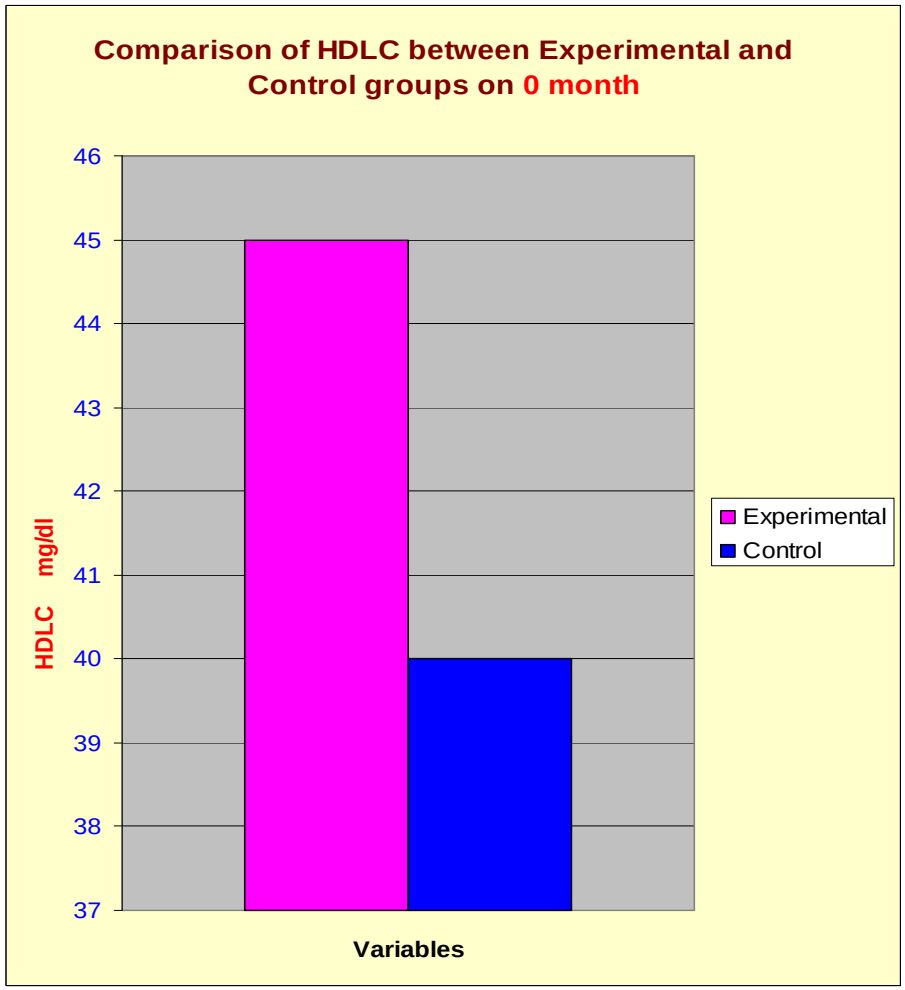
Comparison of different variables between experimental groups and Control Groups

