Egypt. J. Chem. **Vol. 68**, No. 3 pp. 15 - 24 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Biochemical Evaluation of Asymmetric and Symmetric Dimethylargininein Obese Middle-Aged Women as an Early Predictor of Cardiovascular and Renal Diseases: The Effect of Dietary Intervention

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Abstract

Asymmetric dimethyl-L-arginine (ADMA) and symmetric dimethyl-L-arginine (SDMA) are arginine analogues with direct and indirect impacts on nitric oxide synthesis and endothelial dysfunction. The rising global prevalence of obesity carries significant implications for the increased risk of cardiovascular and chronic kidney diseases. **Theobjective** of this study was to evaluate ADMA and SDMA levels among women with various degrees of obesity to predict potential cardiovascular and renal health impairments. Additionally, the study aimed to explore the short-term impact of a balanced, low-caloric regimen on these crucial organs. Seventy-six females with a body mass index (BMI) exceeding 27 kg/m² participated as volunteers. They were categorized into four groups based on their BMI. Anthropometric measurements and dietary history were taken for all volunteers. Blood samples were collected, and laboratory tests were performed, including assessments of renal function and serum levels of ADMA and SDMA. These parameters were reassessed after six weeks of adhering to the low-caloric, balanced, healthy diet. **The findings** demonstrated that morbidly obese individuals were the older and had the highest anthropometric values and had severe accumulation of fat tissue, especially in the visceral area. The elevated anthropometric measurements were accompanied by increased blood glucose, lipid profile and the cardiovascular risk ratio. Significant differences between morbid obese and the other groups were recorded. Basic kidney function indices were within normal range. Significant positive correlations were found between SDMA and both of anthropometric and lipid profile, while positive correlations between ADMA and both of blood pressure, fasting blood glucose, cardiovascular risk factor and estimated glomerular filtration rate. Following a six-week intervention, improvements were observed in all anthropometric measurements and biochemical parameters across all groups with the highest percent decrease was in the SDMA of the morbidly obese group (about 55%). **Conclusion:** The results suggested that obesity, particularly in the elderly, may be linked to an increased risk of metabolic abnormalities. This highlights the significance of adopting healthy eating habits in tackling the obesity problem. Moreover, the results underscored the importance of the ADMA biomarker over the SDMA biomarker as a more reliable indicator of cardiovascular and renal diseases.

Keywords: Novel Biochemical Markers; Asymmetric dimethyl-L-arginine; Symmetric dimethyl-L-arginine; Obesity; Cardiovascular and kidney diseases; Dietary intervention

1 - Introduction

Obesity has emerged as a global epidemic, with predictions indicating 40% rise in its occurrence over the coming decade, this elevating the risk of conditions such as diabetes, cardiovascular disease, and chronic kidney disease (CKD). Among those suffering from obesity, there seems to be a compensatory response involving hyperfiltration, presumably to match the increased metabolic requirements due to higher body weight. However, this increased intraglomerular pressure carries the potential to harm the kidney's structure, thereby amplifying the long-term risk of developing CKD **[1]**.

Asymmetric dimethyl arginine (ADMA) and its counterpart, Symmetric dimethyl arginine (SDMA), are naturally occurring amino acids present in blood plasma, initially identified and characterized in human urine since 1970.These compounds emerge as metabolic by-products resulting from continuous protein modification processes within the cytoplasm of all human cells. They maintain a close relationship with L-arginine, an amino acid that is conditionally essential. Their interaction with L-arginine affects the generation of nitric oxide (NO), a crucial compound for normal endothelial function, thereby influencing cardiovascular well-being. These substances stand as the most potent internally produced inhibitors of nitric oxide synthase (NOS), notably elevated in individuals with end-stage renal disease (ESRD). Studies have linked their increased presence to

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Received date 11 May 2024; revised date 17 July 2024; accepted date 28 July 2024
DOI: 10.21608/EJCHEM.2024.288740.9697

significant forecasts regarding cardiovascular outcomes and mortality among patients undergoing dialysis **[2]**. More recently, ADMA and SDMA have recognized as indicators of oxidative stress **[3].**

Serum creatinine stands as the primary measurement used to estimate glomerular filtration rate (GFR) due to its straightforward clinical applicability. However, it serves as a basic approximation that only rises notably after substantial kidney disease has already set in, influenced by factors beyond the kidneys (extra renal factors). On the other hand, SDMA emerges as a renal biomarker observed in humans, dogs, and cats. It demonstrates correlations with serum creatinine and GFR, presenting as an earlier and more specific indicator for kidney affections. ADMA and potentially SDMA play roles in contributing to hypertension and atherosclerosis among individuals with chronic renal disease. In cases of CKD, concentrations of c(ADMA) and c(SDMA) register significant increases **[4]**. Recent studies have determined that concentrations of ADMA and SDMA hold associations with hospitalization and mortality at the three-month mark, further exacerbated by venous volume overload in acute heart failure (AHF) patients **[5]**.

