

Brief Overview of Dietary Intake, Gut Microbiota, Metabolic Markers and Research Opportunities in Sample of Egyptian Women

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Abstract

Back ground: Metabolic syndrome (MetS) is a phenotype caused by interaction of host intrinsic factors such as genetics and gut microbiome, and extrinsic factors such as diet and lifestyle.

Aim: To demonstrate the interplay of intestinal microbiota with obesity, MetS markers and some dietary ingredients among sample of Egyptian women.

Methods: A cross-sectional study included 115 Egyptian women; 82 obese (59 without MetS and 23 with MetS) and 33 normal weight ones. Anthropometric assessment, 24 hours dietary recall, laboratory evaluation of liver enzymes (AST and ALT), leptin, short chain fatty acids (SCFA), C-reactive protein (CRP), fasting blood glucose (FBS), insulin and lipid profile, in addition to fecal microbiota analysis were done.

Results: The obese women with MetS had the highest significant values of anthropometric and biochemical parameters, and consumed diet high in calories, protein, fat and carbohydrate, low in fiber and micronutrients. Bacteroidetes and Firmicutes was the abundant bacteria among different gut microbiota, with low Firmicutes/ Bacteroidetes Ratio, and insignificant differences between the 3groups. Firmicutes/ Bacteroidetes Ratio significantly correlated positively with total cholesterol and LDL-C and negatively with SCFA.

Conclusion: The dietary factor, dysbiosis and the metabolic product short chain fatty acids have been implicated in causing metabolic defects.

Introduction

Metabolic syndrome (MetS), differently known as insulin resistance, syndrome X, etc., is characterized by WHO as a pathologic condition characterized by abdominal obesity, hyperleptinemia, hypertension, and insulin resistance [1]. In spite of the fact that there's some variety within the definition by other wellbeing care organization, the contrasts are minor. With the effective success in approximately elimination of communicable infectious diseases in most of the world, this modern non-communicable disease (NCD) has gotten to be the major health hazard of advanced world. The two fundamental forces spreading this ailment are the increment intake of high calorie and low fiber fast food and diminish in physical activity due to mechanized transports and sedentary frame of leisure time exercises [2].

Recent clinical and experimental research revealed that the gut microbiota is one of the foremost vital pathogenic variables in MetS [3]. Metabolic syndrome itself could be a phenotype caused by the interaction of host intrinsic variables such as genetics and the gut microbiome, and extrinsic components such as diets and the way of life. Metabolic syndrome is frequently accompanied by an imbalance of the gut microbiota, causes a low-grade inflammatory reaction within the body by wrecking the intestine barrier, creating insulin resistance through metabolites influencing host metabolism and hormone secretion, shaping a vicious circle that advances the persistent progress of MetS. Subsequently, intestinal microbiota may be a potential target for the treatment of MetS [4].

Trillions of microorganisms live in symbiosis within the human body, and are primarily found within the gastrointestinal system, oral mucosa, saliva, skin, conjunctiva, and vagina [5]. The number of the microorganisms; that occupy the gastrointestinal tract (i.e., gut microbiota); be around 1×10^{14} [6] and play a basic role in intestinal homeostasis, development, and protection against pathogens. In addition, their presence within the intestine is related to immunomodulatory and metabolic responses [7].

Gut microbiota comprises of microbes, yeasts, and viruses. Intestine bacteria are more than 1000 species that have related to six overwhelming phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia. The phyla Firmicutes and Bacteroidetes are the foremost common bacteria representing 90% of the gut microbiota [8].

Firmicutes bacteria are Gram-positive one. It plays a key role in the nutrition and metabolism of the host through SCFA synthesis. Through their metabolic products, Firmicutes bacteria are indirectly connected with other tissues and organs and regulate hunger and satiety. In contrast, Bacteroidetes bacteria are Gram-negative and associated with immunomodulation. Their components, lipo-poly-saccharides and flagellin, interact with cell receptors and enhance immune reactions through cytokine synthesis. Increased or decreased Firmicutes / Bacteroidetes (F/B) ratio are associated with the development of obesity or irritable bowel disease (IBD) [9].

Firmicutes, due to their negative influence on glucose and fat metabolism, are commonly referred to as bad gut microbes [10]. Accumulating evidence proved that short chain fatty acids (SCFA) play a critical role in supporting the intestine and metabolic health. The SCFA acetic acid, propionate and butyrate are the main metabolites produced by the microbiota in the large intestine through the anaerobic fermentation of indigestible carbohydrates; they play a crucial role to gastrointestinal health [11].

Microbial SCFA generation is basic for intestine integrity by controlling the luminal pH and mucus production. They are the main energy source for epithelial cells and speculated to play a key role in mucosal immune function. SCFA moreover straight forwardly modulate the metabolic health of the host through multiple neurochemical pathways related to energy expenditure, glucose homeostasis, appetite regulation, and immune-modulation [12].

