JETROMI
Journal of Endocrinology, Tropical Medicine, and Infectiouse Disease

# Effect of Lifestyle Modification and Metformin on Fetuin-A and Transforming Growth Factor-ß (TGF- ß) in Metabolic Syndrome

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 ISSN: XXXX-XXXX e-ISSN: XXXX-XXXX DOI:XXX.XXX/XXXXX.X.X
Journal Homepage: https://talenta.usu.ac.id/jetromi

**Abstract.** Fetuin-A is a liver-synthesized protein that is secreted into serum. Transforming growth factor-β (TGF-β) is a polypeptide member of the TGF-β superfamily of cytokines. The purpose of this study is to evaluate the effects of lifestyle modification and metformin on fetuin-A and Transforming Growth Factor-ß (TGF- ß) in metabolic syndrome (MetS). Forty MetS subjects were randomly assigned to treatment with placebo (n=20) or metformin (n=20) in addition to lifestyle modification for 12 weeks. All 40 participants completed the study. After 12 weeks, both groups had significant reductions in weight, body mass index (BMI), waist circumference (WC), systolic blood pressure (SBP) and diastolic blood pressure (DBP) (all p<0.001). The placebo group also had significant improvement in fasting plasma glucose (FPG) and C-reactive protein (CRP) (p<0,001 ; p<0.05 respectively). Weight, BMI, WC, FPG, 2-hour postprandial glucose (2h-PPG), high density lipoprotein cholesterol (HDL-C), triglycerides (TG), fetuin-A and TGF- ß in the metformin group decreased significantly compared to the placebo group. Reduction of plasma fetuin-A was significantly associated with TG in the metformin group. Lifestyle modification and treatment with metformin for 12 weeks improved cardio-metabolic risk factors in MetS and reduced fetuin-A levels.

**Keyword:** Metabolic syndrome, lifestyle modification, fetuin-A, TGF- ß

***Abstrak.*** *Fetuin-A adalah protein yang disintesis hati yang disekresikan ke dalam serum. Transforming growth factor-β (TGF-β) adalah anggota polipeptida dari superfamili TGF-β sitokin. Tujuan dari penelitian ini adalah untuk mengevaluasi pengaruh modifikasi gaya hidup dan metformin pada fetuin-A dan Transforming Growth Factor-ß (TGF- ß) pada sindrom metabolik (MetS). Empat puluh subyek MetS secara acak ditugaskan untuk pengobatan dengan plasebo (n = 20) atau metformin (n = 20) di samping modifikasi gaya hidup selama 12 minggu. Semua 40 peserta menyelesaikan studi. Setelah 12 minggu, kedua kelompok mengalami penurunan berat badan yang signifikan, indeks massa tubuh (BMI), lingkar pinggang (WC), tekanan darah sistolik (SBP) dan tekanan darah diastolik (DBP) (semua p <0,001). Kelompok plasebo juga mengalami peningkatan yang signifikan dalam glukosa plasma puasa (FPG) dan protein C-reaktif (CRP) (masing-masing p <0,001; p <0,05). Berat badan, BMI, WC, FPG, glukosa postprandial 2 jam (2h-PPG), kolesterol lipoprotein densitas tinggi (HDL-C), trigliserida (TG), fetuin-A dan TGF-ß pada kelompok metformin menurun secara signifikan dibandingkan dengan kelompok plasebo. Pengurangan fetuin-A plasma secara bermakna dikaitkan dengan TG pada kelompok metformin. Modifikasi dan pengobatan gaya hidup dengan metformin selama 12 minggu meningkatkan faktor risiko kardio-metabolik dalam MetS dan mengurangi kadar fetuin-A.*

**Kata Kunci:** *Sindrom metabolik, modifikasi gaya hidup, fetuin-A, TGF- ß.*

Received [date month year] | Revised [date month year] | Accepted [xx Month xxxx]

