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To assess the effect of Metformin on Serum Ghrelin in obese patients

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Introduction: Overweight was defined as a BMI ≥23 kg/m^2 but <25 kg/m^2 for both genders (based on the World Health Organization Asia Pacific Guidelines) with or without Abdominal Obesity (AO). Generalized Obesity (GO) was defined as a BMI ≥ 25 kg/m^2 for both genders (based on the World Health Organization Asia Pacific Guidelines) with or without AO. Ghrelin is synthesized as a pre-prohormone, in the epithelial cells lining the fundus of the stomach, with smaller amounts produced in the placenta, kidney, pituitary and hypothalamus. Ghrelin is an appetite-stimulating hormone that increases growth hormone secretion and food intake in humans. Furthermore, ghrelin regulates several physiological processes, including glucose metabolism, insulin secretion, gastric emptying, cell proliferation, learning and memory, stress and anxiety.

Material and Methods: All the obese patients attending outpatient department (OPD) of Medicine. Subjects willing to participate and written informed consent was obtained from each participant before study. Subjects were screened for selection criteria. Baseline evaluation included recording of demographic details, BMI, medical history, general and systemic examination and laboratory investigations, which included complete haemogram, hepatic and renal function tests and routine urine analysis. The eligible patients were enrolled as randomization. Metformin was given at a daily dose of 1.0 gm BD for 3 months. Follow-up visits were scheduled at the end of every month for 3 months for assessment, including measurement of weight and general and systemic examination.

Results: Total 141 diabetic patients selected, 89 were male (63.12%) while 52 were female patients (36.87%). There was no statistically significant difference (0.231). The mean of Ghrelin (pg/ml) is 311.42±29.53 at baseline (pg/ml) and after 3rd month 279.43±19.34 (pg/ml). Serum Ghrelin within same group showed significant decreased over a period of 3 months ($p< 0.013$). The mean of Total Cholesterol level was 215.97±11.05 mg/dl at baseline, 172.52±10.38 mg/dl after 3rd month. Triglycerides levels within same group showed significant reduction over a period of 3 months ($p< 0.05$). The mean of HDL Cholesterol level was 39.13±3.89 mg/dl at baseline, 43.10±4.33 mg/dl after 3rd month.

Conclusion: Based on the above description of the effect of metformin in modulating AMPK and involving the IR regulation, as well as the pharmacological property of rosiglitazone as one of the compounds of thiazolidinedione, acting as a selective agonist of PPAR-γ, such combination may significantly promote the synthesis and secretion of adiponectin contributing to increasing level of adiponectin and improving insulin sensitivity.

Keyword: Serum Ghrelin, Metformin, Lipid Profile

Introduction:

Overweight was defined as a BMI ≥23 kg/m^2 but <25 kg/m^2 for both genders (based on the World Health Organization Asia Pacific Guidelines) with or without Abdominal Obesity (AO). [(1)] Generalized Obesity (GO) was defined as a BMI ≥ 25 kg/m^2 for both genders (based on the World Health Organization Asia Pacific Guidelines) with or without AO. AO was defined as a Waist Circumference (WC) ≥ 90 cm for men and ≥ 80 cm for women with or without GO. [(2)] Isolated Generalized Obesity (IGO) was defined as a BMI ≥ 25 kg/m^2 with waist circumference of < 90 cm in men and < 80 cm in women. Isolated Abdominal Obesity (IAO) was defined as a WC of ≥ 90 cm in men or ≥ 80 cm in women with a BMI< 25 kg/m^2. [(3)]

The presence of obesity is associated with adverse effects on health including metabolic complications in which numerous cytokines and hormones are involved. Obesity is associated with a higher risk of developing chronic diseases which includes diabetes, hypertension, osteoarthritis, and coronary artery disease (CAD) and moreover epidemiologic studies have found that obese adults have significantly higher mortality as compared with non-obese adults [(4)].

