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Metformin's hypolipidemic action in obese rats and its influence on leptin hormone levels

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Abstract

Metformin, an old medicine with magical properties, has received a lot of attention in recent years because of its newly discovered beneficial effects. Our study was designed to evaluate the Metformin effect on obese rats. Twenty (20) adult female rats weight and age (250 ± 50 gm > years 3 month respectively), fed on rich carbohydrate and lipid diet until they have (450 ± 50 gm) after that treatment with metformin through divided into three periods, the lipid profile and serum leptin levels were measured at each period. At the control group of experiment and at end of each duration (30, 60 and 90 day) the blood sample was collected from the rats who participated in this study. The results showed that Metformin reduced the Total cholesterol and LDL cholesterol concentrations triglyceride and very- low density lipoprotein, on the other side it participated in elevation in High density lipoprotein. In addition, Metformin feeding in the obese rats are associated with significant decrease in serum leptin levels. In conclusion, the results of this study, documented the Metformin has diverse effects through enhancement the lipid profile and decreased leptin hormone value.

Keyword: Leptin hormone, Metformin, Lipid profile.

Introduction

Obesity has risen to the top of the global illness burden. Obesity is a long-term illness that increases the chance of developing other comorbidities. The recent study of adult Body mass index (BMI) shows that the occurrence of obesity increased from 3.2% to 10.8% in Men and 6.4% to 14.9% in women. The global occurrence of morbid obesity is 0.64% and 1.6% in men and women, respectively. Obesity is also linked with complications, including cardiovascular disease, diabetes, hypertension, and stroke (Kahan and Zvenyach 2016). Obesity is related with high plasma concentrations of triglycerides, cholesterol, LDL and low concentrations of HDL. Overweight, obesity, and dyslipidemia, on the other hand, are all modifiable risk factors for cardiovascular disease. (Hernández-Lepe et al. 2017). Metformin has no effect on weight and can even help you lose weight by lowering your food consumption. (Golay 2008). Metformin is a biguanide drug that has been used to treat type 2 diabetes mellitus for more than 60 years. Metformin has been proven to reduce death rates in diabetic patients in the United Kingdom Prospective Diabetic Study (UKPDS), and current studies suggest it may also help with cancer, obesity, nonalcoholic fatty liver disease (NAFLD), metabolic syndrome and polycystic ovary syndrome (PCOS). Also, it has been shown help reduce the weight gain caused by antipsychotic drugs. Metformin has lately been intensively examined, and preliminary evidence shows that it reduces hepatocyte triglyceride buildup and inhibits liver carcinogenesis in people with NAFLD. Metformin has also been demonstrated to decrease visceral fat, limit extracellular matrix remodeling in white adipose tissue (WAT), and prevent obesity-induced inflammation in investigations. However, there is clinical evidence that metformin may be used to treat NAFLD, metabolic syndrome, cancer or to prevent hepatocellular carcinoma in NAFLD patient (Zhou et al. 2018).

Metformin reduced gastrointestinal absorption of carbohydrates, recovers glycemic control involves reduce glucose production by liver, enhancing peripheral insulin sensitivity, and blocking glucose absorption from gastrointestinal (Viollet et al. 2012). In addition it reduced insulin and leptin resistance, (Glueck et al. 2001), the decrease of plasma ghrelin

(Kusaka et al. 2008), and induction of lipolysis and Anorexia by activation of glucagon–like peptide 1 (GLP-1) (Mannucci et al. 2001). Also it reduces hepatic and skeletal muscle lipid depots through increased decreased lipid synthesis and fat oxidation (Mannucci et al. 2001).

On the other hand, metformin is able indirectly interact with many enzymes [e.g. mitochondrial electron transport chain complex I,AMPK, glycerol 3-phosphate dehydrogenase (mGPD)], which lead to a great diversity of probable effects of the drug. Metformin accumulation occurs mostly in the intestine, but also in the stomach, liver, kidney, and to a lesser amount in muscle, according to research on metformin characteristics. Given that these organs are most exposed to high quantities of metformin, the buildup of metformin in the colon and stomach is unsurprising. These concentrations are at least 10 times greater than those seen in the liver, suggesting that the gut is likely a key site of action (Gormsen et al. 2016). In fact, metformin's effects in the gut may be quite different from those in the liver (Van Stee, de Graaf, and Groen 2018).

