**MicroRNAs modulation and clinical outcomes at 1 year of follow-up after excision of subcutaneous abdominal fat in overweight patients with pre-diabetes treated with metformin vs. placebo**

Celestino Sardu, MD, MSc, PhD1\*; Maria Consiglia Trotta, MD2; Gorizio Pieretti, MD3; Gianluca Gatta, MD4 ; Giuseppe Ferrara, MD3; Giovanni Francesco Nicoletti, MD3; Michele D’ Amico, MD2; Giuseppe Paolisso, MD1; Raffaele Marfella, MD, PhD1.

1.Department of Medical, Surgical, Neurological, Metabolic and Aging Sciences, University of Campania “Luigi Vanvitelli”, Naples, Italy; 2. Department of Precision Medicine, University of Campania “Luigi Vanvitelli”, Naples, Italy; 3. Department of Plastic Surgery, University of Campania “Luigi Vanvitelli”, Naples, Italy; 4. Department of Radiology, University of Campania “Luigi Vanvitelli”, Naples, Italy.

**Running title:** microRNAs, overweight, pre-diabetes, sirtuins, microRNAs and cardiac performance.

**Clinical research trial number:** NCT03439592.

**Word count:** 4695.

**\*Corresponding author:**

Celestino Sardu, MD, MSc, PhD

Piazza Miraglia, 2; 80138, Naples. Italy.

Telephone: +39 0815665110;

fax: +39 0815095303.

email: drsarducele@gmail.com

**METHODS**

**Diet therapy after abdominoplasty intervention**

The enrolled patients (normoglycemics vs. prediabetics, and prediabetics treated by metformin vs. placebo) after the intervention of abdominoplasty received the recommended diet therapy. For all the study population we chosen a fixed diet therapy with a recommended daily caloric intake. Moreover, the recommended composition of the dietary regimen was 55% carbohydrates, 30% lipid, and 15% protein as previously reported (1). The mean recommended daily caloric intake was 1300 kcal, ranging from 1250 to 1350 kcal (1).

**Clinical visits, data collection and analysis**

Study population was evaluated during clinical visits 10 days after clinical discharge, and after 6th and 12th month by the treating physician, by telephonic interviews, hospital admissions, and discharge schedules (2). Thus, at follow up visits, physicians (Ce. S, R. M) blinded to study protocol evaluated the clinical status of each patients, and they performed physical examination with collection of vital signs, and review of adverse events. To date, they evaluated the adherence to diet therapy, and they assessed body weight, body mass index (BMI) and any clinical symptom. During the clinical evaluations investigators performed a fasting blood (at least 12 h from last meal) for biochemical peripheral blood assay evaluation at every visit. The peripheral blood at baseline and at follow-up end was used to evaluate microRNAs (miRs) expression in the study population.

Authors (MC. T, M. DA) collected the data prospectively from electronic medical records (EMR), used in clinical setting at participants’ Institutions. Therefore, we used electronic systems for data capture, collection and monitoring, with on-site and real timing data entry. However, the patients’ files were collected in each participating Institution, and then analyzed. Finally, from these data we collected and analyzed clinical characteristics at baseline, inflammatory burden, sirtuin-1 (SIRT1) and microRNAs (miRs) expression at baseline and at follow-up of 12 months. However, the study investigators reported all the events with the potential to be adjudicated as one of the predefined study end points, regardless of the opinion of the investigator. In the case of identification of a suspected unreported event by a reviewer, we asked to the reviewer to make a note back to the investigator.

**Anthropometrics Parameters**

In the study population we evaluated for each patient the height and the weight, and body mass index (BMI) calculated as weight in kilograms divided by the square of height in meters (1). In addition, we calculated the Waist hip ratio (WHR) as waist circumference in centimeters divided by hip circumference in centimeters, and as index of central obesity (1). Finally, in all obese patients we evaluated insulin blood levels, and the homeostasis model for the assessment of insulin resistance (HOMA-IR), (1).

**Clinical and Laboratory Parameters**

For each enrolled patients at baseline and for all follow-up duration, laboratory assessment consisted of a complete blood count, blood chemical analysis, coagulation testing, evaluation of liver and renal function, and measures of electrolytes, C-reactive protein. However, authors collected venous blood in EDTA-coated tubes during hospitalization before abdominoplasty intervention, and at follow-up of 12 months.