Ghrelin is synthesized as a pre-prohormone, in the epithelial cells lining the fundus of the stomach, with smaller amounts produced in the placenta, kidney, pituitary and hypothalamus. [(5)] Ghrelin is an appetite-stimulating hormone that increases growth hormone secretion and food intake in humans. [(6)] Furthermore, ghrelin regulates several physiological processes, including glucose metabolism, insulin secretion, gastric emptying, cell proliferation, learning and memory, stress and anxiety. [(7)] Ghrelin contributes to long- term energy homeostasis by increasing body weight and adiposity, presumably through a reduction of lipid oxidation.[(8)] Blood concentrations of ghrelin are lowest shortly after consumption of a food, and then gradually rise during the fast and reach the peak just prior to the next meal. [(9)] Ghrelin concentrations in blood are reduced in obese humans as compared to lean subjects. [(10)]

Metformin, a biguanide class of oral hypoglycemic agents, is the first line drug for the treatment of type 2 diabetes mellitus. [(11)] Metformin is used clinically for the treatment of obesity and diabetes, and its mechanism of actions include the following: (1) lowers plasma glucose levels by inhibiting gluconeogenesis in liver, (2) decreasing the intestinal absorption of glucose, and (3) improving insulin sensitivity by increasing peripheral glucose uptake and utilization. [(12)] Additionally, metformin has a variety of pleiotropic effects including improved lipid and cholesterol metabolism, decreased inflammation and inhibition of cell growth. [(13)] (4) Increases plasma levels of glucagon-like peptide 1 (GLP-1) is a member of the incretin family of peptide hormones release incretin from the gut in response to ingested glucose. It induces insulin release from pancreatic β-cells, retards gastric emptying, inhibits glucagon release from α cell, and produces a feeling of satiety [(14)].

Clinically, it has been proposed that a combination of changes in lifestyle modification with pharmacological approaches could be a more effective strategy for the management of obesity. In addition, unlike their relatively lean counterparts, the obese patients require specific dosing for a curative response to treatment. On these lines, we hypothesized that

weight control interventions in conjunction with Metformin therapy could have a significant positive impact on the management of obesity. By implicating pharmacological and dietary interventions to contain adiposity, we have explored the therapeutic outcome of obese Patients.

Material and Methods:

Study Design:

- Prospective, randomized.

Study center:

- Study was conducted in obese patients attending the outpatient department of Medicine in Index Medical College and Hospital over a period of 2 years.

Duration of study:

- Total duration of the study was 2 Years and sample collected.

Inclusion Criteria:

- Male or female patients 18 years and above.
- Patients with obesity (BMI > 25 kg/m^2).
- Patient willing to give informed written consent form.

Exclusion Criteria:

- Patients with history of Alcohol intake & Smoking.
- Patients with known history of Diabetes and hypertension.
- Patients with severe cardiac, liver and renal disease.
- Patients with GIT diseases.
- Patients with a history of lactic acidosis
- Patients with hypothyroidism and hyperthyroidism
- Patients taking vitamin B_{12}, folate, steroid, oral contraceptives & hormone replacement therapy
- Pregnant and breast-feeding females.
- Patients with polycystic ovarian disease.

Methodology

Subjects:

- All the obese patients attending outpatient department (OPD) of Medicine.
- Subjects willing to participate and written informed consent was obtained from each participant before study.
- Permission from treating consultant was obtained for subjects to participate in the study.
- Subjects were screened for selection criteria. Baseline evaluation included recording of demographic details, BMI, medical history, general and systemic examination and laboratory investigations, which included complete haemogram, hepatic and renal function tests and routine urine analysis. The eligible patients were enrolled as randomization.

Treatment:
Metformin was given at a daily dose of 1.0 gm BD for 3 months (Tab. Glycomet SR, USV Pharmaceutical Limited).

Follow-up Visits:
Follow-up visits were scheduled at the end of every month for 3 months for assessment, including measurement of weight and general and systemic examination.

Sample collection:

- Samples of venous blood was collected from a forearm vein, at baseline and after 3 months of Metformin.
- Blood samples were centrifuged at 3000 rpm for 15 min to separate the serum, and serum was stored at 4 °C for further assays.

Biochemical Parameters:
The following laboratory investigation was performed on sample of obese patients before and after Metformin therapy.

Procedure:
Blood samples were collected by vein puncture under all aseptic precautions from subjects using disposable syringes in fasting condition and Post Meal were collected in tubes containing sodium fluoride as an anticoagulant.

Ghrelin is estimated ELISA.