New findings indicated that managing weight could potentially safeguard kidney function and enhance cardiovascular well-being. Weight loss has the potential to regulate elevated blood pressure and decrease the risks associated with diabetes and heart disease. The act of shedding excess weight carries numerous benefits for general health and contributes to the enhancement of kidney function, as highlighted in recent research by Nawaz *et.al.* **[6]**.

This study aimed to assess the detrimental impacts of obesity on cardiovascular and renal health by exploring the serum ADMA and SDMA levels as novel biomarkers. Additionally, it sought to examine the immediate impact of nutritional intervention through a balanced, low-caloric diet on the cardiovascular and kidney functions of individuals exhibiting diverse levels of obesity.

2. METHODOLOGY

2.1. Study Design

This short-term study was carried out at the Nutrition department of the National Research Centre (NRC). All participants were briefed about the study's objectives, and their participation was secured through written consent. The research protocol received approval from the "Ethical Committee" of the "National Research Centre" *(Registration Number 19-180)*, ensuring compliance with the principles outlined in the **Helsinki Declaration**.

2.2. Subjects

Seventy-six females with body mass index (BMI) more than 27 kg/m² were included as volunteers in this study with their age ranged from 32 to 64 years old, and their mean BMI was 34.9±0.6 kg/m². They were all enrolled in a program for losing weight in the Nutrition Department, National Research Centre. **Exclusion criteria:** Obese patients on pharmacological treatment, known to have renal failure or thyroid dysfunction effects.

2.3. Diet Therapy

All participants have followed a low caloric balanced regimen (1000-1100 KCal/day) rich in fruits and vegetables for six weeks. All the subjects were examined at baseline (initial visit), and the end of the study (Final visit) with weekly follow up.

2.4. Clinical Examination

Full medical history including important renal symptoms; in addition to clinical examination and**Blood pressure** measurement by cuff sphygmomanometer were taken.

2.5. Anthropometric Measurements

Anthropometric parameters in the form of height and body weight were taken to calculate the body mass index (BMI) = weight in kg/square height in meters. Waist circumference (minimal waist), hip circumference and waist to hip ratio (WHR) were taken as a guide to visceral adiposity **[7]**. Body fat (BF) as a per cent of body weight and the basal metabolic rate were measured using Geratherm Body Fitness (B-5010), German. All measurements were taken by the same researcher to assure accuracy.

2.6. Dietary consumption data

Dietary data were collected using **the 24-hour dietary intake recall**. Portion size and the amount of food left for each subject were recorded in order to estimate the individual food intake items. Snacks consumed in between meals were also documented. The total dietary intake was calculated using the computer application (Nutrisurvey, 2007) to transform the food consumed into nutrients. The total calorie intake during a 24-hour period was calculated. To determine each individual's nutritional pattern, the percentages of protein, carbohydrates, and fats in relation to recommended daily allowance (RDA) were determined. The number of fresh fruits and vegetables consumed every day was recorded.

2.7. Blood Sampling and Biochemical Analysis

Blood samples were obtained on the day of clinical examination after an overnight fast.**The following laboratory parameters were assessed:**

1. **Fasting blood glucose (FBG)** was determined in fresh samples using the glucose oxidase method **[8]**.
2. **Lipid profile** including: Serum total cholesterol (TC), High-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) were done using; cholesterol proceed No 1010, StanBioLiquicolor**[9]**. HDL-C proceed No 0599 StanBioLiquicolor**[10]**, and triglycerides proceed No 2100, (Enzymatic method) **[11]** respectively. Low density lipoprotein- cholesterol (LDL-C) was

calculated according to the Friedewald equation **[12]**. Risk ratio (TC/ HDL-C) and non-HDL-C (TC- HDL-C) were calculated to predict cardiovascular affection.