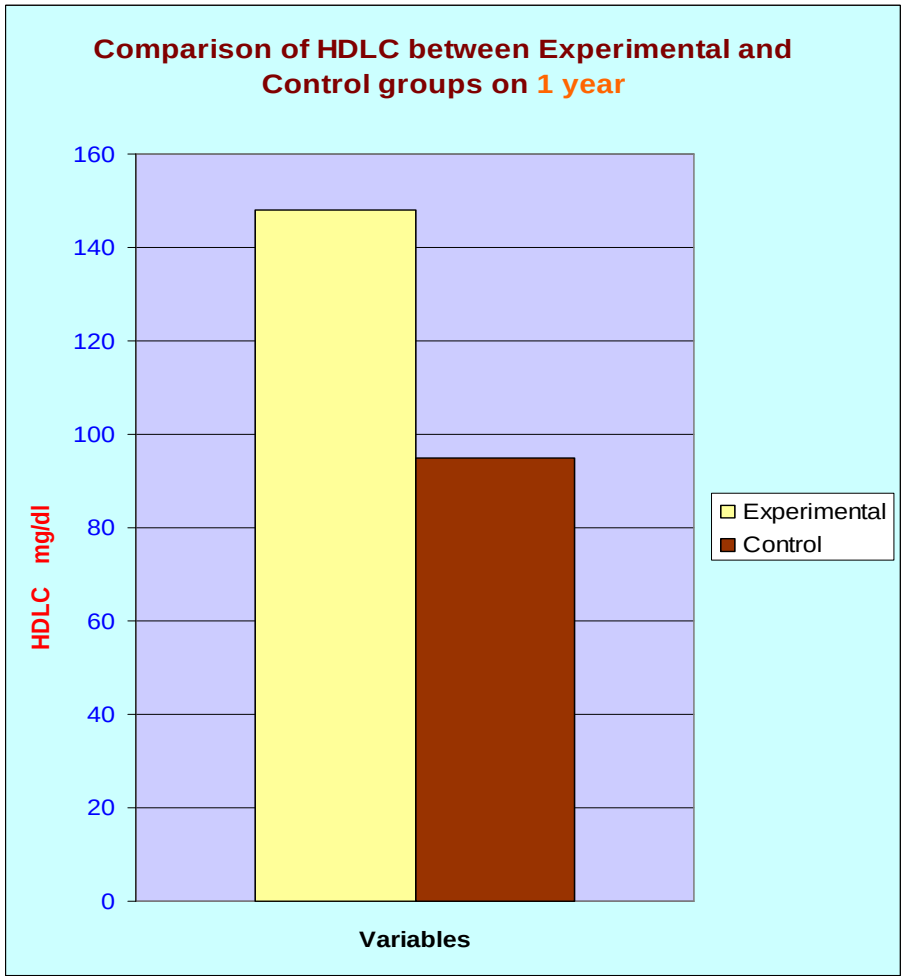


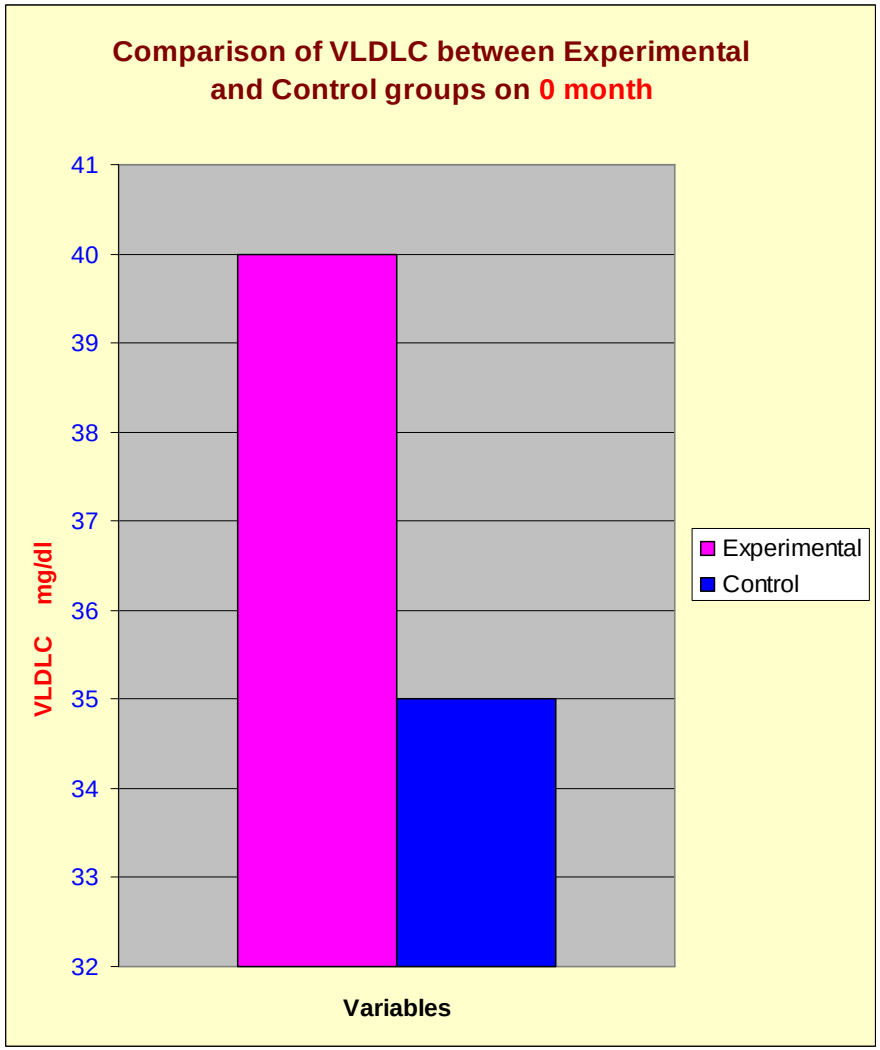


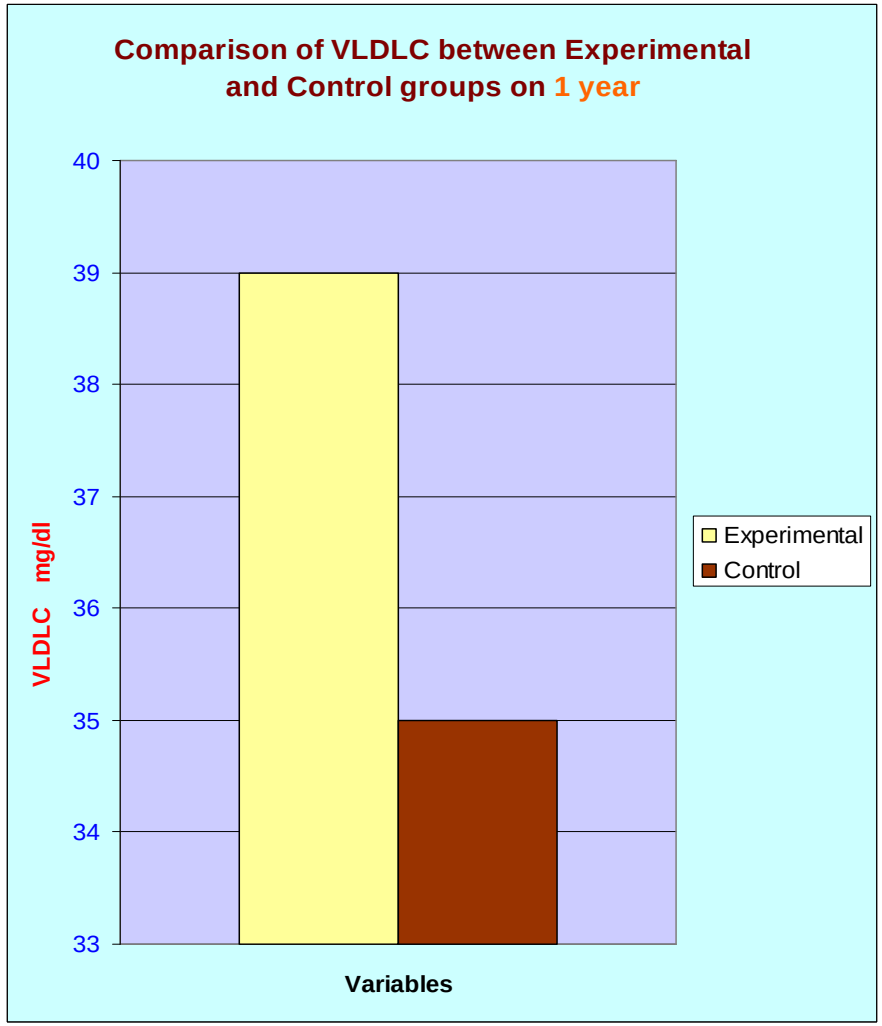

Comparison of LDLC between Experimental and Control groups on 0 month
200
180
160
140
120
100
80
60
40
20
0
LDLC mg/dl
Experimental
Control
Variables