Safety parameters:

- Adverse Events Patients was advised to contact their Principal investigator if any adverse events occurred. The nature, time of onset, and severity of the event, the treatment needed, and any relation to the assigned study regimen was recorded. All serious adverse events were reported to the sponsor.

Statistical Analysis:

- The collected data was compiled in MS Excel sheet for analysis in Statistical Package for the Social Sciences (SPSS) version 25th was applied.
- The qualitative data was represented in the form of frequencies and percentage also represented in visual impression like bar diagram, pie diagram etc.
- p value was check at 0.05 % level of significance.

RESULTS

Graph 1: Distribution of Gender

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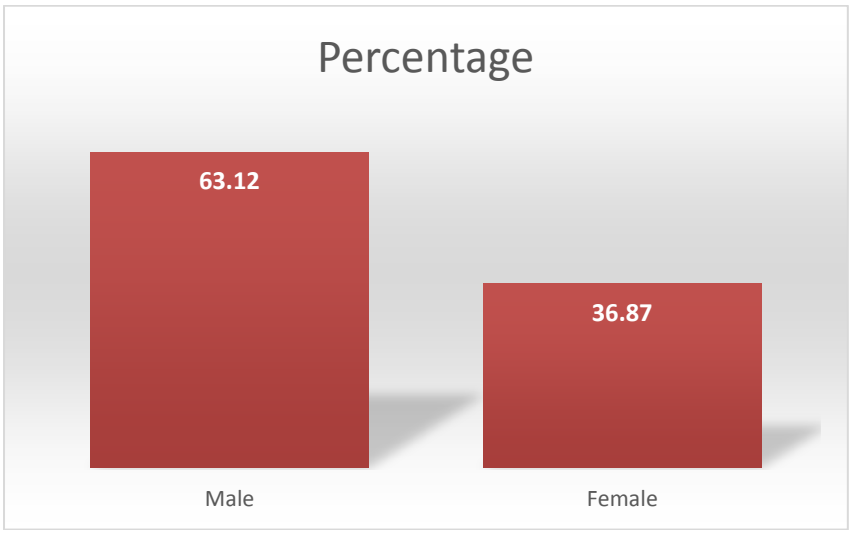
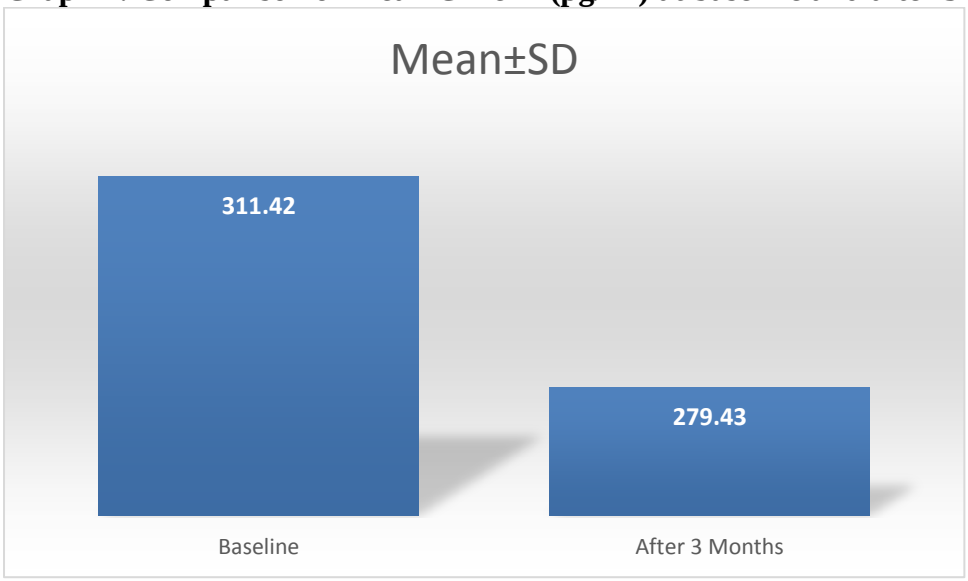