**Sample size calculation and data collection**

For this study we calculated a sample size with 25 participants for each group, with estimated 80% power to detect a change of 0.015 between the mean reported study outcomes of the metformin-treated and placebo-treated groups of patients with pre-diabetes, at a 5% level of significance. A 20% Loss due to early withdrawals and/or non-evaluable measurements was assumed and, combined with the effect of stratification on analysis, resulted in the requirement to recruit at last 20 patients per treatment group. The randomization technique followed a computing generating code in a proportion of 2:1.

**RESULTS**

**1. Clinical characteristics of obese pre-diabetics treated by hypocaloric diet added to metformin at 12 months of follow up vs. baseline.**

At 12 months follow up as compared to baseline, the 28 **obese patients with pre-diabetes treated by hypocaloric diet added to metformin** experienced a statistical significant reduction of BMI (31.2±0.5 vs. 33.9±2.8 Kg/m2; p<0.05), WHR (0.78±0.003 vs. 0.91±0.008; p<0.05), HOMA-IR (4.1±0.28 vs. 5.1±0.72, p<0.05), glucose blood values (5.45± 0.13 vs. 6.64± 0.14 mmol/L, p<0.05), and an increase of insulin blood values (22.9±1.6 vs. 19.8±1.9 µU/ml). **Supplementary table 1**. These patients showed a statistical significant reduction of cholesterol values (4.04±0.76 vs. 4.49±0.87 mmol/L), and triglycerides values (1.66±0.72 vs. 1.89±0.44 mmol/L, p<0.05). **Supplementary table 1**. In these patients the metformin therapy added to hypocaloric diet induced a statistical significant reduction of miR-195, and of miR-27 (104.63±25.41 vs. 198.82±48.99; 62.53±29.71 vs. 116.89±40.31, p<0.05) respectively, and of all inflammatory /oxidative stress markers. **Supplementary table 1, and supplementary figure 1.**

Finally, the metformin therapy induced the significant reduction of Intima Media wall Tickness (IMT, 0.85±0.12 vs. 1.02±0.15 mm, p <0.05), myocardial performance index (MPI, 0.35±0.03 vs. 0.59±0.03, p <0.05), left ventricle mass (LV, 131.8±21.8 vs. 217.4±32.65 RAMSg, p <0.05) and LV mass indexed for body surface area (LV mass/BSA, 56.13±16.18 vs. 94.11±22.13 g/m2, p <0.05), and for height (LV mass/h, 46.63±13.24 vs. 70.11±17.45 m2, p<0.05), and of other morphologic parameters of echocardiography as reported in supplementary table 1.

**2. Obese pre-diabetics treated by hypocaloric diet added to placebo at baseline vs. 12 months of**

**follow up.**

The 27 obese patients with pre-diabetes treated by hypocaloric diet added to placebo at 12 months of follow up as compared to baseline experienced a significant reduction of cholesterol (4.15±0.90 vs. 4.52±0.88 mmol/L, p value <0.05), and of triglycerides blood values (1.70±1.24 vs. 1.86±0.55 mmol/L, p value <0.05). **Supplementary** **table 2**.

**3. Obese normoglycemics treated by hypocaloric diet at baseline vs. 12 months of**

**follow up.**

The 28obese normo-glycemics patients (NG) experienced at 12 months of follow up vs. baseline condition a significant reduction of cholesterol blood levels (4.13±0.79 vs. 4.56±0.91 mmol/L, p value <0.05), of miR-195 (34.51±12.26 vs. 62.18±8.31, p<0.05) and miR-27 (39.48±32.56 vs. 93.46±43.76, p<0.05) and of all inflammatory /oxidative stress markers. **Supplementary table 3, and supplementary figure 1.**

In addition, these patients experienced at 12 months of follow up vs. baseline condition a significant reduction of septum thickness (10.1±1.8 vs. 13.2±2.3 mm, p<0.05), posterior wall thickness (8.4±1.2 vs. 11±1.2 mm, p<0.05), MPI (0.36±0.04 vs. 0.57±0.03, p<0.05), LV mass (126.4±21.3 vs. 201.6±39.3 g, p<0.05), LV mass/BSA (56.59±13.83 vs. 92.08±20.87 g/m2, p<0.05), LV mass/h (44.73±11.28 vs. 69.15±17.10 m2, p<0.05). **Supplementary table 3.**