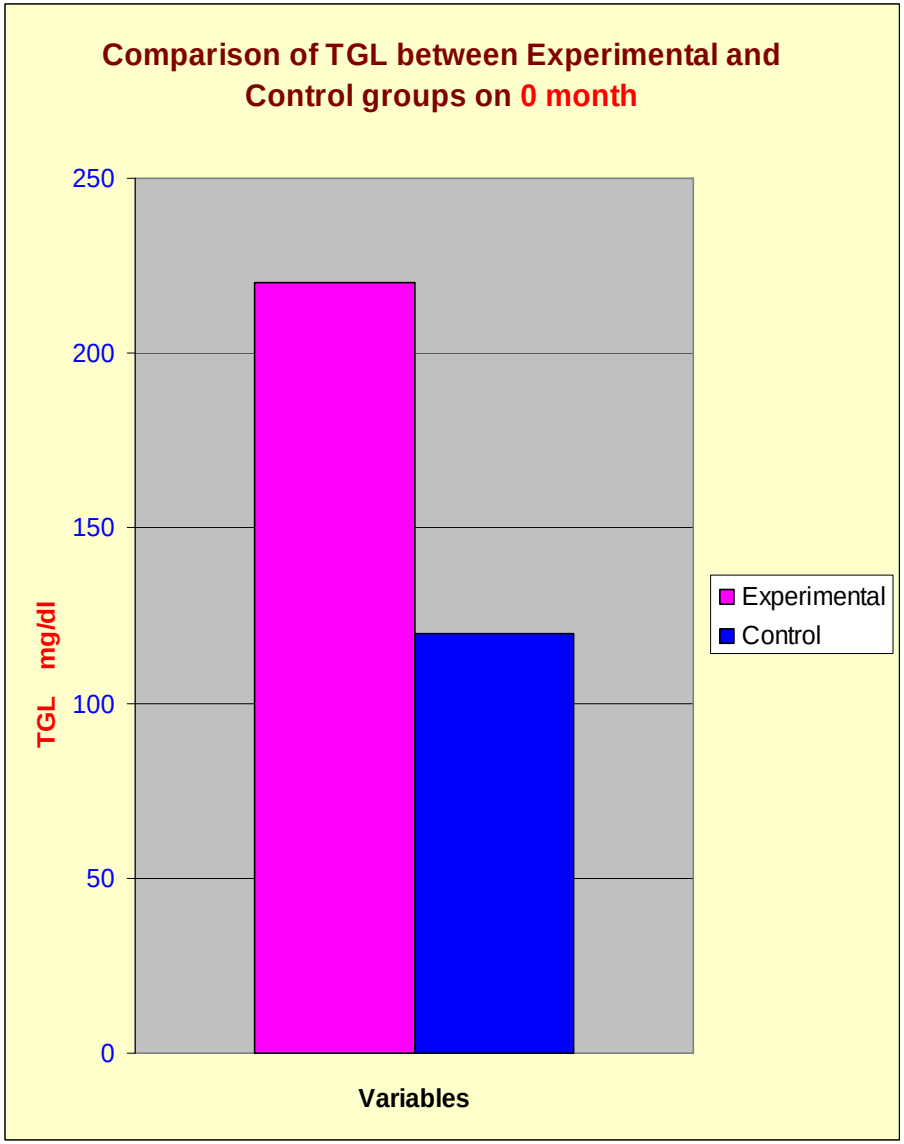

Comparison of LDLC between Experimental and Control groups on 1 year
54
52
50
48
46
44
42
40
LDLC mg/dl
Experimental
Control
Variables

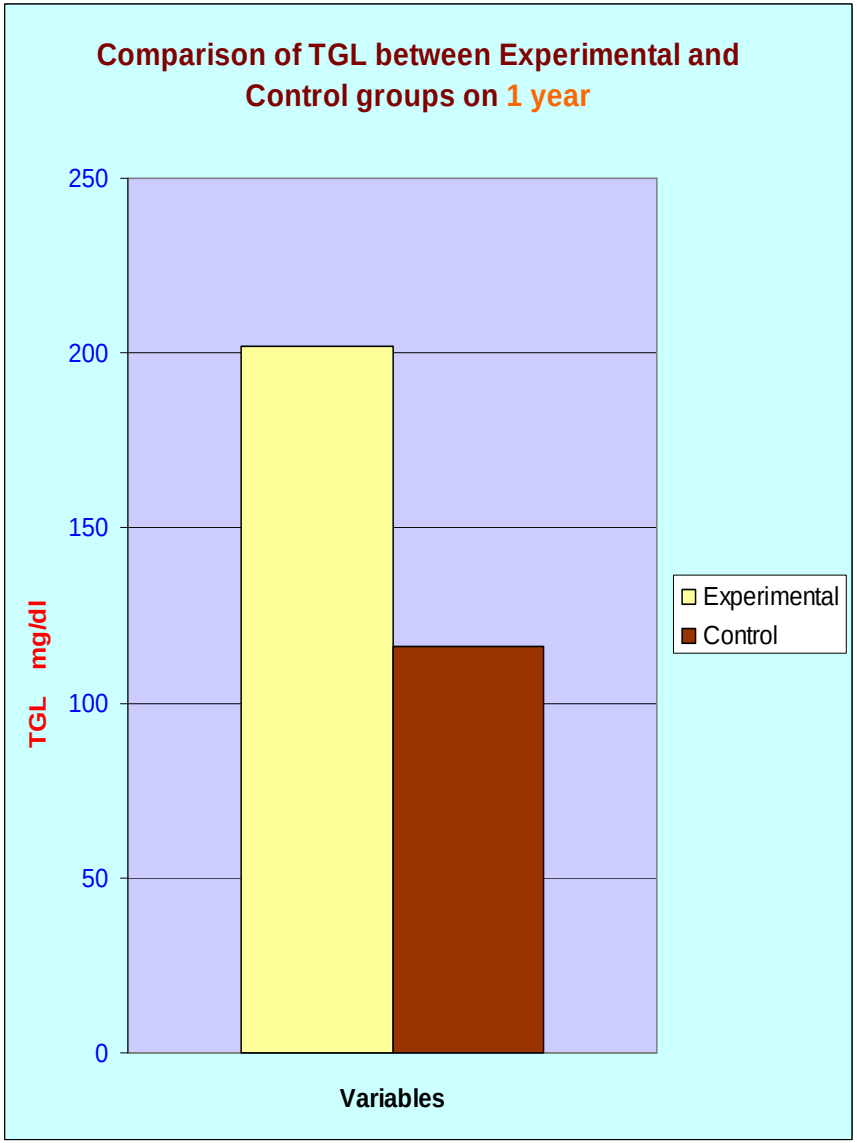

Comparison of HDLC between Experimental and Control groups on 0 month
46
45
44
43
42
41
40
39
38
37
HDLC mg/dl
Experimental
Control
Variables