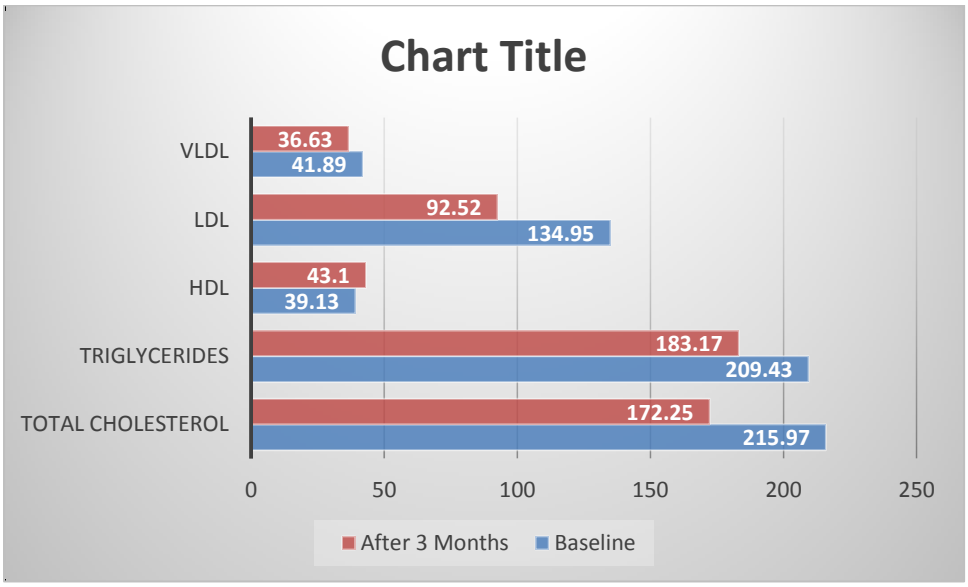
The **Graph** 1 reflects those 141 diabetic patients selected, 89 were male (63.12%) while 52 were female patients (36.87%). There was no statistically significant difference (0.231).

Graph 2: Comparison of Mean Ghrelin (pg/ml) at baseline and after 3 months:



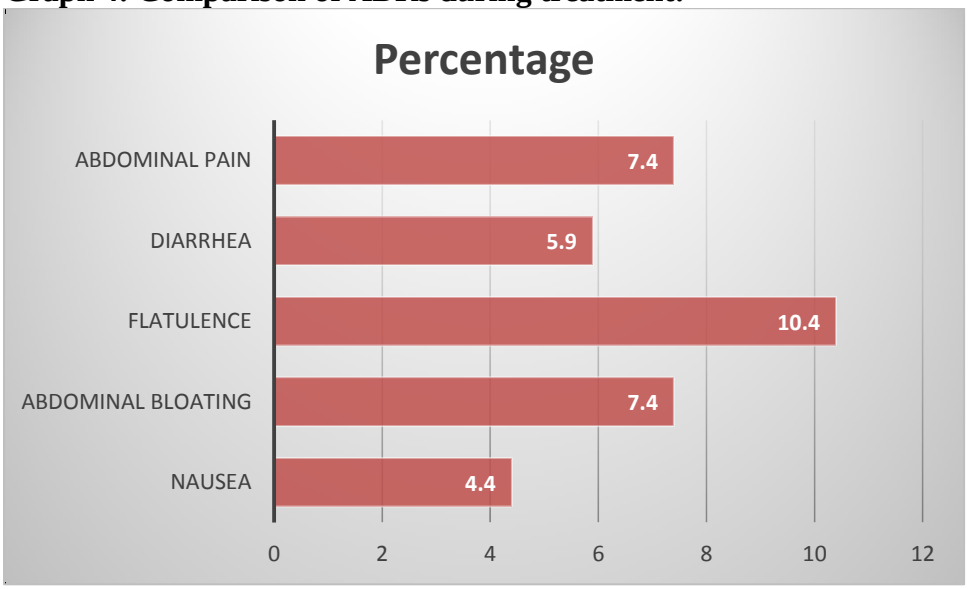
In **Graph 2**, the mean of Ghrelin (pg/ml) is 311.42±29.53 at baseline (pg/ml) and after 3rd month 279.43±19.34 (pg/ml). Serum Ghrelin within same group showed significant decreased over a period of 3 months ($p < 0.013$).