**DISCUSSION**

Obese patients with pre-diabetes vs. normoglycemics over-expressed inflammatory/oxidative stress molecules at level of adipose tissue and of peripheral blood. This pro-inflammatory pattern was linked to different baseline expression of sirtuin-1( SIRT1), miR-195 and miR-27 al level of adipose tissue in obese patients with pre-diabetes vs. normoglycemics. Notably, this trend was confirmed by analysis of peripheral blood samples for the evaluation of circulating inflammatory/oxidative stress molecules, miR-195 and miR-27. Intriguingly, metformin therapy vs. placebo added to hypocaloric diet, significantly reduced the inflammatory/oxidative stress burden, with significant down regulation of miR-195 and miR-27. This study result has been previously reported in animal models and in cellular lines of induced obesity and diabetes (3, 4, 6). Intriguingly, metformin therapy induced these molecular and epigenetic effects, and significant modifications of intima-media thickness (IMT), left ventricle mass (LVM), and myocardial performance index (MPI), (p<0.05). However, we might speculate that the metformin therapy by the regulation of the glucose homeostasis and insulin resistance, might play a relevant anti-inflammatory/oxidative stress effect, linked to the significant down regulation of miR-195 and miR-27. Finally, all these metabolic, molecular, cellular and epigenetic effects induced by metformin therapy, caused a significant reduction of IMT, LVM and MPI.

To date, metformin is a regulator of multiple and complex systems at the molecular, cellular and epigenetic levels, affecting metabolism and inflammation, and which might also affect the function of the human cardiovascular system (1, 2). As first, we might speculate that all these metabolic and inflammatory/oxidative stress pathways are linked to each other (1). Thus, it is not surprisingly that hyperglycemia and insulin resistance could cause an over-expression of inflammatory/oxidative stress molecules, with reduction of SIRT1 of adipose tissue (1). To date, these pathway could activate and promote intra-cellular signaling that interfere with atherosclerotic processes via vascular adaptive response to changes in flow, wall tension, or lumen diameter (1, 2). Subsequently, these processes could cause an activation of smooth muscle cell hyperplasia and fibrocellular hypertrophy, with medial hypertrophy and increase in IMT and myocardial mass as evidenced by higher values of LVM (1, 2). On other hand, metformin therapy could control and reduce these pathways, causing a significant reduction of IMT, LVM and MPI as observed in metformin vs. placebo arm of treatment for obese patients with pre-diabetes. Furthermore, these effects observed in obese patients with pre-diabetes, were not evidenced in absence of metformin therapy (placebo arm of treatment). Thus, we might speculate that these effects induced by metformin therapy might cause a significant reduction of miR-195 and miR-27. Moreover, miR-195 and miR-27 might be involved in a cross talking between inflammation/oxidative stress and glucose homeostasis/insulin resistance, and then implied in the regulation of multiple cellular adaptive processes as atherosclerosis, cellular hypertrophy, and hyperplasia (3-6). Notably, we would remark that these anti-inflammatory/oxidative effects, and the down regulation of miR-195 and miR-27 were not seen in obese with pre-diabetes in the placebo arm of treatment. Thus, at 12 months of follow-up as compared to baseline condition, these patients did not experience significant reduction of inflammatory/oxidative stress markers, they did not show significant reduction of miR-195 and miR-27, and they did not show the significant reduction of IMT, LVM and MPI. On the contrary, all these affects could be seen in obese normoglycemics treated with hypocaloric diet therapy via the significant down regulation of miR-195 and miR-27. Furthermore, taken together our study results might suggest that hyperglycemia and insulin resistance in obese with pre-diabetes could result in the over expression of inflammatory/oxidative stress markers and of circulating miR-195 and miR-27. Notably and clinically relevant, the metformin therapy could reduce the metabolic and inflammatory/oxidative distress in obese with pre-diabetes (1), via the significant reduction of circulating miR-195 and miR-27 at 12 months of follow-up. Finally, in our study we showed that in obese patients with pre-diabetes the metformin reduced the expression of circulating miR-195 and miR-27, leading to the significant reduction of IMT, LVM and MPI. However, the metformin showed metabolic and anti inflammatory/oxidative effects, and was a down regulator of circulating miR-195 and miR-27. To date, we might say that in obese patients with pre-diabetes miR-195 and miR-27 could be evaluated as markers and epigenetic regulators of multiple cardiovascular adaptive processes, implied in IMT, LVM and MPI. Therefore, our study provides evidences that have not been fully elucidated before. Indeed, it is well known that dietary interventions could induce a significant modulation of miRs (7). Notably, the miRs could be expressed at level of tissues and peripheral blood, because they are produced in tissues and relapsed in emetic current (7). Furthermore, in obese patients before and after dietary interventions, and surgical interventions, there is a significant correlation between the adipose tissue expression of miRs, and the circulating values of miRs (7). However, the evaluation of the circulating miRs is relevant, because it represents a valid opportunity and a non invasive strategy for diagnosis of clinical status, and to monitor the response to therapeutic approach (in our case the hypocaloric diet and the metfromin therapy) in a selected population of patients. In this setting, we evidenced for miR-195 and miR-27 a relevant regulative role in glucose homeostasis/insulin resistance, inflammatory/oxidative stress pathways, and in cardiovascular adaptive processes as evaluated by IMT, LVM and MPI.