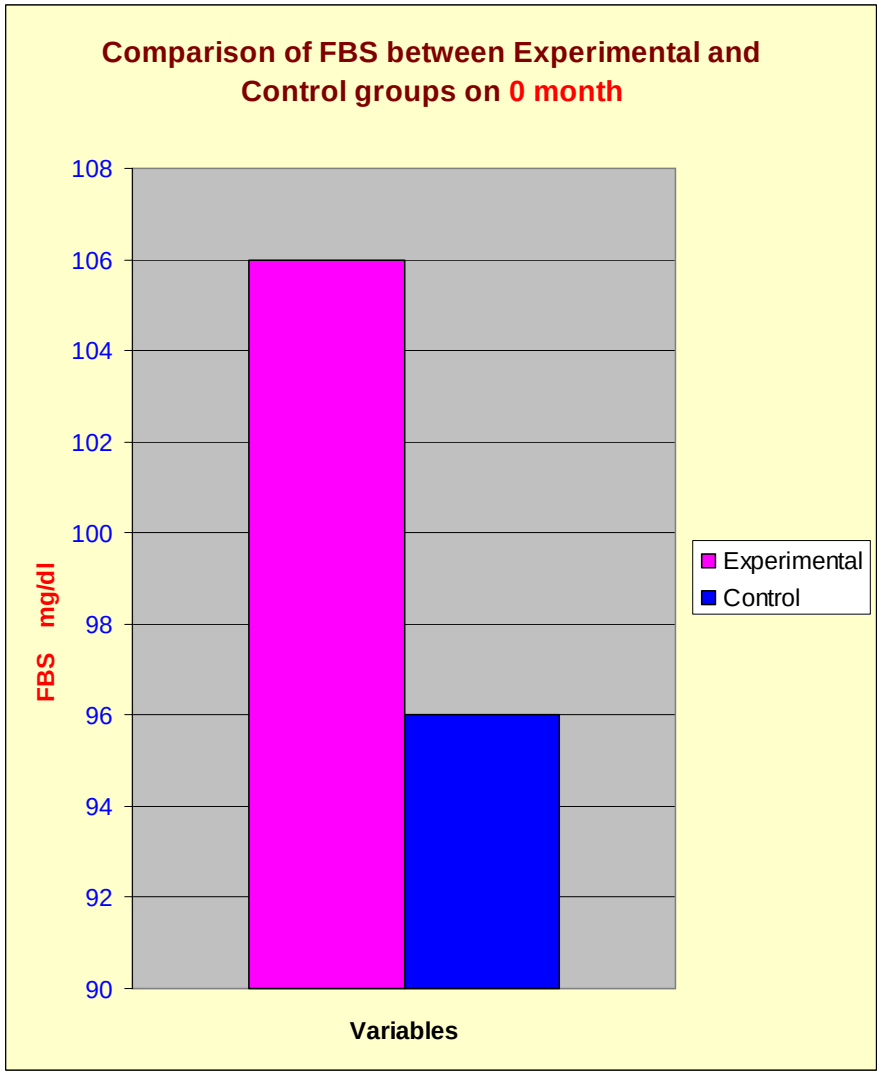

Comparison of HDLC between Experimental and Control groups on 1 year
160
140
120
100
80
60
40
20
0
HDLC mg/dl
Experimental
Control
Variables

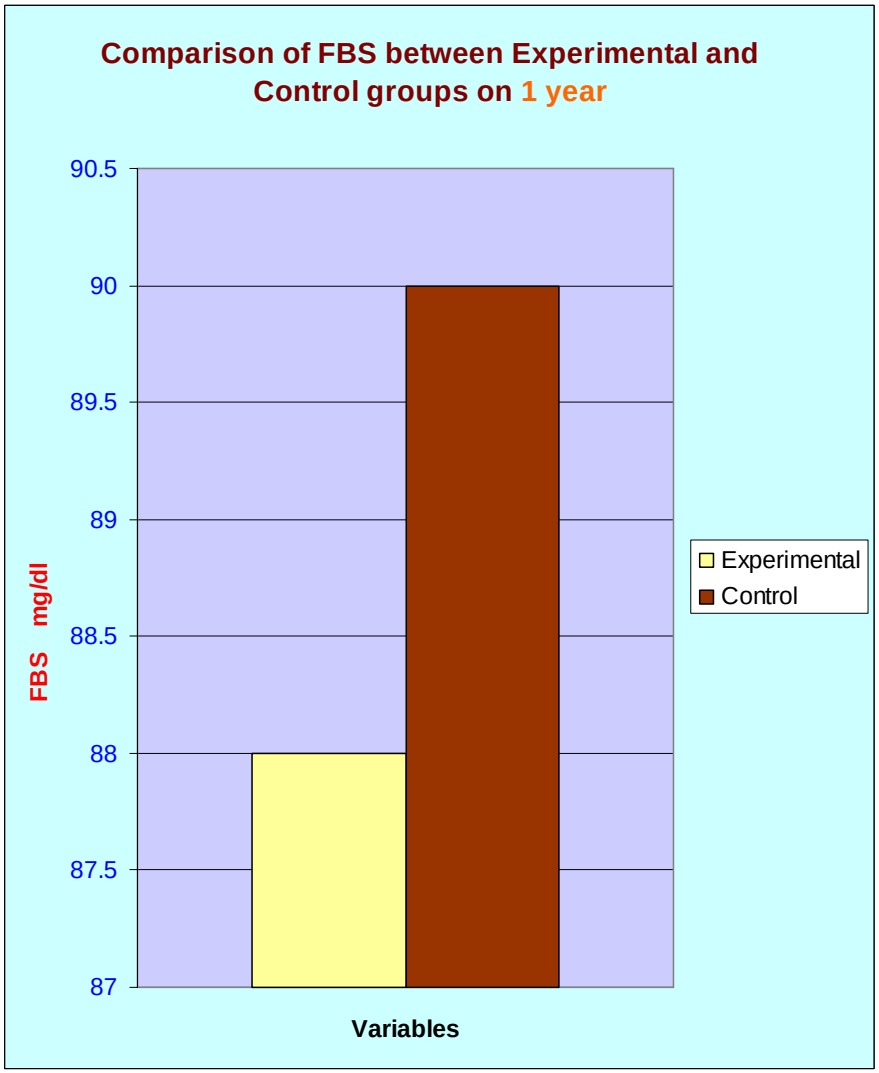

Comparison of VLDLC between Experimental and Control groups on 0 month
41
40
39
38
37
36
35
34
33
32
VLDLC mg/dl
Experimental
Control
Variables