Leptin, a hormone produced by adipose cells, is an important component in the body's homeostatic weight-control loop (Inui 1999). Leptin suppresses food intake and increases energy expenditure through neuropeptidergic effector molecules in the hypothalamus (Inui 1999). It is manufacture primarily by white adipose tissues and released as a (16 kilo Dalton [kDa] protein) (Ahima and Flier 2000). Excessive eating and obesity are reduced in mice and people who are given leptin (Cowley et al. 2001). Leptin concentrations rise with obesity and tend to fall with weight reduction; however the responsiveness of leptin levels to changes in body weight varies greatly. (Van Rossum et al. 2000). Leptin is linked to insulin resistance, especially in female (Panarotto et al. 2000). Hyperleptinemia, alone or in combination with hyperinsulinemia, may play a key role in the development of the insulin resistance syndrome's cardiovascular risk factors (Zimmet et al. 1999).

Material and Methods

Study design: Twenty (20) adults female rats' weight and age (250 ± 50 gm > years 3 month respectively), they were housed in cages and placed in room at (21-24 C°) with air ventilation and light / dark cycle of (12:12h/day). The experimental rats divided randomly in two groups, G1 consider as control group and G2 consider as treated group. The two group first time was fed on a rich carbohydrate and lipid diet until they have (450 ± 50 gm) after that G1 group continue with same diet but G2 treatment with metformin through diet at (100 mg/kg body weight) the lipid profile and serum leptin levels were measured at three time of experimental (three period) (30, 60 and 90 day) the blood sample was collected from the rats who participated in this study.

Serum Preparation: At the begin of experiment and at end of each duration (30, 60 and 90 day), blood sample was collected from two animal group, (3 ml) of blood by cardiac puncture technique Blood samples were placed in non-heparinized tubes and allowed to stand for 10 minutes before being centrifuged for 15 minutes at 3000 RPM to get serum, which was then frozen in securely sealed vials for subsequent chemical analysis at -20°c.

Determination of total serum cholesterol concentration: The procedure was described by (Richmond 1973).

Determination of serum triglycerides (TG) concentration: The procedure of evaluation of serum triglycerides was described by(Fossati and Prencipe 1982).

Determination of serum HDL-c concentration: The procedure was described by (Lopes-Virella et al. 1977).

Calculation of serum very low-density lipoprotein cholesterol concentration: VLDL-c concentration was determined by dividing triglycerides values (in mg /dl) on 5 ((Friedewald, Levy, and Fredrickson 1972).

Calculation of serum low-density lipoprotein cholesterol concentration LDL-c concentration:

This formula is only valid when TG concentration not exceeds 400 mg/dl (Friedewald, Levy, and Fredrickson 1972).

Determination of serum leptin concentration: By using the colorimetric method Precision Intra-assay.

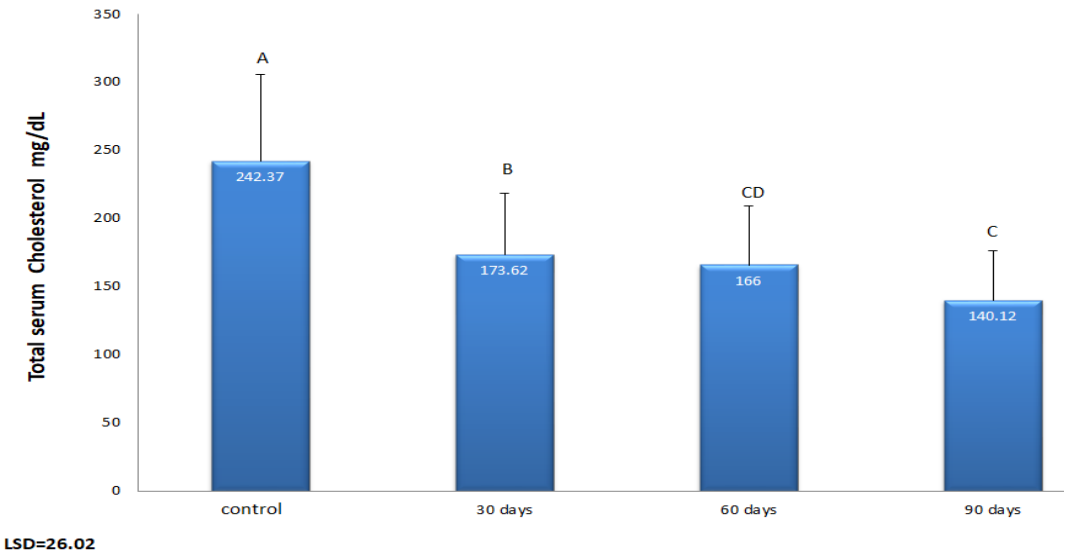
Statistical Analysis: The Statistical Analysis System-SAS (2012) program was used to effect of difference groups in study parameters (SAS, 2012). Statistical analysis of data was performed on the basis of one-Way Analysis of Variance (ANOVA) using a significant level of ($P<0.05$). A specific group differences were determined using least significant differences (LSD) as described by (Snedecor George and Cochran 1973).