**Conclusions**

We might conclude that miR-195 and miR-27 might be used as markers of these adaptive cardiovascular processes, and to monitor at follow-up the effects of hypocaloric diet and metformin therapy in obese patients with pre-diabetes. Finally, we might speculate that in the future specific treatments to target miR-195 and miR-27 expression at level of adipose tissue and in cardiovascular system, could be used to reduce the adaptive cardiovascular processes via the modification of IMT, LVM and MPI, and to improve clinical prognosis in obese normoglycemics and in obese patients with pre-diabetes.

**References**

1. Sardu C, Pieretti G, D'Onofrio N, et al. Inflammatory Cytokines and SIRT1 Levels in Subcutaneous Abdominal Fat: Relationship With Cardiac Performance in Overweight Pre-diabetics Patients. Front Physiol 2018;9:1030.
2. Mohapradeep Mohan, Shaween Al-Talabany, Angela McKinnie, et al. A randomized controlled trial of metformin on left ventricular hypertrophy in patients with coronary artery disease without diabetes: the MET-REMODEL trial. European Heart Journal 2019; 40 (1): 3409–3417.
3. Zheng, D.; Ma, J.; Yu, Y. et al. Silencing of miR-195 reduces diabetic cardiomyopathy in C57BL/6 mice. Diabetologia 2015, 58, 1949–1958.
4. Ahmed FW, Bakhashab S, Bastaman IT, Crossland RE, Glanville M, Weaver JU. Anti-Angiogenic miR-222, miR-195, and miR-21a Plasma Levels in T1DM Are Improved by Metformin Therapy, Thus Elucidating Its Cardioprotective Effect: The MERIT Study. Int J Mol Sci 2018;19(10):3242.
5. Lin Q, Gao Z, Alarcon RM, Ye J, Yun Z. A role of miR-27 in the regulation of adipogenesis. FEBS J 2009;276(8):2348-58.
6. Karolina DS, Tavintharan S, Armugam A, et al. Circulating miRNA profiles in patients with metabolic syndrome. J Clin Endocrinol Metab 2012;97(12):E2271-6.
7. Catanzaro G, Filardi T, Sabato C, et al. Tissue and circulating microRNAs as biomarkers of response to obesity treatment strategies. J Endocrinol Invest 2020 Oct 28. doi: 10.1007/s40618-020-01453-9. In press.

**Supplementary figure and tables’ legend**

**Supplementary figure1.** In this figure the microRNAs (miRs) values ± standard deviation for obese with pre-diabetes under metformin therapy (PDM Met in blue color), obese with pre-diabetes under placebo therapy (PDM pcb in red colour), and obese with normoglycemia (NG in green colour). For each couple of columns for PDM Met, PDM pcb, and NG the first column (on left) is for baseline values, the second column (on the right) is for follow-up values. In the upper part of figure the miR 195 values; in inferior part the miR 27 values. A.U. is for arbitrary units.

miR 195: \* is for p<0.05 comparing baseline vs. follow-up for PDM Met; \*\*is for p<0.05 comparing baseline vs. follow-up for NG.