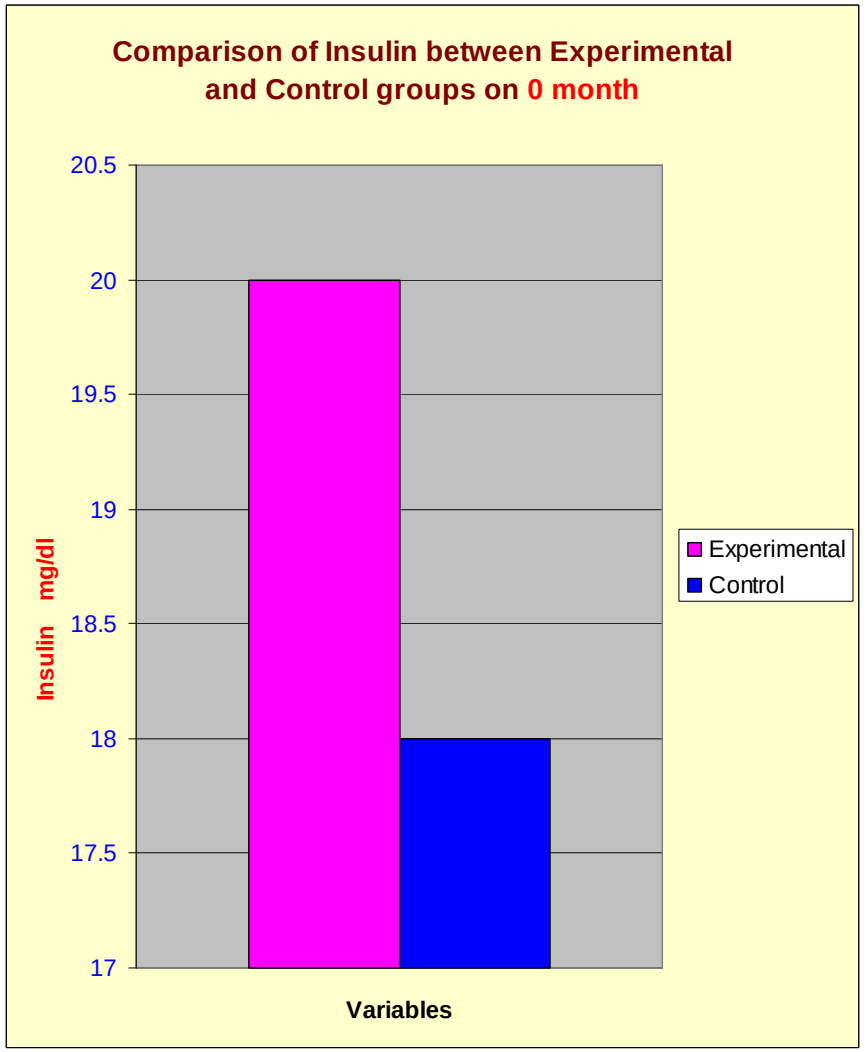

Comparison of VLDLC between Experimental and Control groups on 1 year
40
39
38
37
36
35
34
33
VLDLC mg/dl
Experimental
Control
Variables

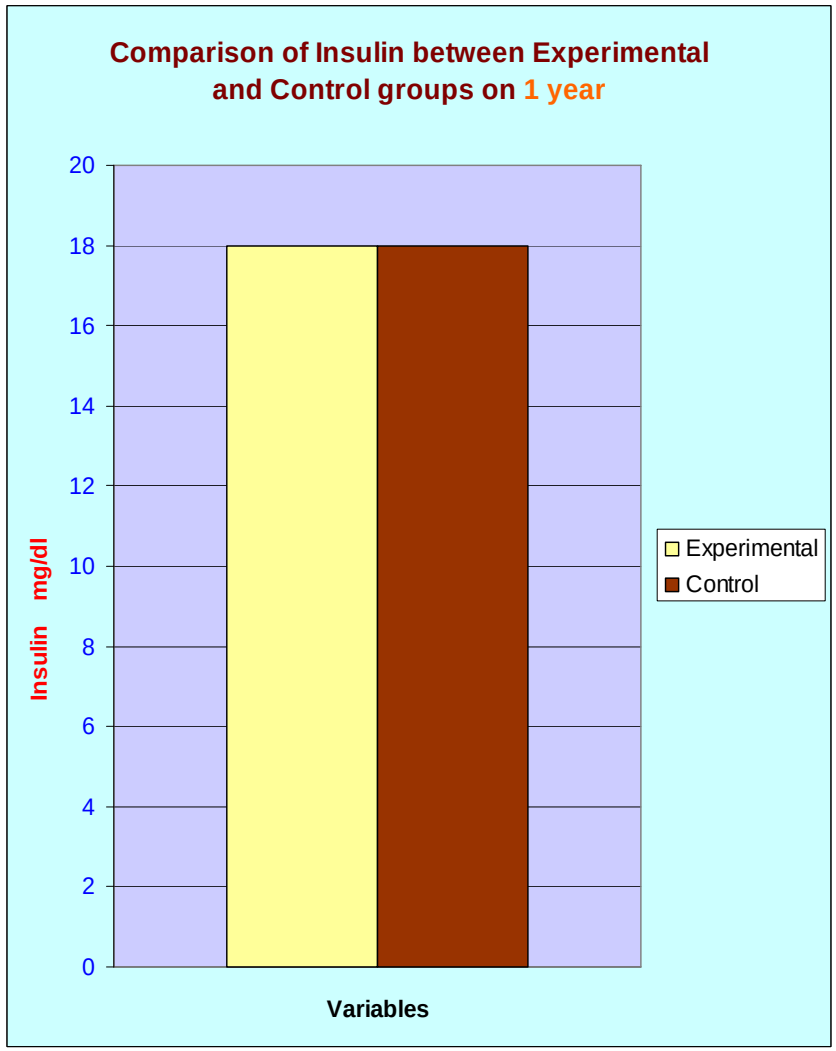

Comparison of TGL between Experimental and Control groups on 0 month
250
200
150
100
50
0
TGL mg/dl
Experimental
Control
Variables


Comparison of TGL between Experimental and Control groups on 1 year
250
200
150
100
50
0
TGL mg/dl
Experimental
Control
Variables


Comparison of FBS between Experimental and Control groups on 0 month
108
106
104
102
100
98
96
94
92
90
FBS mg/dl
Variables
Experimental
Control


Comparison of FBS between Experimental and Control groups on 1 year
90.5
90
89.5
89
88.5
88
87.5
87
FBS mg/dl
Variables
Experimental
Control


Comparison of Insulin between Experimental and Control groups on 0 month
20.5
20
19.5
19
18.5
18
17.5
17
Insulin mg/dl
Experimental
Control
Variables


Comparison of Insulin between Experimental and Control groups on 1 year
20
18
16
14
12
10
8
6
4
2
0
Insulin mg/dl
Experimental
Control
Variables

DISCUSSION

The study shows that atorvastatin increases insulin sensitivity in normal subjects. Compared with placebo, treatment with atorvastatin (10 mg/day) resulted in significant reduction in the HOMA index. In addition, significant reductions in total and LDL cholesterol concentrations were observed in the atorvastatin group. It thus corroborates previous findings that though uncertain, statin therapy can affect insulin resistance syndrome.[14].