3. **Routine kidney biomarkers:** Serum Creatinine was estimated by kinetic method using the kit supplied by ErbaLachemas.r.o., Karásek 1d, 621 00 Brno, CZ. REF/BLT00020 **[13]**. Urea was estimated by colorimetric method **[14]**. Blood urea nitrogen (BUN) was calculated, where BUN (mg/dL) = Serum urea (mg/dL) *0.467. Conversion factor derived by 28/60=0.467 [MW of urea=60, MW of urea nitrogen=28 (14*2)].
4. **Creatinine clearance** was assessed from serum creatinine by using the following formula (COCK CROFT – GAULT EQUATION): Estimated Creatinine Clearance = (140-Age) x Weight in Kg / (72x Serum Creatinine in mg/ dL) multiply by 0.85 for females **[15]**. Normal creatinine clearance is 88–128 mL/min for healthy women and 97–137 mL/min for healthy men.
5. **Estimated glomerular filtration rate** (eGFR) was calculated by the abbreviated MDRD (modification of diet in renal disease) equation, Where eGFR=186 x (Creatinine mg/dL)-1.154x (Age)-0.203 x (0.742 if female). Equation generally provides more accurate estimation at GFR >60 mL/min/1.73 m2**[16, 17]**.
6. Two established competitive ELISA's were used for measuring serum concentrations of **ADMA (expressed in ng/ml) and SDMA(expressed in µmol/ L)** according to manufacturer's guidelines supplied by Human SDMA& ADMA ELISA Kit, EIAab Science Inc. China **[18, 19].** This ELISA kit used the Sandwich-ELISA method. The Micro ELISA strip plate included in the kit was pre-coated with an antibody specific to ADMA or SDMA. Standards or samples were introduced into the appropriate wells of the Micro ELISA strip plate and allowed to bind with the specific antibody. Next, an HRP-conjugated antibody that targets ADMA or SDMA was added to each well of the Micro ELISA strip plate and incubated. Unbound components were washed away, and then TMB substrate solution was added to each well. Only, wells containing ADMA or SDMA and the HRP-conjugated antibody turned blue and later changed to yellow upon the addition of the stop solution. Optical density (OD) was measured spectrophotometrically at 450 nm, with the OD value being directly proportional to the ADMA or SDMA concentrations. Their concentrations in the samples can be determined by comparing their OD to a standard curve.

2.8. Statistical Analysis

The data were presented as mean ±SE. Group comparisons were conducted utilizing the one-way analysis of variance (ANOVA) test, followed by post-hoc testing. Within-group comparisons before and after the intervention were assessed using paired-sample t-tests and percent changes [(X2-X1) *100/X1]. The correlation between variables was examined through the calculation of the correlation coefficient (r). Statistical significance was established at a P value<0.05. The analysis was performed using SPSS window software version 17.0 (SPSS Inc., Chicago, IL, USA, 2008).

3. RESULTS

Following the initial visit, the volunteers were categorized based on their body mass index (BMI) into four distinct groupsaccording to the World Health Organization (WHO) classification **[20]**, detailed in **Table 1:**

- **Group 1:** Classified as overweight, with a BMI ranging from 25.0 to 29.9 (kg/m²)
- **Group 2:** Classified as mildly obese, with a BMI ranging from 30.0 to 34.9 (kg/m²).
- **Group 3:** Classified as moderately obese, with a BMI ranging from 35.0 to 39.9 (kg/m²).
- **Group 4:** Classified as morbidly obese, with a BMI of 40.0 or higher (kg/m²).

Table 1: Classification of cases according to their body mass index (BMI) (kg/m2) [20]

Group	Nomenclature (No.& % of total)	Mean ± SE of BMI (minimum-maximum)	P-Value
1	Overweight (no. =12, 15.8%)	28.6±0.1 (28.5-29.2)	0.001
2	Mildly obese (no. =28, 36.8%)	32.3±0.3 (30.6-34.7)	
3	Moderately obese (no. =20, 26.3%)	36.8±0.2 (35.8-38.2)	
4	Morbidly obese (no. =16, 21.1%)	42.2±0.5 (40.1-44.7)	
	Total (no. =76, 100%)	34.9±0.6 (28.3-44.7)	

Table (2) showed the mean± SE values of the age, anthropometric parameters and blood pressure during the initial and final visits, along with the percentage changes between the two visits. The overweight subjects were the younger group, while the older group was categorized as morbidly obese.

During the initial visit, significant differences were observed among the four groups regarding weight and BMI. Notably, the study revealed that irrespective of their overweight or obese status, all groups exhibited markedly high percentages of body fat,

alongside minimal waist circumferences of moderately and morbidly obese exceeding the recommended values. The individuals with morbid obesity showed the highest values among the studied groups.
Following the dietary intervention, the current study observed a reduction in all anthropometric measurements, particularly notable among individuals categorized with morbid obesity. The most substantial decrease was noted in body weight, reaching a maximum decline of -10.6% when comparing values before and after the intervention.