Graph 3: Comparison of Mean Total Cholesterol level at baseline and after 3 months:



In **Graph 3** the mean of Total Cholesterol level was 215.97±11.05 mg/dl at baseline, 172.52±10.38 mg/dl after 3rd month. Triglycerides levels within same group showed significant reduction over a period of 3 months ($p< 0.05$). The mean of HDL Cholesterol level was **39.13±3.89** mg/dl at baseline, **43.10±4.33** mg/dl after 3rd month. The mean of LDL Cholesterol level was 134.95±11.28 mg/dl at baseline, 92.52±10.91 mg/dl after 3rd month. The mean of VLDL Cholesterol level was 41.89±3.50 mg/dl at baseline, 36.63±3.63 mg/dl after 3rd month ($p< 0.05$).

Graph 4: Comparison of ADRs during treatment:



Most common adverse drug reaction reported were related to gastrointestinal disturbances. Gastrointestinal adverse drug reactions were Nausea in 3 (4.4%) patients, Abdominal blotting in 5 (7.4%) patients, Flatulence in 7 (10.4%) patients, Diarrhea in 4 (5.9%) patients, Abdominal pain in 5 (7.4%) patients.

Discussion

In this study, the mean of **Ghrelin (pg/ml)** is 311.42±29.53 at baseline (pg/ml) and after 3rd month 279.43±19.34 (pg/ml). Serum **Ghrelin** within same group showed significant decreased over a period of 3 months ($p< 0.013$). The observed ghrelin concentrations is accompanied by a decrease in insulin concentrations. [15] although the mechanism of this is unknown, it has been observed that insulin decreases and ghrelin increases in response to treatment with growth hormone. [16]

Similarly, insulin is known to inhibit growth hormone signaling via the growth hormone receptor, whereas ghrelin is the endogenous agonist of the growth hormone secretagogue receptor. [17] There is thus considerable cross-talk between the insulin and growth hormone signaling pathways. [18]

The present study revealed a significant decrease in serum ghrelin concentration in obese patients observed in metformin treated group. The explanation for such finding seems to be a little bit difficult, since conflicting reports are available regarding the influence of obesity, T2DM and even antidiabetic agents on serum ghrelin concentration. In study of Sharifi et al. (2013) concluded that ghrelin concentrations decrease prior to the onset of hyperglycemia and are more related to the fat pad of the body that was came in agreement of our study results. [19]

Other study reported an inverse relationship between ghrelin levels, BMI and waist circumference. [20] Furthermore, plasma concentrations of ghrelin inversely associated with food intake (acute effect) and obesity (chronic effect). [21]

According to previous studies, ghrelin is one of the factors that are involved in appetite regulation and acts as an appetite stimulating factor to pass starvation messages to brain. So its reduction in obesity can be considered as a defense mechanism of body to decrease appetite. [22] Another explanation we speculate that reduction of serum ghrelin concentration in metformin treated group due to direct effect of metformin treatment on synthesis and release of ghrelin from the stomach, i.e., lead to greater reduction of serum ghrelin in metformin treated group. [23]

A recent study by Gagnon et al. (2013) reported that metformin inhibits stomach proghrelin mRNA production and ghrelin secretion an effect mediated through AMPK phosphorylation. The results of the present study are relatively comparable with this finding and this could explain loss of weight in people treated with metformin. One of the possible pathways by which Metformin inhibits ghrelin secretion is through AKT phosphorylation, as AMPK activation has been shown to increase AKT activity. [24]

In our study the mean fasting blood glucose level at baseline was 96.54 mg/dl with SD of 8.53 mg/dl and after 3 months 71.62±7.35 mg/dl. These was statistically significant difference in mean Fasting Blood Glucose level at baseline versus after 3 months (p=0.025). The mean of PPBG level was 134.45±13.24mg/dl at baseline, followed by 103.63±15.43 mg/dl after 3rd month, PPBG levels within same group showed significant reduction over a period of 3 months. These was statistically significant difference in mean Post Prandial Blood Glucose level at baseline versus 3rd month of study period (P=0.017). While comparing with other studies metformin Suppress hepatic gluconeogenesis by activation of enzyme (AMP activated protein kinase) lowers plasma glucose levels by inhibiting gluconeogenesis in liver. [25]