Results and Discussion

Effect of Metformin on total cholesterol concentration: The effects of Metformin administration in overweight female rats in control group and treated group after 30, 60, 90 days of experiment on serum concentration of TC conc. are

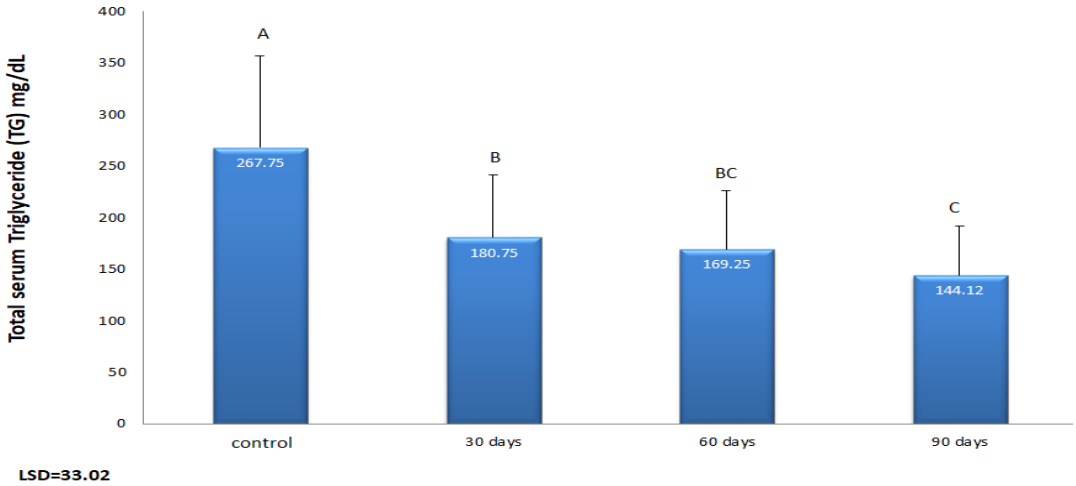
clarified in histograms (1). Significant decrease (P<0.05) in serum TC conc. was observed after 60 day compared to the value in control and after 30 days of treatment. Through the duration of experiment the mean value of TC conc. were (242.37), (242.37) (166) and (140.12) for control group, 30 days, 60 days and 90 days respectively.

Effect of Metformin on triglycerides (TG) concentration: The histogram (2) also pointed to elevation the value of triglycerides (TG) concentration in rats serum of control group (267.75), comparing to significant lowering (P<0.05) in TG conc. In G2 group, concentration was observed in 90, 60 days and 30days, with mean values of (144.12), (169.25) and (180.75) respectively.



Histogram (1): Effect of Metformin on total cholesterol concentration in serum animal

Values are expressed as mean ± SE. n= 10 / each group. Control: animal without treatment, 30 days: rats received 100 mg/ kg Metformin for 30 days. 60days: rats received 100 mg/ kg Metformin for 60 days. 90days: rats received 100 mg/ kg Metformin for 90 days. Various capital letters denote significant differences (P<0.05) between groups.

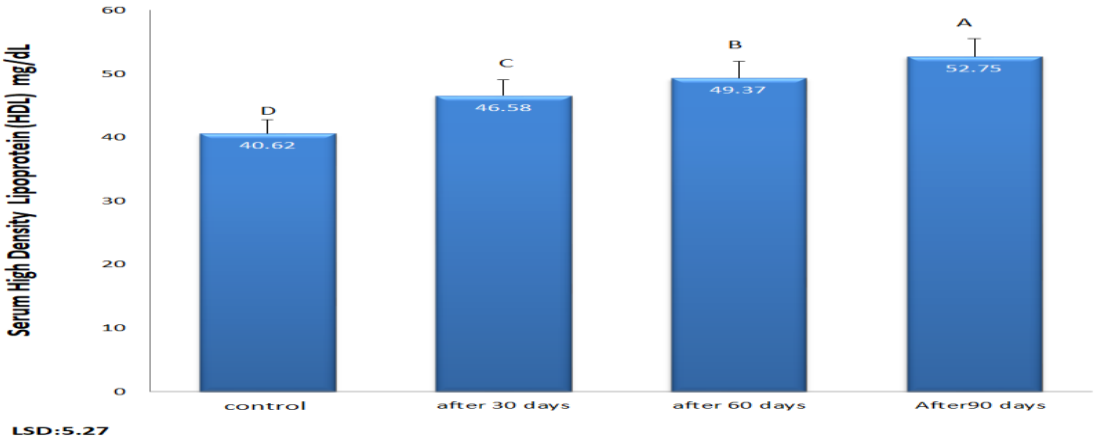


Histogram (2): Effect of Metformin on triglycerides (TG) concentration:

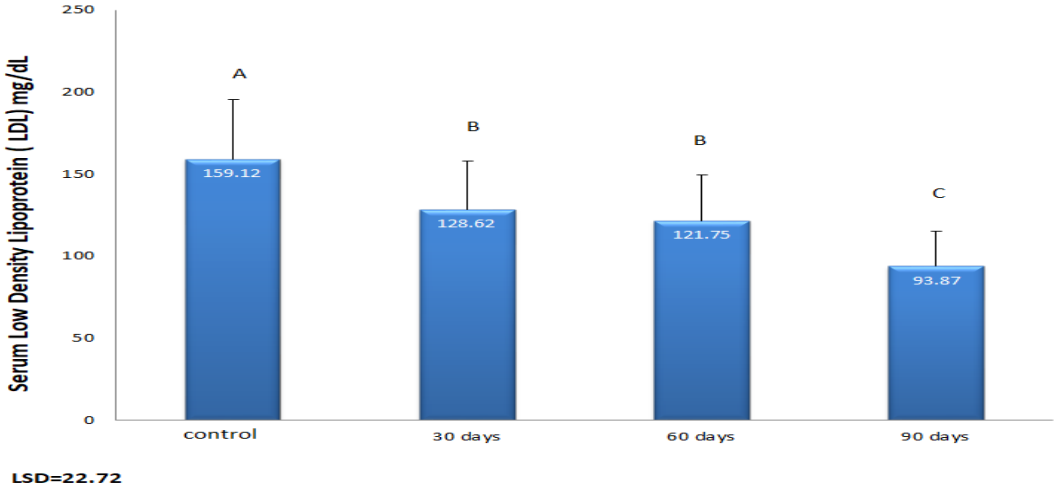
Values are expressed as mean ± SE. n= 10 / each group. Control: animal without treatment, 30 days: rats received 100 mg/ kg Metformin for 30 days. 60days: rats received 100 mg/ kg Metformin for 60 days. 90days: rats received 100 mg/ kg Metformin for 90 days. Various capital letters denote significant differences (P<0.05) between groups.

Effect of Metformin on High density lipoprotein cholesterol (HDL-c) concentration in serum animal: The histogram (3) pertaining to the mean value of High-density lipoprotein cholesterol (HDL-c) concentration in the female rat's serum for different treated duration. Significant increase (P<0.05) in HDL-c concentration in rat's serum was observed in 90 days from treated compared to duration 60 days, and 30 days. The values of serum HDL-c concentration through the experiment duration were (40.62) (46.58) (49.37) and (52.75) for control, 30 days, 60 days and 90 days respectively.

Effect of Metformin on very low-density lipoprotein cholesterol concentration in serum: The histogram (4) also pointed to tend to elevation the value of very low-density lipoprotein cholesterol concentration (VLDL-c) in rat's serum in control group (53.52), comparing to significant lower (P<0.05) in VLDL-c concentration was observed in of 90, 60 days and 30days, with mean values, (30.01), (33.92) and (35.02) respectively.



Histogram (3): Effect of Metformin on High density lipoprotein cholesterol (HDL-c) concentration in serum: Values are expressed as mean ± SE. n= 10 / each group. Control: animal without treatment, 30 days: rats received 100 mg/ kg Metformin for 30 days. 60days: rats received 100 mg/ kg Metformin for 60 days. 90days: rats received 100 mg/ kg Metformin for 90 days. Various capital letters denote significant differences (P<0.05) between groups.

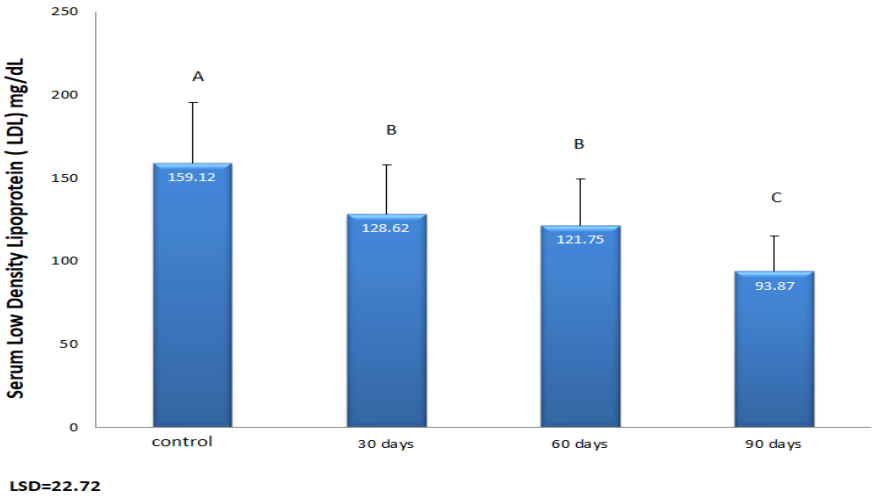


Histogram (4): Effect of Metformin on very low-density lipoprotein cholesterol concentration (VLDL-c) concentration in serum animal