Table (3) illustrates the mean values of biochemical parameters during the initial and final visits, alongside the percentage changes observed between these two visits. The study revealed that elevated BMIwas accompanied with elevated blood glucose levels, alterations in the lipid profile, and an increase in the cardiovascular risk ratio (TC/HDL-C). Notably, significant differences were observed between the morbidly obese group and the other groups regarding risk ratio and HDL-C. However, despite these metabolic changes, fundamental kidney function indicators, including creatinine, urea, creatinine clearance, and eGFR, remained within normal ranges.
After dietary therapy, the study also demonstrated a significant decrease in most of the biochemical markers across all groups, including blood glucose and lipid profile. However, the most pronounced reduction among the biochemical parameters was observed in the kidney function marker SDMA, as it decreased by 55.2% in women with morbid obesity when compared to the other kidney function parameters, while ADMA exhibited a decrease of 19.1% among the overweight participants.

The study's baseline findings revealed a noteworthy positive correlation coefficient between serum SDMA and several factors including weight, MWC, W/HR, TC, and TG, while ADMA exhibited positive correlations with SBP, DBP, FBG, TG, risk ratio and eGFR **(Table 4)**. These results align with the observed changes in blood pressure throughout the study, where a decrease in anthropometric and biochemical measures, including ADMA,corresponded to a significant reduction in both systolic and diastolic blood pressure post intervention.

The comparison between the macronutrients of the low caloric regimen and the habitual diet consumed by the obese volunteers before intervention was shown in **table (5).** Data revealed that there was higher intake of calories, protein, total fat, saturated fatty acids (SFAs) and cholesterol, and low monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) before intervention which was prominent among morbidly obese women (106.9, 160.4, 138.2, 17.8, 114.8, 4.7 and 3.4 percent of the recommended daily allowance (RDAs) respectively).
After intervention the finding demonstrated that the hypocaloric regimen decreased the intake of protein, carbohydrate and total fat, and proved healthy distribution of fatty acids; the MUFAs and PUFAs.

4. DISCUSSION

Addressing overweight and obesity continues to be an enduring public health challenge at the national level. Obesity is associated with various detrimental health consequences, such as increased risks of cardiovascular and metabolic conditions, several types of cancer, disability, mortality, and, more recently highlighted, increased susceptibility to COVID-19 infections and associated complications **[21, 22, 23].**
From a clinical standpoint, the obesity phenotype isn't uniform but rather represents a spectrum encompassing varying levels of metabolic unhealth. Metabolically unhealthy obesity frequently aligns with the traits of metabolic syndrome. The connections between obesity and CKD are complex, involving bidirectional, intricate, and multi-layered interactions. This complexity might find explanation through shared pathophysiological pathways, such as chronic inflammation, increased oxidative stress, and hyper-insulinemia. Additionally, common clusters of risk factors and associated conditions, like insulin resistance, hypertension, and dyslipidemia, further contribute to this intricate relationship **[24]**.

4.1. Anthropometric Evaluations

In the current study, regarding the age distribution, the overweight subjects were the younger group, while the older group was categorized as morbidly obese. The obtained results are in accordance with a previous study addressing the impact of age on the correlation between BMI and mortality **[25]**. Blüher'sresearch **[25]** highlighted the association between weight and various demographic and socioeconomic factors. Specifically, among women, the mean BMI exhibited an increase from 23.8 among individuals aged 30-to-44, to 25.2 among those aged 55 to 64, subsequently declining in older age categories **[25]**.
All groups exhibited markedly high percentages of body fat, alongside minimal waist circumferences of moderately and morbidly obese exceeding the recommended values. The individuals with morbid obesity showed the highest values among the studied groups. The study's findings underscore the prevalence of substantial fat accumulation, particularly visceral fat tissue, which has been linked to chronic alterations and diseases affecting various organ systems **[26]**.
Caballero **[27]** highlighted that the relationship between BMI and disease risk is not linear. Over 60% of the global disease burden linked to obesity affects individuals with a BMI ≥30, that constituting roughly 10% of the global population of overweight or obese individuals. Simultaneously, there was a notable decrease in reported blood pressure values, with the greatest percentage reduction observed in SBP (23.1%) in morbidly obese individuals.