## 1 Introduction

The Metabolic Syndrome (MetS) represents a combination of cardio-metabolic risk factor determinants including central adiposity, insulin resistance, glucose intolerance, dyslipidemia, non-alcoholic fatty liver disease (NAFLD) and hypertension. It is rapidly increasing in prevalence worldwide as a consequence of the obesity epidemic. As a result, MetS will have a considerable impact on the global incidence of cardiovascular disease and type 2 diabetes (T2DM) (Bruce et al., 2009). Insulin resistance is thought to be the primary underlying abnormality leading to MetS (Reaven, 1988)

Fetuin-A (also known as human protein alpha-2-Heremans-Schmid-glycoprotein, AHSG) and other circulating proteins have been shown to be involved in the regulation of insulin sensitivity. Fetuin-A is a liver-synthesized protein that is secreted into serum. It can bind the insulin receptor and inhibit insulin signaling in skeletal muscle and hepatocytes, inhibiting insulin

signal transduction and resulting in insulin resistance in the target tissues (Srinivaset al., 1993). In humans, higher levels of fetuin-A are associated with higher TG, low-density lipoprotein cholesterol (LDL-C), BMI, and insulin resistance (Stefanet al., 2006). Higher fetuin-A concentrations were associated with accumulation of visceral adipose tissue, a major component of the MetS (Ix et al., 2009). The link between fetuin-A, obesity, insulin resistance, NAFLD, and MetS in humans is less clear. Some studies in adults have reported significant associations between fetuin-A, NAFLD and insulin resistance (Mori et al., 2006). Most of these studies were cross-sectional and limited by many confounders. Longitudinal studies are preferable to clarify these metabolic relationships.

Transforming growth factor-β (TGF-β) is a polypeptide member of the TGF-β superfamily of cytokines. The TGF-β superfamily consists of TGF-β, activins, inhibins, growth differentiation factors, and bone morphogenetic proteins (BMPs). The TGF-β superfamily proteins share common sequences and motifs to exert their various biological actions, including cell growth, differentiation, proliferation, migration, adhesion, apoptosis, and extracellular matrix (ECM) production. Metabolic syndrome is mostly characterized as visceral fat obesity with multiple cardiovascular risk factors, including elevated blood pressure, hyperglycemia, and dyslipidemia. Therefore, an understanding of the molecular mechanism by which visceral obesity is promoted is essential for preventing cardiovascular events in individuals with MetS (Ken-ichiet al., 2011).

Lifestyle modifications (LSM) to address overweight, physical inactivity and an atherogenic diet have been recommended as a foundation for the management ofMetS(Eckelet al., 2005). However, LSM alone is often unable to achieve clinically meaningful weight loss (UKPDS, 1998).

Metformin, a biguanide oral antidiabetic agent, has been shown to reduce weight, hyperinsulinemia and hyperglycemia in adult patients with T2D. It is recommended as first-line pharmacotherapy in overweight and obese T2D patients (Sharoffet al., 2010). While metformin has been found to attenuate the insulin-sensitizing effect of exercise, it has been found to have beneficial effects on inhibition of platelet aggregation, antioxidant activity, weight reduction, lipid parameters (total cholesterol, HDL-C, LDL-C and TG) and arterial hypertension (Gluecket al., 2001, Wulffeléet al., 2004, Pasqualiet al., 2000). Metformin can be given safely to euglycemic patients, as it does not induce hypoglycemia (Linet al., 2000). Furthermore, in ob/ob mice, a model of hepatic steatosis, metformin reversed hepatomegaly, hepatic fat accumulation and ALT abnormalities by reducing hepatic tumor necrosis factor-α (TNF-α) expression (WHO, 2004).

The aim of this study was to assess the effect of LSM on cardio-metabolic risk factors, fetuin-A and TGf-ß levels with or without metformin in relation to improvement of insulin sensitivity in patients with the MetS.