Values are expressed as mean ± SE. n= 10 / each group. Control: animal without treatment, 30 days: rats received 100 mg/ kg Metformin for 30 days. 60days: rats received 100 mg/ kg Metformin for 60 days. 90days: rats received 100 mg/ kg Metformin for 90 days. Various capital letters denote significant differences (P<0.05) between groups.

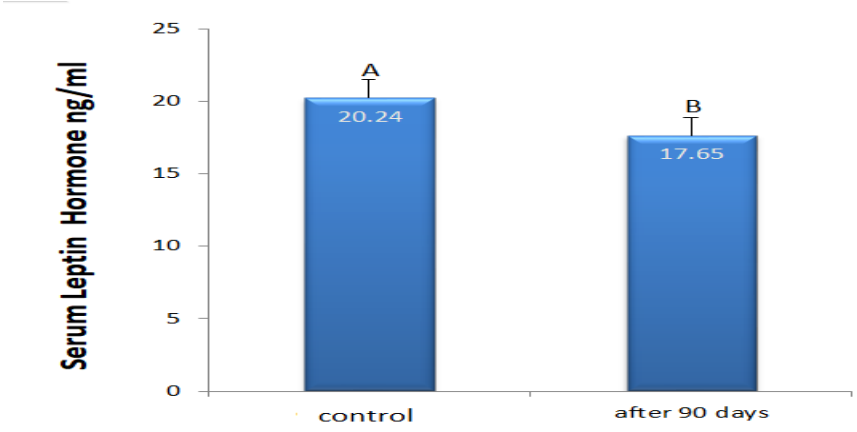
Effect of Metformin on low-density lipoprotein cholesterol concentration LDL-c concentration in serum: The effects of Metformin feeding in rats after 90 , 60 , 30 days on serum concentration of low-density lipoprotein cholesterol concentration (LDL-c) compare to control group of experiment are clarified in histograms (5). Significant decrease (P<0.05) in serum LDL-c conc. was observed after 90 day compared to the value in control group and after 30, 60 days. Through the duration of experiment the mean value of LDL-c were (159.12), (128.62) (121.75) and (93.87) for duration control, 30days, 60days and 90days respectively.

Effect of Metformin on leptin concentration in serum: The histogram (6) also pointed to tend to elevation the value of leptin concentration in female rat's serum at control group (20.24), comparing with significant lowering (P<0.05) in leptin concentration was observed in 90 days after begin of experiment, with mean values of (20.24) and (17.65) respectively.



Histogram (5): Effect of Metformin on low-density lipoprotein cholesterol (LDL-c) concentration in serum.

Values are expressed as mean ± SE. n= 10 / each group. Control: animal without treatment, 30 days: rats received 100 mg/ kg Metformin for 30 days. 60days: rats received 100 mg/ kg Metformin for 60 days. 90days: rats received 100 mg/ kg Metformin for 90 days. Various capital letters denote significant differences (P<0.05) between groups.



Histogram (6): Effect of Metformin on leptin concentration in serum animal

Values are expressed as mean ± SE. n= 10 / each group. Control: animal without treatment, 30 days: rats received 100 mg/ kg Metformin for 30 days. 60days: rats received 100 mg/ kg Metformin for 60 days. 90days: rats received 100 mg/ kg Metformin for 90 days. Various capital letters denote significant differences (P<0.05) between groups.