Insulin Resistance refers to the reduction in insulin mediated glucose uptake in insulin sensitive tissues, specifically in the skeletal muscles. As a compensatory response, hyperinsulinemia ensures to maintain normal blood glucose levels. In epidemiological studies, fasting insulin level is commonly used as a surrogate marker of insulin resistance.[14].

In normoglycemic subjects, fasting insulin correlated well with whole body glucose uptake. Although fasting insulin is a reasonable measure of insulin resistance, it is potentially confounded by variability in insulin secretion. Thus the indexes derived from fasting insulin and glucose, such as Homeostasis Model Assessment (HOMA), the Quantitative Insulin Sensitivity Check Index (QUICKI), and the Insulin Sensitivity Index (ISI) developed by Gutt and coworkers, have been more widely used to assess insulin resistance in clinical and population based studies.

Although the role of insulin resistance in the pathophysiology of type 2 diabetes mellitus is well accepted, the relationship between insulin resistance and blood pressure remains controversial. Nearly 40 years ago, Welborn and colleagues observed that non diabetic patients with essential hypertension had significantly higher plasma insulin concentrations than did normotensive individuals.

Statins are the more effective LDL – cholesterol-lowering drugs by about 25% to 60%. In addition, they also increase HDL-cholesterol by about 5% to 10% and decrease triglycerides by about 10% to 30%. The effect on triglycerides is proportional to the decrease in LDL-cholesterol. Pre diabetes and type 2 diabetes are characterized with low grade inflammation. Aggressive lowering of LDL-cholesterol by atorvastatin decreases hsCRP by 42% vs 9.6% with placebo. Several studies have proved that LDL-reduction by statins is associated with improved endothelial function due to enhanced NO release.Besides these actions ,the reduction in risk of development of diabetes is due to improved insulin sensitivity by statins.[12]

Okyima et. al suggest that statins could have some impact on insulin action, and, to estimate the direct effects of statins on insulin secretion from pancreatic beta cells, MIN6 cells were treated with pravastatin, simvastatin or atorvastatin. Basal insulin secretion at low glucose concentration was unexpectedly increased at very high doses of simvastatin or atorvastatin after 24 and 48 hours of incubation, though insulin secretion was apparently decreased by these lipophilic statins.[18].

Yoshitomi et al. assessed the relationship between IR and the changes of lipid profile in patients with hyperlipidaemia treated by atorvastatin. The IR did not affect the degree of reduction in cholesterol by atorvastatin in non-diabetic subjects. The IR may influence hypertriglyceridaemia greater than the effect of atorvastatin in non-diabetic subjects.[20]

It has been suggested that HMG Co-A reductase inhibitors ('statins') may reduce the risk of developing type 2 Diabetes mellitus. Yee et al. designed to evaluate whether use of statins would also delay progression to insulin therapy. After multivariate adjustment, however, statin use was associated with a 10-month delay before newly treated diabetic subjects needed to start insulin treatment.[21]

Poalisso G et al. observed that statins administration was associated with an improvement of insulin resistance and decline in plasma triglyceride concentrations.

This study suggest that statins increase insulin sensitivity even in normoglycemic patients.

CONCLUSION

1. Statins improve insulin sensitivity even in normoglycemics and prevent the progression of IGT to type 2 diabetes.
2. Statins reduce levels of interleukin 6 and TNF through its anti-inflammatory activity. These cytokines inhibit lipoprotein lipase activity and to stimulate lipolysis in adipose tissue. Atorvastatin may therefore interrupt the progression from central obesity to insulin resistance mediated by the adipose tissue derived cytokines.
3. Impaired endothelial function has been shown to correlate with insulin resistance. Atorvastatin by restoring endothelial function, may beneficially affect glucose and insulin transport.
4. Since statins are used for the treatment of hypercholesterolemia in clinical practice, it is important to know their effect on insulin sensitivity. If further studies confirm the observation that statins improve insulin sensitivity and reduce the onset of type 2 diabetes, the perceived benefit of cardiovascular intervention in clinical trials could be greatly increased and the long term cost-benefit analysis of those interventions may be more positive than previous studies have estimated.

BIBLIOGRAPHY

1. Mcfarlane S et al. Pleotropic effects of statins: Lipid reduction and beyond. Journal of clinical endocrinology 2002;879(4); 1451-1458
2. Wolfrum et al. Endothelium dependent effects of statins . Arterioscler Thromb Vasc Biol;2003;23(5).729-736
3. Freeman et al. Pravastatin and development of diabetes.West of Scotland Coronary Prevention Study .Circulation.2001;103;(3);357-362
4. Ikeda et al. Pleotropiceffects of statins on vascular tissue. Current Drug Targets Cardio Haematology Disorder.2001;1(1);51-58
5. Waldman et al . Pleotropic effects of statins. Drugs. 2003;63(2);139-152
6. Shepherd et al.Prevention of coronary artery disease with pravastatin in hypercholesterolemia.1995 NEJM303.1301-1307.
7. Tonolo et al .Additive effects of simvastatin beyond its effect on LDLcholesterol in hypertensive type 2 diabetic patients.2000 Eur J Clin 30;980-987
8. Tedgui et al .Anti-inflammatory mechanisms in vascular wall.Circulation. 2001.88:877 – 887
9. Perticone et al. Effects of atorvastatin on endothelial function of hypercholesterolemia. Atherosclerosis 2000. 152; 511-518.

10. Mcfarlane et al. Insulin resistance and cardiovascular disease.Journal of Clinical Endocrinology 2001. 86; 713-718.

11.Jialal et al.Effect of statin on high sensitive Creactive protein. Circulation.2001. 103; 1933-1935.

12.Albert et al.Effect of Statins on C-reactive protein. PRINCE trial JAMA2001.286; 64-70

13.Yokota et al. Mechanism of preventive effect of statins on diabetic nephropathy. Kidney International journal. 1999; 71; 178-181.

14.Recent advances in our understanding of insulin action and insulin resistance. Le Roith et al. Diabetes Care.2001.24;503-508.

15.Gill et al.Difficult diabetes. Oxford ;Blackwell science ;2001

16. King et al.Global burden of diabetes. Diabetes care.1998.21(9).1414-31.

17.Parhoefer et al. Atorvastatin aids insulin sensitivity in metabolic syndrome. American journal of Cardiology2006;98;66-69.

18.Okajima et al. Effects of statins on insulin secretion from a beta cell line, MIN6 cells.Atheroscler Thromb 2006.13(6);329-335.