## 2 Method

Study subjects who met the 2006 IDF definition of the metabolic syndrome were recruited from the nurse of H. Adam Malik Hospital in Medan, Indonesia. The criteria included central obesity (WC of ≥ 90 cm in men and ≥ 80 cm in women of Asian ethnicity) plus any 2 of the following 4 factors: elevated triglycerides (≥ 150 mg/dL) or specific treatment for this lipid abnormality, reduced HDL-C (< 40 mg/dL in men and < 50 mg/dL in women) or specific treatment for this lipid abnormality, elevated BP blood pressure (SBP ≥130 mmHg or DBP ≥ 85 mmHg) or treatment of previously diagnosed hypertension, and elevated FPG (≥ 100 mg/dL) or previously diagnosed type 2 diabetes (WHO, 2004, IDF, 2006). Exclusion criteria included smoking, known cardiovascular disease or any major illness, and use of medication that could affect laboratory test results. Forty subjects gave their full informed consent to participate and undergo LSM for 12 weeks. They were assigned randomly to treatment with either placebo or metformin. Each participant was advised to take one capsule three times a day after meal. For the placebo group, the capsule contained calcium gluconate 500 mg. For the metformin group, the capsule contained metformin 500 mg. No vitamins or other nutritional supplements were prescribed. Prior to initiation and during the study, all the participants discussed LSM including diet and physical activity with a trained health nurse. To facilitate behavior change, each participant received an instructional leaflet and a diary to record behavioral performance, diet, physical activity, WC and weight. Every week, all participants attended a follow-up meeting for confirmation of compliance and monitoring of any health and safety problems related to behavioral changes and treatment.

### 2.1 Anthropometric and body composition measurements

Baseline anthropometric measures were taken. The following BMI categories appropriate for Asians were used: underweight, BMI < 18.5 kg/m2 ; normal, 18.5 to 22.9 kg/m2; overweight, 23.0 to 24.9 kg/m2; obese class I, 25.0 to 29.9 kg/m2; obese class II BMI ≥ 30.0 kg/m2 (Misraet al., 2007). BMI was measured every week to assess the immediate effect of LSM.

### 2.2 Diet and exercise regimen

For 12 weeks, all subjects followed a weight maintenance diet (total calories per day divided into 55 to 60% carbohydrate, 15 to 20% protein and 20 to 25% fat) and moderate exercise in accordance with recommendations from the Endocrinology Association of Indonesia (Perkeni, 2011). All subjects were free living and consumed self-selected foods from a list of food replacements made according to their individual dietary habits. The dietitian reviewed the participants' diet on a weekly basis to ensure compliance.

The exercise program consisted of moderate aerobic exercise at least 3 times per week, with a minimum of 30 minutes for each session (Perkeni, 2011). Each session included 5 minutes of

warm-up, 20 minutes of main exercises, and 5 minutes of relaxation exercises. Each training session was supervised by a physiotherapist.

### 2.3 Blood Pressure and Blood Sample Analysis

Blood pressure was averaged from two measurements using a mercurial sphygmomanometer after a 10 minute rest. All subjects reported for blood sampling in the morning after an overnight fast. Blood samples were centrifuged for 15 minutes, after which plasma- and serum-containing tubes were stored at -20°C until analysis. Blood glucose was measured by photometer autoanalyzer Modular P800.Plasma HDL-C, LDL-C and TG were measured using ARCHITECT ci8200 (Abbott Diagnostics, USA). High-sensitivity CRP was measured by sensitive immunoassay using Immulite® 1000 Analyzer System (Siemens Healthcare, Germany). HbA1c measurement was done by high-performance liquid chromatography (HPLC) using D-10™ (Bio-Rad, USA). Homeostatic model assessment of insulin resistance (HOMA-IR) was computed using the formula:

$$HOMA\text{-}IR = FPG \times fasting\ serum\ insulin / 22.5$$

Where FPG is expressed in mmol/L and fasting serum insulin in mU/L. Fetuin-A determination was performed by human fetuin-A enzyme immunoassay and TGF-ß also by enzyme immunoassay.

## 3 Statistical Analysis

Data were presented as mean ± SD. Normality assumption of data from the placebo group and metformin group was evaluated and confirmed using Shapiro-Wilk normality test. Differences between and within groups were tested using the dependent t-test and independent sample t-test. Abnormal data were tested using Mann-Whitney U test, Wilcoxon testand Spearman's correlation coefficient test. Two-sided p-values of less than 0.05 were regarded as statistically significant. The data were analyzed using SPSS software.