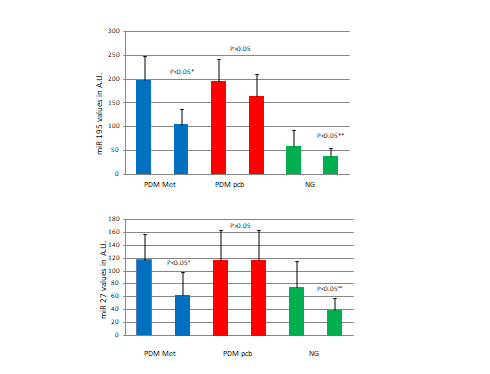
miR 27: ° is for p<0.05 comparing baseline vs. follow-up for PDM Met; °° is for p<0.05 comparing baseline vs. follow-up for NG.

**Supplementary table 1. Clinical characteristics of obese pre-diabetics (group 1) at baseline vs. 12 months of follow up.** In this table are reported the study variables of group 1, 20 pre-diabetics obese patients treated by hypocaloric diet added to metformin. In the study variables (first column to the left) are reported clinical variables, biohumoral markers, echocardiographic and parameters for each group of patients. . We reported fasting glucose and lipid values. Abbreviations: CRP, C reactive protein; g, grams; m, meters; h height; HOMA\_IR, homeostasis model for the assessment of insulin resistance; IL6, interleukine 6; LAD, left atrium diameter; LV, left ventricle; LVEF, left ventricle ejection fraction; LVEDd, left ventricle end diastolic diameter; LVEDs, left ventricle end systolic diameter; MPI, myocardium performance index; TNFα tumor necrosis factor alpha; WHR, waist hip ratio. The symbol ‡ is indicating a p value <0.05 by the comparison of group 1 at baseline vs. group 1 at 12 months follow up

**Supplementary table 2. Clinical characteristics of obese pre-diabetics (group 2) at baseline vs. 12 months of follow up.** In this table are reported the study variables of group 2, 20 pre-diabetics obese patients treated by hypocaloric diet added to placebo. In the study variables (first column to the left) are reported clinical variables, biohumoral markers, echocardiographic and parameters for each group of patients. We reported fasting glucose and lipid values. Abbreviations: CRP, C reactive protein; g, grams; m, meters; h height; HOMA\_IR, homeostasis model for the assessment of insulin resistance; IL6, interleukine 6; LAD, left atrium diameter; LV, left ventricle; LVEF, left ventricle ejection fraction; LVEDd, left ventricle end diastolic diameter; LVEDs, left ventricle end systolic diameter; MPI, myocardium performance index; TNFα tumor necrosis factor alpha; WHR, waist hip ratio. The symbol ǂ is indicating the p value < 0.05 by the comparison of group 2 at baseline vs. group 2 at 12 months follow up.

**Supplementary table 3. Clinical characteristics of obese normoglycemics (group 3) at baseline vs. 12 months of follow up.** In this table are reported the study variables of group 3, 18 normoglycemics obese patients treated by hypocaloric diet. In the study variables (first column to the left) are reported clinical variables, biohumoral markers, echocardiographic and parameters for each group of patients. . We reported fasting glucose and lipid values. Abbreviations: CRP, C reactive protein; g, grams; m, meters; h height; HOMA\_IR, homeostasis model for the assessment of insulin resistance; IL6, interleukine 6; LAD, left atrium diameter; LV, left ventricle; LVEF, left ventricle ejection fraction; LVEDd, left ventricle end diastolic diameter; LVEDs, left ventricle end systolic diameter; MPI, myocardium performance index; TNFα tumor necrosis factor alpha; WHR, waist hip ratio. The symbol symbol ° is indicating the p value < 0.05 by the comparison of group 3 at baseline vs. group 3 at 12 months follow up.