19. Takono et al. Effects of statins on glucose tolerance in type 2 diabetes mellitus.AtherosclerоThromb 2006.13(2);95-100.

20.Yoshitomi et al .Relationship between insulin resistance and effect of atorvastatin in nondiabetic subjects. Atherosclero Thrombosis.2005; 12(1); 9-13.

21.Yee et al. Statin use in type 2 diabetes is associated with a delay in starting insulin.Diab Med. 2004.21(9);962-7.

22.Jick et al.Statins and newly diagnosed diabetes. British journal of clinical pharmacology. 2004; 58(3); 303-9.

PROFORMA

Data collected from the participants

Name : Age : Sex : Occupation :

Address : Weight

BMI				
SBP				
DBP				
Hb				
TC				
DC				
Urea				
Creatinine				
Electrolytes				
LFT				
T.Proteins				
Urine analysis				
ECG				
X-ray chest				
T.Cholesterol				
LDL-C(mg/dl)				
HDL-C(mg/dl)				
VLDL-C(mg/dl)				
TGL(mg/dl)				
FBS				
S.Insulin				

MASTER CHART - EXPERIMENTAL GROUP

S.No.	Name	Age/Sex	BMI	SBP/DBP mmhg	T.cholesterol mg/dl	LDLC mg/dl	HDLC mg/dl	VLDLC mg/dl	TGL mg/dl	FBS mg/dl	S.insulin µU/ml
1	Munusamy	48 / m	26	156 / 100	282	194	48	42	210	105	21
2	Muthu	45 / m	25	160 / 90	285	202	42	46	215	109	20
3	Krishnan	46 / m	23	150 / 110	274	203	41	50	200	98	21
4	Pasupathy	49 / m	24	160 / 100	281	196	39	48	216	102	19
5	Moorthy	46 / m	27	152 / 90	292	194	49	38	215	108	19
6	Benjamin	46 / m	26	162 / 100	271	188	45	44	220	110	20
7	Murugesan	49 / m	26	156 / 90	282	192	44	40	202	98	18
8	James Raja	48 / m	24	160 / 100	275	201	40	46	216	108	22
9	Kalimuthu	45 / m	23	162 / 98	282	192	42	38	218	102	21
10	Ganesan	44 / m	26	166 / 92	268	206	48	36	226	95	19
11	Subbiah	49 / m	24	154 / 90	272	188	42	46	208	97	20
12	Karupiah	45 / m	24	152 / 94	284	202	51	48	220	101	18
13	Rajendran	46 / m	23	160 / 90	268	196	41	52	211	110	19
14	Kuppusamy	49 / m	25	156 / 96	288	188	42	56	210	106	21
15	Ramachandran	48 / m	20	162 / 96	288	192	48	42	206	98	20
16	Lakshmi	49 / F	24	150 / 90	276	182	47	38	210	92	21
17	Kamalammal	49 / F	24	154 / 90	276	196	46	48	217	102	20

S.No.	Name	Age/Sex	BMI	SBP/DBP mmhg	T.cholesterol mg/dl	LDLC mg/dl	HDLC mg/dl	VLDLC mg/dl	TGL mg/dl	FBS mg/dl	S.insulin µU/ml
18	Vijaya	46 / F	26	160 / 90	280	201	48	52	210	105	21
19	Saroja	47 / F	21	154 / 96	296	190	48	48	216	109	20
20	Vasanthi	49 / F	24	160 / 100	274	188	46	38	198	108	21
21	Rajeswari	48 / F	24	150 / 110	278	196	49	36	202	102	19
22	Pushpa	45 / F	26	152 / 100	281	195	52	38	196	108	19
23	Jaya	44 / F	23	156 / 90	284	199	51	44	192	110	20
24	Dayalan	46 / m	24	162 / 110	278	192	46	46	204	102	18
25	Egambaram	48 / m	25	158 / 74	276	204	48	42	201	108	22
26	Munirajan	46 / m	27	164 / 90	284	203	46	42	197	92	21
27	Saraswathy	49 / F	24	162 / 98	288	205	45	38	192	95	19
28	Rajakumari	44 / F	24	160 / 100	292	198	49	40	195	99	20
29	Arockiyam	47 / m	26	160 / 90	282	196	46	46	204	101	21
30	Joseph	44 / m	27	164 / 94	272	204	47	50	203	116	20
31	Mariammal	49 / F	24	150 / 92	278	196	45	48	201	106	19
32	Saritha	47 / F	23	156 / 94	274	194	49	46	195	108	18
33	Govindan	44 / m	25	158 / 92	278	191	48	40	197	102	20
34	Rajathi	48 / F	23	160 / 90	282	192	42	42	191	102	21

MASTER CHART- CONTROL GROUP

S.No.	Name	Age/Sex	BMI	SBP/DBP mmhg	T.cholesterol mg/dl	LDLC mg/dl	HDLC mg/dl	VLDLC mg/dl	TGL mg/dl	FBS mg/dl	S.insulin µU/ml
1	Lakshmi	44 / F	24	160 / 90	132	107	41	49	108	110	20
2	Mani	46 / m	26	154 / 90	121	111	36	52	112	98	16
3	Jayaraman	46 / m	25	160 / 100	112	108	42	51	123	85	15
4	Baskaran	45 / m	24	170 / 90	128	112	42	48	111	94	17
5	Kasi	44 / m	25	160 / 90	126	98	51	46	124	104	18
6	Pitchai	45 / m	24	170 / 110	132	100	54	45	112	106	17
7	Manickam	45 / m	24	150 / 100	127	102	48	49	119	98	19
8	Rathnam	46 / m	25	152 / 98	122	92	46	40	118	96	20
9	Jaya	44 / F	22	156 / 92	128	96	45	46	112	102	14
10	Nagammal	46 / F	23	158 / 94	112	98	49	50	108	104	16
11	Kuppusamy	46 / m	26	152 / 96	124	95	47	58	110	92	18
12	Sivaraman	45 / m	24	162 / 90	121	94	48	52	106	88	17
13	Surnammal	46 / F	21	152 / 100	120	98	45	54	110	94	19
14	Ranganayaki	47 / F	21	156 / 98	136	102	46	58	108	96	16
15	Vijayalakshmi	43 / F	22	162 / 100	120	106	48	48	112	98	17
16	Parvathi	46 / F	21	158 / 98	128	101	47	51	104	89	18
17	Sarasvathy	45 / F	21	156 / 102	132	88	48	42	116	98	18