## 4 Results

All 40 participants completed the study for 12 weeks. Analysis of baseline characteristics showed no significant differences in selected cardio-metabolic risk factors and fetuin-A (Table 1). After 12 weeks, both groups had reductions in weight, BMI, WC, SBP, and DBP. Reduction in CRP was also found in the placebo group; fetuin-A was reduced in the metformin group. Compared to placebo, weight, BMI, WC, FPG, 2h-PPG, HDL-C and TG had decreased significantly in the metformin group (Table 2).

**Table 1** Baseline Characteristic

| Characteristicts | Placebo group (n = 20) Mean (SD) | Metformin group (n = 20) Mean (SD) | P |
|---|---|---|---|
| Age, yr | 40.1 (5.78) | 42.7 (5.2) | 0.149 |
| Weight, kg | 77.6 (11.0) | 81.4 (14.6) | 0.354 |
| BMI, kg/m² | 32.1 (4.1) | 34.2 (5.6) | 0.180 |
| WC, cm | 95.7 (7.3) | 97.9 (11.5) | 0.449 |
| SBP, mmHg | 123.5 (11.4) | 127.0 (20.3) | 0.989 |
| DBP, mmHg | 82.2 (10.5) | 80.8 (11.0) | 0.495 |
| HDL-C, mg/dL | 46.4 (8.5) | 48.9 (16.4) | 0.968 |
| TG, mg/dL | 147.5 (30.6) | 152.3 (66.9) | 0.799 |
| FPG, mg/dL | 83.4 (10.6) | 84.9 (8.9) | 0.341 |
| 2h-PPG, mg/dL | 114.9 (35.4) | 105.1 (22.4) | 0.602 |
| HOMA-IR | 1.13 (0.94) | 1.0 (0.6) | 0.700 |
| CRP, mg/dL | 3.6 (2.5) | 3.9 (2.4) | 0.699 |
| Fetuin-A, µg/mL | 461.4 (74.6) | 459.0 (62.8) | 0.911 |
| TGF-ß1, pg/mL | 47479,34 (6942.01) | 45272.06 (3711.22) | 0.380 |

**Table 2** Change in selected cardio-metabolic risk factors from baseline and at 12 weeks, N= 40

| Parameter | Placebo group (n=20) | | | | Metformin group (n=20) | | | | P |
|---|---|---|---|---|---|---|---|---|---|
| | Baseline | 12 weeks | Defference | P | Baseline | 12 weeks | Defference | P | |
| Weight (SD), kg | 77.6 (11.0) | 75.2 (10.8) | -2.3 | 0.001 ** | 81.4 (14.6) | 77.4 (14.5) | -3.9 | 0.001 ** | 0.001 ** |
| BMI (SD), kg/m² | 32.1 (4.1) | 30.9 (4.1) | -1.1 | 0.001 ** | 342 (5.6) | 32.4 (5.6) | -1.8 | 0.001 ** | 0.002 ** |
| WC (SD), cm | 95.7 (7.3) | 89.9 (7.5) | -5.8 | 0.001 ** | 97.9 (11.5) | 91.8 (10.7) | -6.2 | 0.001 ** | 0.047 * |
| SBP (SD), mmHg | 123.5 (11.4) | 114.0 (8.2) | -9.5 | 0.007 ** | 127.0 (20.3) | 112.8 (8.5) | -14.3 | 0.001 ** | 0.160 |
| DBP (SD), mmHg | 82.2 (10.5) | 69.0 (5.5) | -31.8 | 0.001 ** | 80.6 (11.0) | 67.5 (7.2) | -13.3 | 0.001 ** | 0.089 |
| HDL-C (SD), mg/dL | 46.4 (8.5) | 45.3 (10.0) | -1.1 | 0.628 | 489 (16.4) | 45.5 (9.8) | -2.4 | 0.653 | 0.043 * |
| TG (SD), mg/dL | 147.5 (50.5) | 153.3 (67.9) | 5.8 | 0.634 | 152.3 (66.9) | 149.0 (102.4) | -3.3 | 0.147 | 0.045 |
| FPG (SD), mg/dL | 83.4 (10.6) | 91.7 (20.6) | 8.3 | 0.001 ** | 84.9 (8.9) | 87.7 (10.7) | 2.8 | 0.305 | 0.013 * |
| 2h-PPG (SD), mg/dL | 114.9 (35.4) | 112.5 (37.7) | -2.5 | 0.717 | 105 (22.4) | 102.3 (19.3) | -2.8 | 0.491 | 0.007 ** |
| HOMA-IR (SD) | 1.13 (0.94) | 1.68 (0.25) | -0.5 | 0.610 | 1.03 (0.61) | 1.03 (0.4) | 0 | 0.956 | 1.000 |
| CRP (SD), mg/dL | 3.6 (2.5) | 3.0 (2.2) | -0.6 | 0.048 * | 3.9 (2.4) | 3.5 (1.9) | -0.6 | 0.327 | 0.133 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Fetuin-A (SD), µg/mL | 461.4 (74.6) | 42.6 (84.8) | -34.8 | 0.158 | 459.0 (62.8) | 398.1( 101.4) | -610 | 0.005 ** | 0.477 |
| TGF-ß1 (SD), pg/mL | 47479.34(6942.01) | 47346.69 (6654.11) | -132.65 | 1.000 | 45272.06 (3711.22) | 4458.71 (8232.4) | -1013.35 | 0,661 | 0.353 |