Table (2): Mean± SE of Anthropometric parameters at initial and final visits and % changes between the two visits

Parameters	Initial				Final							
	Overweight (No.= 12)	**Mildly Obese (No.= 28)**	**Moderately Obese (No.= 20)**	**Morbidly Obese (No.= 16)**	**Overweight**	**% Change**	**Mildly Obese**	**% Change**	**Moderately Obese**	**% change**	**Morbidly Obese**	**% Change**
Age (year)	42.3±3.9 a	50.9±2.3 b	53.2±2.1 b	54.3±1.2 b								
Height (cm)	157.0±1.7 a	156.1±1.1 a	158.0±1.3 a	155.5±1.4 a								
Weight (Kg)	70.7±1.3 a	78.8±1.4 b	92.0±1.3 c	102.4±2.7 d	69.3±1.2 e	-1.9	75.3±1.7** e	**-4.4**	87.7±1.8** f	-4.7	91.5±3.9** f	**-10.6**
BMI (Kg/m2)	28.6±0.1 a	32.3±0.3 b	36.8±0.2 c	42.2±0.5 d	28.1±0.1 e	-1.8	30.9±0.3** f	**-4.1**	35.9±0.4** g	-2.3	39.4±0.5** h	-6.6
Body fat (%)	41.9±1.4 a	45.5±1.8 b	49.5±0.9 c	52.7±0.9 d	40.8±2.1** e	-2.8	44.8±1.5** f	-1.7	49.3±0.8** g	-0.5	50.90±1.07** h	-3.3
BMR (Kcal)	1936.3±40.5 a	1966.4±25.6 a	2132.8±32.5 b	2239.8±36.5 c	1889.5±54.6 e	-2.4	1921.0±30.2 e	-2.3	2054.7±22.8 f	-3.7	2057.5±43.7 f	-8.1
MWC (cm)	78.3±1.5 a	86.9±0.9 b	90.2±1.3 b	101.0±1.3 c	72.5±1.3* e	**-7.4**	83.3±0.9** f	-4.1	83.7±0.5** f	**-7.2**	92.8±2.2** g	-8.2
Hip circum. (cm)	108.5±1.2 a	111.7±1.2 a	118.0±0.9 b	129.0±1.8 c	105.5±0.6** e	-2.8	107.8±1.2** e	-3.5	116.2±1.8** f	-1.6	120.5±2.8 f	-6.6
WHR (cm/cm)	0.7±0.0 a	0.8±0.0 b	0.8±0.0 b	0.8±0.0 b	0.6±0.0** e	-4.2	0.8±0.0** f	-1.3	0.7±0.0* g	-6.5	0.8±0.0 f	-1.3
Neck (cm)	31.7±0.4 a	32.6±0.2 b	33.4±0.1 b	37.8±0.5 c	31.0±0.4* e	-2.1	32.3±0.2** f	-1.2	32.5±0.2** f	-2.7	35.5±0.2* g	-5.9
SBP (mmHg)	116.7±1.4 a	115.7±2.7 a	125.0±2.6 b	131.3±0.6 b	115.7±3.3 e	-0.8	120.5±2.4 e	**+4.1**	134.0±4.1* f	**+7.0**	101.0±0.4** g	**-23.1**
DBP (mmHg)	73.3±1.4 a	77.1±1.4 a	82.0±1.6 b	85.0±0.9 b	72.3±2.8 e	-1.4	74.3±1.6 ef	-3.7	80.6±1.9 f	-1.7	76.0±2.9** ef	**-10.6**

BMI: Body Mass Index, BMR: Basal Metabolic Rate, MWC: Minimal Waist Circumference, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure.
***Difference between basal and last mean values of the same group: *Significant at p < 0.05, **Significant at p < 0.01**
(a to d): Difference among groups at the basal visit; **(e to h)** Difference among groups at the last visit. Groups sharing the same initial at the same raw are not statistically significant from each other

Table (3): Mean± SE of biochemical parameters at initial and final visits and % changes between the two visits