S.No.	Name	Age/Sex	BMI	SBP/DBP mmhg	T.cholesterol mg/dl	LDLC mg/dl	HDLC mg/dl	VLDLC mg/dl	TGL mg/dl	FBS mg/dl	S.insulin µU/ml
18	Laksmanan	46 / m	24	162 / 98	128	89	42	46	119	102	19
19	Jyothi	44 / F	22	154 / 98	136	92	41	50	108	96	17
20	Madhavan	45 / m	24	164 / 100	122	78	39	48	106	104	20
21	Rathnammal	46 / F	23	168 / 100	112	79	49	38	105	94	18
22	Subramanian	45 / m	21	170 / 100	108	92	45	44	116	88	16
23	Vinayagamurthy	46 / m	24	160 / 98	118	91	44	40	118	95	20
24	Ragavan	47 / m	23	150 / 90	126	94	40	46	112	94	19
25	Sankaran	44 / m	22	152 / 96	114	88	42	38	106	88	18
26	Chandra	44 / F	21	156 / 100	122	97	48	54	119	96	19
27	Indra	46 / F	22	160 / 90	130	102	47	49	121	98	19
28	Krishnamurthy	46 / m	24	154 / 100	126	88	51	48	118	90	17
29	Pushpa	45 / m	22	152 / 90	118	86	41	52	112	98	15
30	Shanmugam	45 / F	23	162 / 100	128	88	42	38	116	102	20
31	Shekar	46 / m	22	156 / 100	124	92	48	42	115	94	16
32	Arumugam	45 / m	21	152 / 98	128	94	47	48	109	98	18
33	Vel Murugan	46 / m	22	160 / 100	119	93	26	36	112	98	19
34	Ganesan	47 / m	23	162 / 98	122	95	42	44	115	88	18

BMI - **Body Mass Index**
SBP - **Systolic Blood Pressure**
DBP - **Diastolic Blood Pressure**
TGL - **Triglycerides**
LDLC - **Low Density Lipoprotein Cholesterol**
HDLC - **High Density Lipoprotein Cholesterol**
VLDLC - **Very Low Density Lipoprotein Cholesterol**
FBS - **Fasting Blood Sugar**

Table 1:

Anthropometrical, Clinical and Biochemical characters of volunteers

	Experimental Group (34)	Control Group (34)
Age	**45 ± 4**	**43 ± 3**
Males	**31**	**31**
Females	**13**	**13**
BMI	**27.3 ± 12**	**27.5 ± 2.1**
SBP	**154 ± 16**	**146 ± 24**
DBP	**100 ± 12**	**94 ± 8**
T. Cholesterol	**280 ± 20**	**140 ± 20**
LDL	**180 ± 20**	**110 ± 20**
HDL	**45 ± 10**	**40 ± 10**
VLDL	**40 ± 5**	**35 ± 15**
TGL	**220 ± 20**	**120 ± 20**
FBS	**106 ± 6**	**96 ± 6**
Fasting Insulin	**20 ± 5**	**18 ± 5**

Table 2: Values of Blood parameters of Experimental Group

	0mon	1 mon	2mon	3mon	4mon	5mon	6mon	7mon	8mon	9mon	10 mon	11mon
T. cholesterol	2.80 ± 20	276 ± 18	270 ± 16	260 ± 15	256 ± 16	248 ± 18	240 ± 16	234 ± 12	226 ± 11	218 ± 13	212 ± 12	208 ± 10
HDL	45 ± 10	45 ± 12	46 ± 12.	46 ± 13	47 ± 12	48 ± 16	48 ± 8	49 ± 11	50 ± 14	50 ± 10	51 ± 13	51 ± 11
LDL	180 ± 20	178 ± 18	17 ± 12	172 ± 11	172 ± 12	168 ± 18	166 ± 10	164 ± 8	160 ± 12	160 ± 8	158 ± 10	152 ± 8
VLDL	40 ± 5	40 ± 3	40 ± 2	39 ± 6	39 ± 5	39 ± 6	39 ± 5	39 ± 4	39 ± 2	39 ± 4	39 ± 3	39 ± 4
TGL	220 ± 20	218 ± 18	218 ± 16	214 ± 10	214 ± 8	216 ± 8	212 ± 10	210 ± 11	210 ± 12	206 ± 6	204 ± 8	204 ± 8
FBS	106 ± 6	106 ± 5	105 ± 3	103 ± 4	103 ± 3	101 ± 4	98 ± 5	98 ± 6	96 ± 5	93 ± 4	92 ± 5	90 ± 3

12mon
202 ± 12
52 ± 12
148 ± 12
39 ± 3
202 ± 6
88 ± 4

Table 3: Values of Blood parameters of Control Group

	0mon	1mon	2mon	3mon	4mon	5mon	6mon	7mon	8mon	9mon	10 mon	11mon	12mon
T. cholesterol	140 ± 20	138 ± 8	136 ± 16	134 ± 15	132 ± 15	130 ± 16	128 ± 8	125 ± 10	123 ± 8	120 ± 12	117 ± 6	115 ± 6	112 ± 18
HDL	40 ± 10	40 ± 8	41 ± 9	41 ± 7	41 ± 5	42 ± 6	42 ± 7	42 ± 7	42 ± 6	43 ± 8	43 ± 9	43 ± 8	44 ± 9
LDL	110 ± 30	110 ± 12	108 ± 18	108 ± 16	108 ± 14	106 ± 12	106 ± 13	106 ± 13	102 ± 11	100 ± 18	98 ± 16	97 ± 10	95 ± 13
VLDL	35 ± 15	35 ± 9	35 ± 8	35 ± 7	35 ± 8	35 ± 10	35 ± 10	35 ± 8	35 ± 12	35 ± 14	35 ± 11	35 ± 6	35 ± 8
TGL	120 ± 20	120 ± 11	120 ± 8	119 ± 12	120 ± 14	118 ± 12	119 ± 8	116 ± 15	118 ± 8	118 ± 11	110 ± 12	116 ± 12	116 ± 13
FBS	96 ± 10	96 ± 8	94 ± 6	94 ± 5	92 ± 8	94 ± 8	94 ± 5	92 ± 6	92 ± 9	88 ± 8	90 ± 7	90 ± 8	90 ± 6

Table 4: Showing serum insulin and HOMA-IR 2 values of different group

	S. insulin initial value	S. insulin end value	Homeostasis model assessment of insulin resistance 2-IR (initial value)	HOMA - 2 IR end value
Exp Group	**20 ± 5**	**18 ± 3**	**4.3 ± 0.5**	**3.7 ± 0.4**
Control Group	**18 ± 5**	**18 ± 5**	**4.3 ± 0.3**	**4.3 ± 0.2**