## 5 Discussion

Obesity is the most common risk factor for the MetS and NAFLD (Reinehet al., 2008). As suggested by novel evidence, hepatocytes from fatty liver release factors called hepatokines (e.g., fetuin-A, sex hormone-binding globulin) into the circulation that are directly involved in local pathogenesis, systemic inflammation and hepatic insulin resistance (Reavenet al., 1988). Thefetuin-A levels of obese children are apparently similar to those of adults (Ohkawaraet al., 2007). A study by Mori and colleaguesdid not find a significant association between fetuin-A and insulin resistance in type 2 diabetic subjects (Mori et al., 2006).In contrast, other studies demonstrated a relationship between fetuin-A and insulin resistance in adults without T2D (Stefanet al., 2006).Fetuin-A concentrations decreased significantly in obese children after substantial weight loss after 1 year, but was apparently unchanged in those who did not lose weight (Ohkawaraet al., 2007). Our study found that fetuin-A decreased significantly with LSM and metformin treatment for 12 weeks, possibly associated with weight reduction.

A recent systematic review showed a dose-response effect of aerobic exercise on visceral adiposity, but the ability of exercise to reduce visceral adipose tissue was less robust in those with metabolic disorders (Wing et al., 2001). It remains unclear if the same dose-response effect on central adiposity will also be seen in those with MetS. Nevertheless, during weight maintenance, regular exercise still has an important role in abdominal fat loss and may help prevent weight regain in those who have successfully lost weight (Ross et al., 2004). However, even in the absence of weight loss, exercise has been shown toreduce visceral adipose tissue (Stone et al., 2005). Our study demonstrated that weight, BMI and WC decreased significantly in the course of 12 weeks of LSM in both groups.

The National Cholesterol Education Program Adult Treatment Panel III (NCEP:ATP-III) recommends LDL-C reduction as the primary treatment goal for CVD risk reduction. Therapeutic lifestyle changes, particularly improvement in physical activity and weight management, need to be instituted in those individuals with the MetS to address elevated TG and low HDL-C (Kelley, 2007). Although aerobic exercise training has generally been shown to increase HDL-C and decrease TG, its effects on LDL-C has been mixed (Kodamaet al., 2007, Stefanicket al., 1998). Beneficial effects of exercise training on lipids and lipoproteins may have additional impact when combined with dietary modification and weight loss (Wheltonet al., 2002). Our study demonstrated HDL-C and TG did not decrease significantly in the course of 12 weeks of LSM on both groups.

A recent meta-analysis of randomized controlled trials studying the effect of aerobic exercise on BP showed reduction in systolic and diastolic BP by approximately 3.8 and 2.6 mmHg, respectively (Bacon et al., 2004). Although the effect of aerobic exercise on blood pressure is small and not consistently observed in all studies, there may be additional benefit when combined with dietary modification and/or weight loss (Cornieret al., 2008). Our study demonstrated significant reductions in systolic and diastolic BP in both groups in the course of 12 weeks of LSM.