Parameters	Initial				Final							
	Overweight (No.= 12)	Mildly Obese (No.= 28)	Moderately Obese (No.= 20)	Morbidly Obese (No.= 16)	Overweight	% Change	Mildly Obese	% Change	Moderately Obese	% Change	Morbidly Obese	% Change
FBG (mg/dL)	90.3±0.6 a	114.9±9.6 ab	116.6±5.3 b	105.5±4.2 ab	86.7±2.1 e	-4.1	102.4±2.9 f	-10.8	105.0±4.9** f	-10.0	99.5±2.8 f	-5.7
T. Cholesterol (mg/dL)	224.5±2.7 a	210.9±3.83 a	229.4±9.5 a	210.4±5.2 a	199.2±5.0** ef	-11.3	201.9±3.4** ef	-4.3	192.3±4.7** e	-16.2	206.8±5.3** f	-1.7
TG (mg/dL)	78.4±4.1 ab	71.8±2.3 a	91.4±7.1 b	94.6±6.9 b	72.1±4.7**[ef	-8.1	67.4±2.1 e	-6.1	85.1±4.3 fg	-6.9	90.7±7.9** g	-4.1
HDL-C (mg/dL)	68.0±1.4 a	57.0±2.5 b	55.5±2.4 b	40.0±1.3 c	71.3±1.9** e	+4.7	64.1±2.1** f	+12.4	58.0±2.4** f	+4.5	46.3±0.4** g	+15.7
LDL-C (mg/dL)	140.8±2.2 a	139.6±4.7 a	155.6±8.7 a	151.4±5.7 a	113.5±4.5** e	-19.4	124.4±3.7** e	-10.9	117.3±4.9** e	-24.7	142.3±4.8** f	-6.0
Risk ratio (TC/HDL-C)	3.3±0.04 a	4.0±0.3 ab	4.3±0.3 b	5.4±0.3 c	2.8±0.1 e	-15.2	3.2±0.1 ef	**-20.0**	3.5±0.2** f	-18.6	4.5±0.1 g	-16.7
Non-HDL-C	156.5±1.8 a	153.9±4.8 a	173.9±9.9 a	170.4±5.6 a	127.6±4.0** e	-18.5	137.9±3.8** e	-10.5	134.3±5.6** e	-20.7	160.5±4.9** f	-5.8
Creatinine (mg/dL)	0.59±0.03 a	0.58±0.02 a	0.62±0.04 a	0.55±0.03 a	0.65±0.04 e	-5.1	0.42±0.02** f	-27.6	0.57±0.02 e	-8.1	0.56±0.06** e	-9.7
Urea (mg/dL)	34.0±1.1 ab	30.6±1.7 a	37.4±1.6 b	34.4±2.2 ab	31.1±2.4 e	-8.7	29.2±1.1 e	-4.5	32.9±1.9** e	-11.8	30.4±2.6** e	-11.4
BUN (mg/dL)	15.9±0.5 ab	14.3±0.8 a	17.5±0.7 b	16.1±1.1 ab	14.5±1.1 e	-8.7	13.6±0.5 e	-4.5	15.4±0.9** e	-11.7	14.2±1.2** e	-11.3
Creatinine clearance	145.9±13.9 a	145.9±5.7 a	173.1±16.1 ab	194.9±11.4 b	129.0±18.2** e	-11.6	204.0±17.4** f	+39.8	152.1±7.8 ef	-12.2	170.1±14.3* ef	-12.7
eGFR (mL/min/1.73 m2)	124.8±8.4 a	121.3±4.3 a	122.7±10.8 a	127.9±7.3 a	111.3±10.9** e	10.8	187.8±15.4** f	+54.8	117.4±5.1 e	-4.3	133.1±16.8** e	+4.1
ADMA (ng/ml)	116.1±4.1 a	114.1±4.6 a	104.6±4.3 a	106.1±4.2 a	94.0±2.9** ef	-19.1	103.5±3.9** f	-9.3	85.3±5.5** e	-18.5	88.9±2.8** e	-16.2
SDMA (µmol/ L)	1.3±0.0 a	2.4±0.1 b	1.6±0.2 a	2.4±0.1 b	1.2±0.0 e	-7.7	1.3±0.1** e	-46.3	1.3±0.2 e	-20.5	1.1±0.1** e	-55.2

FBG: Fasting Blood Glucose, TG: Triglycerides, HDL-C: High Density Lipoprotein Cholesterol, LDL-C: Low Density Lipoprotein Cholesterol, BUN: Blood Urea Nitrogen, eGFR: Estimated Glomerular Filtration Rate, ADMA: Asymmetric dimethyl arginine, SDMA: Symmetric dimethyl arginine. *&Difference between basal and last mean values of the same group: *Significant at p < 0.05, **Significant at p < 0.01 (a to d)**: Difference among groups at the basal visit; **(e to h)** Difference among groups at the last visit. Groups sharing the same initial at the same raw are not statistically significant from each other

4.2. Novel Biochemical Markers

ADMA and SDMA are recognized as risk factors impacting cardiovascular and renal systems **[28]**. Given ADMA's narrow normal concentration range, even slight increases in its levels may signify elevated cardiovascular risk. Consequently, achieving high analytical precision becomes crucial to differentiate between normal and slightly elevated concentrations **[29]**. Moreover, obesity is acknowledged for inducing changes in lipid biochemistry, culminating in dyslipidemic atherogenesis; a pivotal factor in the onset of cardiovascular events **[30]**.