The effect of Metformin on lipid profile: Multiple CHD risk factors, including the atherogenic insulin resistance syndrome, were found to be closely linked to morbid obesity. (Garnett et al. 2013). Hyperinsulinemia, hyperleptinemia, low HDL cholesterol, high triglycerides, high LDL cholesterol, and high PAI-Fx are all symptoms of hyperinsulinemia and hyperleptinemia. Metformin is a safe and effective way to minimize the risk of coronary heart disease(Malin and Kashyap 2014). In the current study, significant reductions in serum total cholesterol and LDL concentrations were seen after 90 days of Metformin treatment, while there was no significant change in serum total cholesterol concentration in animals after 60 days. The effects of metformin on blood lipid levels demonstrated a significant reduction in total and LDL cholesterol in the metformin-treated senior groups as compared to the placebo groups. LDL cholesterol has largely supplanted total cholesterol as a risk measure and the major therapeutic goal for hyperlipidemias in clinical practice (Solymár et al. 2018; Stone et al. 2014). In our meta-analysis, the decrease in total cholesterol is thought to be related to a decrease in LDL cholesterol. LDL cholesterol has largely supplanted total cholesterol as a risk measure and the major therapeutic goal for hyperlipidemias in clinical practice (Stone et al. 2014). Over a wide range of LDL cholesterol levels, lowering LDL cholesterol has been shown to reduce cardiovascular risk and mortality in a continuous and graded manner (Wadhera et al. 2016). For every 1.0 mmol/L increase in LDL cholesterol, the chance of an acute cardiovascular incident increased by around 40% (Sharrett et al. 2001). Individual outcomes with lower rates per 1.0 mmol/L LDL cholesterol reductions included major coronary events (24%), coronary revascularization (24%), ischemic stroke (20%), and any stroke (15%) (Stone et al. 2014). All-cause mortality was reduced by 10% per 1.0 mmol/L LDL reduction(Stone et al. 2014). Our findings show that metformin treatment may reduce the incidence of major coronary events by 4–5% and all-cause mortality by roughly 2% in elderly populations with carbohydrate metabolic abnormalities, based on these epidemiologic findings. A variety of processes could explain these positive improvements in blood lipid profiles (Malin and Kashyap 2014), some of which can also contribute to the well-known weight reducing effect of metformin. Metformin decreases ectopic lipid depots (liver and skeletal muscle) via increasing fat oxidation and lowering lipid synthesis (Malin and Kashyap 2014). In our meta-analysis, the decrease in total cholesterol is thought to be related to a decrease in LDL cholesterol. LDL cholesterol has largely supplanted total cholesterol as a risk measure and the major therapeutic goal for hyperlipidemias in clinical practice (Stone et al. 2014). LDL cholesterol reduction has been shown to lower cardiovascular risk and mortality over a wide range of LDL cholesterol levels in a continuous and graded manner (Wadhera et al. 2016). For every 1.0 mmol/L increase in LDL cholesterol, the chance of an acute cardiovascular incident increased by nearly 40% (Sharrett et al. 2001). All-cause mortality was reduced by 10% per 1.0 mmol/L LDL reduction(Stone et al. 2014). Our findings show that metformin treatment may reduce the incidence of major coronary events by 4–5% and all-cause mortality by roughly 2% in elderly populations with carbohydrate metabolic abnormalities, based on these epidemiologic findings. A variety of processes could explain these positive improvements in blood lipid profiles. (Malin and Kashyap 2014) some of which may potentially contribute to metformin's well-known weight-loss impact. Metformin decreases ectopic lipid depots (liver and skeletal muscle) via increasing fat oxidation and lowering lipid synthesis (Malin and Kashyap 2014).

After 30, 60, and 90 days of treatment, the results of the current study revealed a reduction in triglycerides (TG) and very low-density lipoprotein cholesterol (VLDL-c) concentrations in serum animals. Metformin is the first-line medication for type 2 diabetic treatments. Metformin decreases plasma VLDL triglyceride levels in addition to its well-known antihyperglycemic effects (TG). It had an effect primarily due to a decrease in VLDL-TG, with a minor increase in HDL. Metformin had no effect on hepatic VLDL-TG production, VLDL particle composition, or hepatic lipid composition, but it did increase clearance of glycerol tri[(3)H]oleate-labeled VLDL-like emulsion particles into brown adipose tissue selectively (BAT). Metformin-treated mice had lower BAT bulk and lipid droplet content, indicating enhanced BAT activation. In addition, BAT had higher levels of AMP-activated protein kinase 1 (AMPK1) expression and activity, as well as HSL and mitochondrial content. In T37i differentiated brown adipocytes, therapeutic doses of metformin enhanced AMPK and HSL activity and promoted lipolysis. Overall, our findings point to BAT as a key factor in metformin's TG-lowering impact, as it promotes VLDL-TG absorption, intracellular TG lipolysis, and subsequent mitochondrial fatty acid oxidation. As a result, targeting BAT as a future

therapeutic method for dyslipidemia treatment could be investigated (Geerling et al. 2014).