**Supplementary figure 1.**



**Supplementary table 1. Obese pre-diabetics treated by hypocaloric diet added to metformin (Pre-DM plus metformin) at baseline vs. 12 months of follow up.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Study variables** | **Pre-DM plus MET patients (n 28) at baseline** | **Pre-DM plus MET patients (n 28) at 12 months of follow up** | **P value** |
| ***Clinical variables*** |  |  |  |
| BMI (Kg/m2) | 33.9±2.8 | 31.2±0.5 | <0.05‡ |
| Systolic arterial pressure (mmHg) | 136±13.2 | 129±2.3 | / |
| Diastolic arterial pressure (mmHg) | 83±3.1 | 78±2.8 | / |
| Heart rate (beats for minute) | 72±9 | 66±8 | / |
| WHR | 0.91±0.008 | 0.78±0.003 | <0.05‡ |
| HOMA-IR | 5.1±0.72 | 4.1±0.28 | <0.05‡ |
| Insulin(µU/ml) | 19.8±1.9 | 22.9±1.6 | <0.05‡ |
| Glucose (mmol/L) | 6.64± 0.14 | 5.45± 0.13 | <0.05‡ |
| Cholesterol (mmol/L) | 4.49±0.87 | 4.04±0.76 | <0.05‡ |
| HDL(mmol/L) | 1.78±0.44 | 1.81±0.44 | / |
| LDL (mmol/L) | 3.32±0.63 | 3.10±0.53 | / |
| Triglycerides(mmol/L) | 1.89±0.44 | 1.66±0.72 | <0.05‡ |
| Creatinine (mmol/L) | 101.5±4.1 | 103.6±6.4 | / |
|  |  |  |  |
| ***Biohumoral markers*** |  |  |  |
| CRP (mmol/L) | 1.04± 0.48 | 0.48± 0.28 | <0.05‡ |
| IL6(pg/ml) | 4.22±0.45 | 3.29±0.31 | <0.05‡ |
| TNFα (pg/ml) | 6.95±0.59 | 5.14±0.38 | <0.05‡ |
| Nitrotyrosine (nmol/l) | 5.214±0,702 | 2.148±0.348 | <0.05‡ |
| miR 195 | 198.82±32.18 | 104.54±32.71 | <0.05‡ |
| miR 27 | 116.89±40.31 | 62.53±35.34 | <0.05‡ |
|  |  |  |  |
| ***Echocardiographic parameters*** |  |  |  |
| Intima-media tickness (mm) | 1.02±0.15 | 0.85±0.12 | <0.05‡ |
| LVEDd (mm) | 56±5.8 | 54±3.3 | / |
| LVESd (mm) | 35±4.9 | 31±5.4 | / |
| LVEF (%) | 52±7 | 57±5 | / |
| LAD (mm) | 45±6 | 44±2 | / |
| Septum (mm) | 14±2.9 | 10.2±2.3 | <0.05‡ |
| Posterior wall (mm) | 12±1.3 | 8±1.1 | <0.05‡ |
| MPI | 0.59±0.03 | 0.35±0.03 | <0.05‡ |
| LV mass (g) | 217.4±32.65 | 131.8±21.8 | <0.05‡ |
| LV mass/BSA (g/m2) | 94.11±22.13 | 56.13±16.18 | <0.05‡ |
| LV mass/h (m2) | 70.11±17.45 | 46.63±13.24 | <0.05‡ |

**Supplementary table 2. Obese pre-diabetics treated by hypocaloric diet added to placebo (Pre-DM plus placebo) at baseline vs. 12 months of follow up.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Study variables** | **Pre-DM plus placebo patients**  **(n 27) at baseline** | **Pre-DM plus placebo patients (n 27) at 12 months of follow up** | **P value** |
| ***Clinical variables*** |  |  |  |
| BMI (Kg/m2) | 33.8±2.9 | 32.1±0.8 | / |
| Systolic arterial pressure (mmHg) | 135±12.6 | 124±11.3 | / |
| Diastolic arterial pressure (mmHg) | 84±2.1 | 76±2.3 | / |
| Heart rate (beats for minute) | 72±10 | 71±7 | / |
| WHR | 0.91±0.007 | 0.88±0.006 | / |
| HOMA-IR | 5.0±0.69 | 5.0±0.67 | / |
| Insulin(µU/ml) | 20.0±1.8 | 20.2±1.6 | / |
| Glucose (mmol/L) | 6.63± 0.18 | 6.67± 0.19 | / |
| Cholesterol(mmol/L) | 4.52±0.88 | 4.15±0.90 | <0.05ǂ |
| HDL(mmol/L) | 1.79±0.36 | 1.84±0.38 | / |
| LDL(mmol/L) | 3.33±0.59 | 3.17±0.52 | / |
| Triglycerides(mmol/L) | 1.86±0.55 | 1.70±1.24 | <0.05ǂ |
| Creatinine (mmol/L) | 101.8±3.8 | 98.6±3.5 | / |
|  |  |  |  |
| ***Biohumoral markers*** |  |  |  |
| CRP (mmol/L) | 1.09±0.49 | 0.56±0.28 | / |
| IL6(pg/ml) | 4.36±0.43 | 4.11±0.25 | / |
| TNFα (pg/ml) | 6.92±0.54 | 6.52±0.49 | / |
| Nitrotyrosine (nmol/l) | 5.20±0.64 | 4.52±0.38 | / |
| miR 195 | 196.41±46.67 | 164.52±47.44 | / |
| miR 27 | 116.81±40.31 | 98.66±30.28 | / |
|  |  |  |  |
| ***Echocardiographic parameters*** |  |  |  |
| Intima-media tickness (mm) | 1.03±0.17 | 1.02±0.17 | / |
| LVEDd (mm) | 56±5.1 | 55±3.1 | / |
| LVESd (mm) | 33±4.6 | 32±4.1 | / |
| LVEF (%) | 52±7 | 54±8 | / |
| LAD (mm) | 43±6 | 43±2 | / |
| Septum (mm) | 14±2.7 | 13.3±2.2 | / |
| Posterior wall (mm) | 12±1.8 | 10.6±1.4 | / |
| MPI | 0.58±0.03 | 0.49±0.05 | / |
| LV mass (g) | 218.3±42.3 | 181.2±31.4 | / |
| LV mass/BSA (g/m2) | 93.54±21.88 | 79.81±16.83 | / |
| LV mass/h (m2) | 70.34±17.62 | 63. 24±14.43 | / |