In this study, the mean of HbA1c levels was 5.91±0.14% at baseline, 4.32±0.17 after 3rd month. HbA1c levels within same group showed significant reduction over a period of 3 months ($p< 0.0001$). Improving insulin sensitivity by increasing peripheral glucose uptake and utilization by up-regulating expression of glucose transporter GLUT-4 in muscle and skeletal muscle and decreasing the intestinal absorption of glucose. Metformin increases plasma levels of glucagon-like peptide 1 (GLP-1) inhibiting glucagon release from α cell, and produces a feeling of satiety. [26]

Advantages of metformin are non-hypoglycaemic weight loss promoting has potential to prevent macrovascular as well as microvascular complications of diabetes, no acceleration of β cell exhaustion/ failure in type 2 DM. Antihyperglycaemic efficacy (HbA1c reduction by 0.8–1.2%) equivalent to other oral drugs. [27]

In this study the mean of Total Cholesterol level was 215.97±11.05 mg/dl at baseline, 172.52±10.38 mg/dl after 3rd month. Triglycerides levels within same group showed significant reduction over a period of 3 months ($p< 0.05$). The mean of HDL Cholesterol level was 39.13±3.89 mg/dl at baseline, 43.10±4.33 mg/dl after 3rd month. The mean of LDL Cholesterol level was 134.95±11.28 mg/dl at baseline, 92.52±10.91 mg/dl after 3rd month. The mean of VLDL Cholesterol level was 41.89±3.50 mg/dl at baseline, 36.63±3.63 mg/dl after 3rd month ($p< 0.05$).

Moreover it was considered that adiponectin elevated insulin sensitivity via the stimulation of glucose utilization and increasing free fatty acid oxidation based on the adenosine monophosphate-activated protein kinase (AMPK) signal pathway, besides, PPAR-γ response element (PPRE) is present in the promoter region of adiponectin gene, suggesting an important role of PPAR-γ in regulating the synthesis of adiponectin gene. [28]

Conclusion

Metformin remains equivocal as a primary treatment for obesity and as a weight loss agent. It may serve as an adjunct therapy for those patients who are at high risk for metabolic complications or are experiencing other sequelae of obesity. Its mechanistic effects on central hypothalamic signaling, incretin secretion and alteration of the gut microbiome establish attractive areas of research in identifying new targets for obesity treatment. The long-term impacts of metformin use on aging and sarcopenia have yet to be elucidated, but they may also provide important insights into optimizing body composition with age. In the meantime, metformin will continue to serve as a mainstay treatment in the management of T2D and confer multiple metabolic effects beyond glycemic control.

Reference:

1. Patterson ZR, Khazall R, MackayH, Anisman H, Abizaid A. Central ghrelin signaling mediates the metabolic response of C57BL/6 male mice to chronic social defeat stress. Endocrinology 2013; 154:1080–91.
2. Theander-Carrillo C, Wiedmer P, Cettour-Rose P , Nogueiras R , Perez-Tilve D, Pfluger P, etal. Ghrelin action in the brain controls adipocyte metabolism. J Clin Invest. 2006; 116: 1983–93.
3. Healy JE, Bateman JL, Ostrom CE, Florant GL. Peripheral ghrelin stimulates feeding behavior and positive energy balance in a sciurid hibernator. Horm Behav. 2011; 59:512–9.