In this study, metformin intake resulted in a decrease in the value of LDL cholesterol in all periods compared to the value before treatment (The effect of metformin on weight and other metabolic parameters and outcomes in obese nondiabetic animals). Oscar and his colleagues speculate that the increased levels of HDL seen in metformin-treated animal reflect a lower degree of oxidative stress and quiet inflammation during the trial period than in placebo-treated animal. Over time, a continuous reduction in oxidative stress and quiet inflammation has been linked to weight loss. Total cholesterol is divided into high-density lipoproteins and low-density lipoproteins. HDL makes up around 20% of total plasma cholesterol and is linked to a lower risk of cardiovascular disease, whereas ox-LDL is a measure of oxidative stress and is linked to a higher risk of metabolic disease and CVD (Viteri et al. 2017).

The effect of Metformin on leptin concentration:

After 90 days of using metformin, there was a small drop in leptin levels in serum individuals, according to our data (Komori et al. 2004) and after treatment with metformin, total calorie intake was dramatically lowered as a result of reduced consumption of food. This indicates that the medication may have an appetite suppressing impact (Komori et al. 2004). Obese rats had higher serum leptin levels, which is consistent with earlier observations on obese rats. This could be because the rat is resistant to leptin's effects (Patanè et al. 2000). It's also been claimed that BMI is one of the key determinants regulating leptin levels, and that as obese rat lose weight, their leptin levels drop. (Carlsen et al. 1998).

Conclusions

We may deduce from the findings that metformin has a variety of effects by acting on several healthy biomarkers such as lipid profile and leptin hormone. Metformin reduces total cholesterol and LDL cholesterol concentrations, as well as triglyceride and very-low density lipoprotein, in obese rats, preventing cardiovascular disease. On the other hand, it contributes to an increase in high density lipoprotein. Metformin consumption is linked to a considerable reduction in serum leptin levels in obese animal.

References

Ahima, Rexford S, and Jeffrey S Flier. 2000. “Leptin.” *Annual review of physiology* 62(1): 413–37.

Carlsen, E et al. 1998. “Effect of the Introduction of Total Mesorectal Excision for the Treatment of Rectal Cancer.” *British Journal of Surgery* 85(4): 526–29.

Cowley, Michael A et al. 2001. “Leptin Activates Anorexigenic POMC Neurons through a Neural Network in the Arcuate Nucleus.” *Nature* 411(6836): 480–84.

Fossati, Plero, and Lorenzo Prencipe. 1982. “Serum Triglycerides Determined Colorimetrically with an Enzyme That Produces Hydrogen Peroxide.” *Clinical chemistry* 28(10): 2077–80.

Friedewald, William T., Robert I. Levy, and Donald S. Fredrickson. 1972. “Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, without Use of the Preparative UltracentrifugeFriedewald, William T Levy, Robert I Fredrickson, Donald S.” *Clinical chemistry* 18(6): 499–502.

Garnett, Sarah P et al. 2013. “Optimal Macronutrient Content of the Diet for Adolescents with Prediabetes; RESIST a Randomised Control Trial.” *The Journal of Clinical Endocrinology & Metabolism* 98(5): 2116–25.

Geerling, Janine J et al. 2014. “Metformin Lowers Plasma Triglycerides by Promoting VLDL-Triglyceride Clearance by Brown Adipose Tissue in Mice.” *Diabetes* 63(3): 880–91.

Glueck, C J et al. 2001. “Metformin Reduces Weight, Centripetal Obesity, Insulin, Leptin, and Low-Density Lipoprotein Cholesterol in Nondiabetic, Morbidly Obese Subjects with Body Mass Index Greater than 30.” *Metabolism-Clinical and Experimental* 50(7): 856–61.

Golay, Alain. 2008. “Metformin and Body Weight.” *International Journal of Obesity* 32(1): 61–72.

Gormsen, Lars C et al. 2016. “In Vivo Imaging of Human 11C-Metformin in Peripheral Organs: Dosimetry, Biodistribution, and Kinetic Analyses.” *Journal of Nuclear Medicine* 57(12): 1920–26.

Hernández-Lepe, Marco Antonio et al. 2017. “Double-Blind Randomised Controlled Trial of the Independent and Synergistic Effect of Spirulina Maxima with Exercise (ISESE) on General Fitness, Lipid Profile and Redox Status in Overweight and Obese Subjects: Study Protocol.” *BMJ open* 7(6): e013744.

Inui, Akio. 1999. “Cancer Anorexia-Cachexia Syndrome: Are Neuropeptides the Key?” *Cancer research* 59(18): 4493–4501.

Kahan, Scott, and Tracy Zvenyach. 2016. “Obesity as a Disease: Current Policies and Implications for the Future.” *Current obesity reports* 5(2): 291–97.