Insulin resistance is another core component of MetS that requires careful attention. Weight loss and LSM can lead to clinically meaningful improvements in insulin sensitivity and should be considered the primary therapeutic options for treating insulin resistance. The difficulties and frustrations associated with weight loss efforts and LSM have driven the demand for using pharmaceutical agents that target insulin resistance in a more direct fashion. The exact role for these agents is less clear. Several randomized controlled trials have shown that agents targeting insulin resistance can help prevent the progression to T2D in individuals with impaired glucose tolerance (IGT). These studies did not directly target individuals with the MetS. It is unclear whether these agents truly prevent progression to T2D or simply treat glucose intolerance or mild hyperglycemia. In addition, studies have not clearly shown whether these agents improve cardiovascular outcomes. As with weight loss medications, the goals for the use of agents targeting insulin resistance must be clear (Hennigeet al., 2008). Our study demonstrated HOMA-IR did not decrease significantly in both groups.

Fetuin-A induces low grade inflammation, which is also associated with MetS and an atherogenic lipid profile (Reinehret al., 2008, Ridker, 2001). Inflammation assessed by elevated CRP measurements has been linked to excess cardiovascular risk and MetS (Ridkeret al., 2003, Gonzálezet al., 2006). CRP is a general marker of inflammation, making it suitable to assess in individuals with the metabolic syndrome. Elevated levels of CRP are associated with increased WC, insulin resistance, BMI and hyperglycemia; and in the presence of more components of the MetS (Deepaet al., 2006, Guldikenet al., 2007, Bahiaet al., 2006, Gonzálezet al., 2006, Clearfield, 2005). Because MetS has been linked with a greater chance of future cardiovascular events, CRP levels may be an important independent predictor of unfavorable outcomes in those already with MetS (Van Dielenet al., 2004). There are, however, no currently recommended direct therapies targeting inflammation. LSM and weight loss result in decreased CRP concentrations, as does the treatment of the other associated comorbidities such as dyslipidemia, elevated blood pressure, insulin resistance and hyperglycemia (Devaraj, 2007, Knowleet al., 2002). Our study observed CRP decreased significantly only in the placebo group.

It has not been determined how the Pro 10 variant form of the TGF-β1 protein is linked to visceral adiposity and elevated levels of circulating insulin, there is a possibility that TGF-β1 is involved in the insulin resistance with obesity. Since macrophage infiltration into adipose tissue causes insulin resistance and since coculture experiments with human adipocytes and

macrophages have shown that downstream effectors of TGF-β such as PAI-1, collagen VI, and phosphorylated Smad were increased in both macrophages and adipocytes, TGF-β has the potential for increasing insulin resistance (Ken-ichi, 2011).

In experimental animal studies, Samad et al. reported enhancement of gene and protein expression of TGF-β1 in two strains of genetically obese mice (ob/oband db/db) compared with that in lean mice(Samad, 1997) and Raju et al. showed that an obese state increases levels of TGF-β1 but not TGF-β2 in platelets of Zucker rats, recognized as an experimental model of Mets (Raju, 2006). Moreover, Sciarretta et al. showed that serum levels of inflammatory markers, including Creactiveprotein, tumor necrosis factor-alpha and TGF-β, in hypertensive patients with MetS were significantly higher than those in patients without MetS (Ken-ichi, 2011).

## 6 Conclusion

LSM decreased CRP, human fetuin-A concentrations, TGF-ß and selected cardio-metabolic risk factors in this 12-week study. These findings raise the possibility that fetuin-A may directly promote the MetS phenotype in humans and there is possibility that TGF-ß is involved in the insulin resistance with obesity. The selected cardio-metabolic factors significantly improved with metformin to the same degree as with the LSM. Longitudinal and larger scale studies are needed to evaluate the direction of the observed associations, the regulatory factors that alter serum fetuin-A concentrations, its effects on cardiovascular events, and the long-term effects of metformin on selected cardio-metabolic risk factors.

## Acknowledgments

The authors gratefully acknowledge that the present research is supported by Ministry of Research and Technology and the Higher Education Republic of Indonesia, Research and Community Service, Universitas Sumatera Utara.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper

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