Concurrently, CKD has established associations with atherosclerotic cardiovascular disease (ASCVD) risk, particularly among individuals with diabetes. The altered metabolism of accumulating solutes in CKD, such as ADMA, SDMA, and trimethylamine N-oxide (TMAO), serves as a potential reflection of pathways linking CKD with ASCVD **[31]**.

A positive significant correlation emerged between ADMA levels and both systolic and diastolic pressure post-intervention. These findings coordinate with Riccioni et al. **[32] and Qin et al. [33]**, who highlighted ADMA as an endogenous nitric oxide synthase (NOS) inhibitor, known to mediate endothelial dysfunction and atherosclerosis. Circulating ADMA levels have been associated with various cardiovascular risk factors such as hypercholesterolemia, arterial hypertension, diabetes mellitus, hyperhomocysteinemia, aging, and smoking **[32, 33]**.

Table (4): Correlation coefficient between both of mean values of ADMA and SDMA and those of anthropometric measurements and other biochemical parameters at the initial and final visits for all overweight and obese volunteers (no. = 76).

Parameters	ADMA		SDMA	
	r value			
	Initial	Final	Initial	Final
Age (year)	N.S.	N.S.	N.S.	N.S.
Weight (kg)	N.S.	N.S.	**.270***	N.S.
BMI (kg/m2)	**-.275***	**-.285***	N.S.	N.S.
BF (%)	N.S.	**-.281***	N.S.	N.S.
BMR (Kcal)	N.S.	**-.338***	**.244***	N.S.
MWC (cm)	N.S.	N.S.	**.228***	N.S.
Hip (cm)	**-.226***	**-.442****	N.S.	N.S.
WHR (cm)	N.S.	**.343***	**.273***	N.S.
SBP (mmHg)	**.407****	**.355****	N.S.	N.S.
DBP (mmHg)	**.338****	**-.229***	N.S.	N.S.
FBG (mg/dL)	**.353****	N.S.	N.S.	N.S.
T. Cholesterol (mg/dL)	**-.294***	N.S.	**.455****	N.S.
TG (mg/dL)	**.257***	N.S.	**.433****	N.S.
HDL (mg/dL)	N.S.	N.S.	**.383****	**.283***
LDL (mg/dL)	**-.333****	N.S.	N.S.	N.S.
Non-HDL (mg/dL)	**-.347****	N.S.	**-.250***	N.S.
Risk ratio (T. chol./HDL)	**.271***	NS	**N.S.**	**.255***
Creatinine (mg/dL)	**-.360****	**-.506****	**-.247***	N.S.
Urea (mg/dL)	N.S.	**-.333****	N.S.	N.S.
Creatinine Clearance	N.S.	N.S.	N.S.	N.S.
eGFR (mL/min/1.73 m2)	**.359****	**.388****	N.S.	N.S.
ADMA (ng/ml)	-	-	**.358****	**.361****
SDMA (µmol/ L)	**.358****	**.361****	-	-

Numbers presented in this table are the value of r =correlation coefficient.
*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level

4.3. Dietary Assessment

Data showed that before the intervention, morbidly obese women had a higher intake of calories, protein, total fat, saturated fatty acids (SFAs), and cholesterol, while their consumption of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) was lower.

After the intervention, the results indicated that the hypocaloric regimen led to a reduction in the consumption of protein, carbohydrates, and total fat. It also showed a healthier distribution of fatty acids, specifically increasing the intake of MUFAs and PUFAs.

Studies by Zhang et al. **[34]**and Mantzouranis et al. **[35]** shed light on the adverse effects of excessive protein consumption on macrophages and its potential role in atherosclerosis development. Moreover, research by Maki et al. **[36]** highlighted that diets high in saturated fatty acids (SFAs) are linked to increased risk of ASCVD, primarily due to their impact on elevating low-density lipoprotein cholesterol (LDL-C) levels. Dietary recommendations from health authorities encourage limiting SFA intake, particularly for individuals with clinical ASCVD, dyslipidemia, or diabetes mellitus. Guasch-Ferré et al. **[37]** indicated that replacing SFAs, trans fats, or refined carbohydrates with MUFA-Ps could lead to significantly lower mortality rates. Overall, these findings endorse current dietary guidelines advocating for the substitution of animal fats with unsaturated plant oils to prevent chronic diseases and premature deaths.