Komori, T et al. 2004. "Metformin Ameliorates Treatment of Obese Type 2 Diabetic Patients with Mental Retardation; Its Effects on Eating Behavior and Serum Leptin Levels." *Experimental and clinical endocrinology & diabetes* 112(08): 422–28.

Kusaka, I, S Nagasaka, H Horie, and S Ishibashi. 2008. "Metformin, but Not Pioglitazone, Decreases Postchallenge Plasma Ghrelin Levels in Type 2 Diabetic Patients: A Possible Role in Weight Stability?" *Diabetes, Obesity and Metabolism* 10(11): 1039–46.

Lopes-Virella, Maria Fernanda, Pamela Stone, Shelton Ellis, and John A Colwell. 1977. "Cholesterol Determination in High-Density Lipoproteins Separated by Three Different Methods." *Clinical chemistry* 23(5): 882–84.

Malin, Steven K., and Sangeeta R. Kashyap. 2014. "Effects of Metformin on Weight Loss: Potential Mechanisms." *Current opinion in endocrinology, diabetes, and obesity* 21(5): 323–29.

Mannucci, Edoardo et al. 2001. "Effect of Metformin on Glucagon-like Peptide 1 (GLP-1) and Leptin Levels in Obese Nondiabetic Subjects." *Diabetes care* 24(3): 489–94.

Panarotto, Daniel, Jean-Luc Ardilouze, Daniel Tessier, and Pierre Maheux. 2000. "The Degree of Hyperinsulinemia and Impaired Glucose Tolerance Predicts Plasma Leptin Concentrations in Women Only: A New Exploratory Paradigm." *Metabolism-Clinical and Experimental* 49(8): 1055–62.

Patanè, Giovanni et al. 2000. "Metformin Restores Insulin Secretion Altered by Chronic Exposure to Free Fatty Acids or High Glucose: A Direct Metformin Effect on Pancreatic Beta-Cells." *Diabetes* 49(5): 735–40.

Richmond, W. 1973. "Preparation and Properties of a Cholesterol Oxidase from Nocardia Sp. and Its Application to the Enzymatic Assay of Total Cholesterol in Serum." *Clinical chemistry* 19(12): 1350–56.

Van Rossum, Elisabeth F C et al. 2000. "Leptin Responses to Weight Loss in Postmenopausal Women: Relationship to Sex-Hormone Binding Globulin and Visceral Obesity." *Obesity research* 8(1): 29–35.

Sharrett, A Richcv et al. 2001. "Coronary Heart Disease Prediction from Lipoprotein Cholesterol Levels, Triglycerides, Lipoprotein (a), Apolipoproteins AI and B, and HDL Density Subfractions: The Atherosclerosis Risk in Communities (ARIC) Study." *Circulation* 104(10): 1108–13.

Snedecor George, W, and William G Cochran. 1973. *Statistical Methods*. Iowa State University Press.

Solymár, Margit et al. 2018. "Metformin Induces Significant Reduction of Body Weight, Total Cholesterol and LDL Levels in the Elderly – A Meta-Analysis." *PLoS ONE* 13(11): 1–13.

Van Stee, Mariël F, Albert A de Graaf, and Albert K Groen. 2018. "Actions of Metformin and Statins on Lipid and Glucose Metabolism and Possible Benefit of Combination Therapy." *Cardiovascular diabetology* 17(1): 1–22.

Stone, Neil J et al. 2014. "2013 ACC/AHA Guideline on the Treatment of Blood Cholesterol to Reduce Atherosclerotic Cardiovascular Risk in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines." *Journal of the American College of Cardiology* 63(25 Part B): 2889–2934.

Viollet, Benoit et al. 2012. "Cellular and Molecular Mechanisms of Metformin: An Overview." *Clinical science* 122(6): 253–70.

Viteri, Oscar A et al. 2017. "Potential of Metformin to Improve Cardiac Risk in Postpartum Women with Gestational Diabetes." *Frontiers in medicine* 4: 180.

Wadhera, Rishi K et al. 2016. "A Review of Low-Density Lipoprotein Cholesterol, Treatment Strategies, and Its Impact on Cardiovascular Disease Morbidity and Mortality." *Journal of clinical lipidology* 10(3): 472–89.

Zhou, Joseph, Scott Massey, Darren Story, and Lixin Li. 2018. "Metformin: An Old Drug with New Applications." *International Journal of Molecular Sciences* 19(10): 1–15.

Zimmet, Paul, E J Boyko, G R Collier, and Maximilian de Courten. 1999. "Etiology of the Metabolic Syndrome: Potential Role of Insulin Resistance, Leptin Resistance, and Other Players." *Annals of the New York Academy of Sciences* 892(1): 25–44.