**Supplementary table 3. Obese normoglycemics treated by hypocaloric diet (NG).**

|  |  |  |  |
| --- | --- | --- | --- |
| **Study variables** | **NG patients (n 28) at baseline** | **NG patients (n 28) at 12 months of follow up** | **P value** |
| ***Clinical variables*** |  |  |  |
| BMI (Kg/m2) | 33.7±2.4 | 31.5±0.7 | / |
| Systolic arterial pressure (mmHg) | 131±12.8 | 124±7.6 | / |
| Diastolic arterial pressure (mmHg) | 85±2.1 | 75±2.2 | / |
| Heart rate (beats for minute) | 69±8 | 66±5 | / |
| WHR | 0.91±0.001 | 0.81±0.001 | / |
| HOMA-IR | 4.1±0.25 | 4.1±0.28 | / |
| Insulin(µU/ml) | 22.5±1.9 | 23.1±1.7 | / |
| Glucose (mmol/L) | 5.31± 0.52 | 5.31 ± 0.48 | / |
| Cholesterol (mmol/L) | 4.56±0.91 | 4.13±0.79 | <0.05° |
| HDL(mmol/L) | 1.82±0.41 | 1.78±0.39 | / |
| LDL (mmol/L) | 3.16±0.55 | 3.11±0.52 | / |
| Triglycerides (mmol/L) | 1.64±0.32 | 1.62±0.41 | / |
| Creatinine (mmol/L) | 83.2±2.9 | 74.2±1.7 | / |
|  |  |  |  |
| ***Biohumoral markers*** |  |  |  |
| CRP (mmol/L) | 0.85±0.36 | 0.46±0.12 | <0.05° |
| IL6 (pg/ml) | 3.49±0.38 | 3.12±0.38 | <0.05° |
| TNFα (pg/ml) | 5.56±0.92 | 4.72±0.71 | <0.05° |
| Nitrotyrosine (nmol/l) | 1.89±0.24 | 0.92±0.11 | <0.05° |
| miR 195 | 62.18±8.31 | 38.14±17.17 | <0.05° |
| miR 27 | 93.46±43.76 | 39.48±18.15 | <0.05° |
|  |  |  |  |
| ***Echocardiographic parameters*** |  |  |  |
| Intima-media tickness (mm) | 0.88±0.14 | 0.81±0.13 | / |
| LVEDd (mm) | 54±4.2 | 53±4.5 | / |
| LVESd (mm) | 31±5.8 | 29±4.8 | / |
| LVEF (%) | 54±5 | 56±6 | / |
| LAD (mm) | 42±2 | 41±2 | / |
| Septum (mm) | 13.2±2.3 | 10.1±1.8 | <0.05° |
| Posterior wall (mm) | 11±1.2 | 8.4±1.2 | <0.05° |
| MPI | 0.57±0.03 | 0.36±0.04 | <0.05° |
| LV mass (g) | 201.6±39.3 | 126.4±21.3 | <0.05° |
| LV mass/BSA (g/m2) | 92.08±20.87 | 56.59±13.83 | <0.05° |
| LV mass/h (m2) | 69.15±17.10 | 44.73±11.28 | <0.05° |