Table (5): The macronutrient contents of the low caloric regimen and the traditional (habitual) diet consumed by the overweight and obese volunteers.

Nutrient intake	Mean value± SE % of RDA					RDAs
	Low caloric regimen	Habitual diet				
		overweight	Mildly obese	Moderately obese	Morbidly obese	
Energy (kcal)	1074.3±15.3 48.8	1959.8±8.8**a 89.1	2130.7±5.3**b 96.9	2241.1±6.2**c 101.9	2350.7±4.5**d 106.9	2200
Protein (g)	44.2±5.9 88.4	64.1±1.8**a 128.1	78.9±2.3**b 157.7	79.6±4.2**c 159.1	80.2±2.8**d 160.4	50
Carbohydrates (g)	124.5±6.3 41.5	244.7±4.5**a 81.56	268.8±3.8**b 89.6	279.8±5.3**c 93.3	289.8±3.2**d 96.6	300
Fat (g)	44.4±2.3 63.4	80.6±2.3**a 115.1	82.2±4.1**b 117.5	89.3±6.4**c 127.6	96.8±3.1**d 138.2	70
SFAs (g)	9.0±1.1 7.6	36.2±11.4**a 16.6	39.2±14.1**b 16.5	43.2±10.4**c 17.3	46.6±13.6**d 17.8	Not more than 7% of Total Calorie
MUFAs (g)	15.7±2.1 13.2	13.5±2.3**a 6.2	13.1±3.1**b 5.5	12.9±3.2**c 5.2	12.4±3.7**d 4.7	12%-14% of Total Calories
PUFAs (g)	11.7±1.2 9.81	9.3±3.6*a 4.3	9.2±1.0*b 3.9	9.1±1.1*c 3.6	8.9±4.7*d 3.4	6%-8% of Total Calories
Cholesterol (mg)	105.3±5.5 52.7	222.0±13.2**a 111.0	215.3±14.2**b 107.7	218.9±12.2**c 109.4	229.6±14.1**d 114.8	200
Dietary fiber (g)	21.8±0.3 87.0	14.7±2.3*a 58.9	13.3±1.4*b 53.4	12.8±2.3*c 51.0	11.9±1.3*d 47.6	25

SFAs: Saturated **F**atty **A**cids**, MUFAs: M**onounsaturated **F**atty **A**cids**, PUFAs: P**olyunsaturated **F**atty **A**cids**.**
a: Low caloric regimen vs. overweight, b: Low caloric regimen vs. Mildly obese, c: Low caloric regimen vs. Moderately obese and **d: Low caloric regimen vs. Morbidly obese. *Significant at P≤ 0.05 **Highly Significant at P≤ 0.001**

5. CONCLUSION

The outcomes of this study suggested a potential association between obesity and an elevated risk of metabolic abnormalities, particularly in older individuals. Furthermore, the research revealedvariations in the correlation between the inert molecules SDMA and ADMA and the pathological and metabolic changes associated with obesity. The results indicated that concentrations of SDMA were not linked to blood pressure, blood glucose levels, or the examined renal parameters; instead, they exhibited a significant correlation with obesity parameters and lipid profiles. On the other hand, ADMA distinctly displayed an association with renal and cardiovascular indices, specifically blood pressure, serum lipid levels, risk factors, and eGFR.This underscores the significance of the ADMA parameter over SDMA as an indicator of cardiovascular and renal diseases.The majority of these associations diminished after intervention with a hypocaloric, healthy, balanced diet, leading to weight loss and improvement in biochemical parameters. This highlights the crucial role of appropriate nutrition in addressing the issue of obesity.

6. Conflict of interest

We have no conflicts of interest to disclose or to declare.

7. Formatting of funding sources
This project was financial supported by National Research Centre, Egypt with ID “1205020”.

8.Acknowledgments
We express our thanks to our institute "National Research Centre; Egypt “Authors arealso grateful to the volunteers’ females who participated in this study.

9. Ethics approval
The research protocol received approval from the "Ethical Committee" of the "National Research Centre" *(Registration Number 19-180)*, ensuring compliance with the principles outlined in the **Helsinki Declaration**. Each volunteer woman was required to sign a written informed consent form after being fully informed about the study's purpose.

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