4. Foster-Schubert KE, et al. Acyl and total ghrelin are suppressed strongly by ingested proteins, weakly by lipids, and biphasically by carbohydrates. J Clin Endocrinol Metab. 2008; 93: 1971–9.
5. Gale SM, Castracane VD, Mantzoros CS. Energy homeostasis, obesity and eating disorders: recent advances in endocrinology. J Nutr. 2004; 134(2): 295-8.
6. American Diabetes Association. Standards of medical care in diabetes 2014. Diabetes Care. 2014; 37 (1): S14-80.
7. Liu W, Yang XJ: The Effect of Metformin on Adolescents with Type 1 Diabetes: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Int J Endocrinol 2016;3854071.
8. Pernicova I, Korbonits M. Metformin mode of action and clinical implications for diabetes and cancer. Nat Rev Endocrinol. 2014; 10: 143-56.
9. Maida A., Lamont BJ., Caox, Drucker DJ., Metformin regulates the incretin receptors axis via a pathway dependent on peroxisome proliferator-activated receptors alpha in mice. Diabetologia. 2011 54: 339-49.
10. Kahan S and Zvenyach T. Obesity as a disease: current policies and implications for the future. Curr Obes Rep 2016; 5: 291–297.
11. van Vliet-Ostaptchouk JV, Nuotio ML, Slagter SN, et al. The prevalence of metabolic syndrome and metabolically healthy obesity in Europe: a collaborative analysis of ten large cohort studies. BMC Endocr Disord 2014; 14: 9.
12. Jensen MD, Ryan DH, Apovian CM, et al. 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American college of cardiology/American heart association task force on practice guidelines and the obesity society. Circulation 2014; 129: S102–S138.
13. Zhu H and Wang L. Interpretation of 2016 American association of clinical endocrinologists (AACE) and American college of endocrinology (ACE) clinical practice guidelines for comprehensive medical care of patients with obesity. Chin J Diabetes 2017; 25: 10–13.
14. Bluher M. Are metabolically healthy obese individuals really healthy? Eur J Endocrinol 2014; 171: R209–R219.
15. Kujawska-Luczak M, Stankowiak-Kulpa H, Swora-Cwynar E, et al. Obesity - should we revise indications for treatment with metformin? Prz Menopauzalny 2014; 13: 115–121.
16. Apovian CM, Aronne LJ, Bessesen DH, et al. Pharmacological management of obesity: an endocrine society clinical practice guideline. Clin Endocrinol Metab 2015; 100: 342–362.
17. Sherafat-Kazemzadeh R, Yanovski SZ and Yanovski JA. Pharmacotherapy for childhood obesity: present and future prospects. Int J Obes (Lond) 2012; 37: 1–15.
18. McDonagh MS, Selph S, Ozpinar A, et al. Systematic review of the benefits and risks of metformin in treating obesity in children aged 18 years and younger. JAMA Pediatr 2014; 168: 178–184.
19. O'Connor EA, Evans CV, Burda BU, et al. Screening for obesity and intervention for weight management in children and adolescents: evidence report and systematic review for the US preventive services task force. JAMA 2017; 317: 2427–2444.
20. Graff SK, Mario FM, Ziegelmann P, et al. Effects of orlistat vs. metformin on weight lossrelated clinical variables in women with PCOS: systematic review and meta-analysis. Int J Clin Pract 2016; 70: 450–461.
21. Firouzjaei A, Li GC, Wang N, et al. Comparative evaluation of the therapeutic effect of metformin monotherapy with metformin and acupuncture combined therapy on weight loss and insulin sensitivity in diabetic patients. Nutr Diabetes 2016; 6.

22. Naderpoor N, Shorakae S, de Courten B, et al. Metformin and lifestyle modification in polycystic ovary syndrome: systematic review and meta-analysis. Hum Reprod Update 2015; 21: 560–574.
23. Mauras N, DelGiorno C, Hossain J, et al. Metformin use in children with obesity and normal glucose tolerance–effects on cardiovascular markers and intrahepatic fat. J Pediatr Endocrinol Metab 2012; 25: 33–40.
24. Sharifi F, Yamini M, Esmaeilzadeh A, et al. Acylated ghrelin and leptin concentrations in patients with type 2 diabetes mellitus, people with prediabetes and first degree relatives of patients with diabetes, a comparative study. J Diab Metab Disord. 2013; 12(1): 51.
25. Monti V, Carlson JJ, Hunt SC, et al. Relationship of ghrelin and leptin hormones with body mass index and waist circumference in a random sample of adults. J Am Diet Assoc. 2006; 106(6): 822-8.
26. Hansen TK, Dall R, Hosoda H, et al. Weight loss increases circulating levels of ghrelin in human obesity. Clin Endocrinol (Oxf). 2002; 56: 203-6.
27. Coll AP, Farooqi IS, O'Rahilly S. The hormonal control of food intake. Cell. 2007; 129: 251-262.
28. Leclerc GM, Leclerc GJ, Fu G, et al. AMPK-induced activation of Akt by AICAR is mediated by IGF-1R dependent and independent mechanisms in acute lymphoblastic leukemia. J Mol Signal. 2010; 5: 15-27.