This study aimed to identify the interplay of the intestinal microbiota with obesity, metabolic markers and some dietary ingredient; especially the fat content; among a sample of Egyptian women.

Subjects And Methods

A cross-sectional study included 115 Egyptian women, with ages ranged between 25 and 60 years; mean age 41.62 ± 10.70 years. They were recruited and randomly chosen, from all employees and workers; of all categories; of the “National Research Centre”, Egypt. A written informed consent was obtained from all participants after being informed about the purpose of the study. This research paper was derived from a cross-sectional survey of a project funded by National Research Centre (NRC) Egypt, 2019–2022 entitled “Gut Microbiota in Obesity and Metabolic syndrome among obese women: Interactions of the Microbiome, Epigenetic, Nutrition and Probiotic Intervention.” (12th Research Plan of the NRC), which was approved from

"Ethics Committee of NRC" (Registration Number is 19/236) . All methods were performed in accordance with the relevant guidelines and regulations.

Methods:

For each participated woman, blood pressure, anthropometric measurements, 24 hour dietary recall, laboratory investigations and microbiota analysis were done.

- ***Blood pressure***

Blood pressure was measured using the standardized mercury sphygmomanometer with a suitable cuff size. It was measured on the left arm while the participated women were sitting relaxed for 5 minutes. Two readings were obtained and the average was recorded. Systolic blood pressure (SBP); determined by the onset of the "tapping" korotkoff sounds (K1), while the fifth korotkoff sound (K5), or the disappearance of korotkoff sounds, as the definition of diastolic blood pressure (DBP) were recorded.

- ***Anthropometric measurements***

Body weight, height, neck, hip and waist circumferences were measured, following the recommendations of the "**International Biological Program**" [13]. Body weight (Wt) was determined to the nearest 0.01 kg using a Seca Scale Balance, with the woman wearing minimal clothes and with no shoes. Body height (Ht) was measured to the nearest 0.1 cm using a Holtain portable anthropometer. Circumferences was measured using non-stretchable plastic tape; approximated to the nearest 0.1 cm. Neck Circumference was measured at a point mid-way between the collarbone and chin in the middle of the neck while Standing or sitting with a straight back. Waist circumference (WC) was measured at the midpoint between the lower curvature of the last fixed rib and the superior curvature of the iliac crest, with the woman in an upright standing position and their arms alongside the body, feet together, and abdomen relaxed. Hip circumference was measured at the maximum extension of the buttocks measuring the largest diameter above the symphysis pubis overlapping the apex of the buttocks. Waist/ hip ratio [WC/ Hip C in cm] and Body mass index (BMI) [BMI: weight (in kilograms) divided by height (in meters squared)] were calculated. The participated women were all chosen as obese; as their BMI $\geq 30 \text{ Kg/m}^2$. The participant women were classified according to their BMI into 2 groups: 30 women with normal BMI (BMI=18<25 Kg/m²) and 82 obese women (BMI $\geq 30 \text{ Kg/m}^2$).

- ***Dietary recalls***

Information from each participant about her usual pattern of food intake was obtained. Data was collected by means of dietary interview consisting of 24 h recall that repeated for 3 days, and a food frequency questionnaire. Analysis of food items was done using World Food Dietary Assessment System, (**WFDAS**), **USA, University of California** [14].

- ***Blood sampling and laboratory investigations***

In the morning, venous blood samples (after 12-hour fasting) were drawn from the participated women, using venipuncture. Biochemical parameters were performed on fasting sera that were stored at -70 C° until

used for assessment of liver enzymes: Aspartate amino-transferase (AST) and Alanine amino-transferase (ALT), leptin, Short Chain Fatty Acids (SCFA), C-reactive protein (CRP), fasting blood glucose (FBG), insulin, and lipid profile. All were done in the laboratory of "Medical Excellence Research Center MERC" which is a part of "NRC", Egypt.

Serum concentrations of AST and ALT were determined using the automated clinical chemistry analyzer Olympus AU 400 analyzer (<https://www.mybiosource.com>).

The assay of human Leptin in serum was performed by ELIZA method, using kits of BioLegend, Inc., (San Diego – USA), according to the method of **Considine et al.** [15].

Human Short Chain Fatty Acids (SCFA) were assessed in serum using Enzyme Linked Immuno-sorbent Assay (ELISA) kits; Catalog Number: MBS7269061 according to the method described by **den Besten et al [16]**.

Fasting blood glucose (FBG) level was measured using the automated clinical chemistry analyzer Olympus AU 400 analyzer. Serum insulin was assessed using Enzyme Immunoassay Test Kit Catalog No. E29-072(**Immunospec Corporation**).

The assay of the serum CRP was performed by Enzyme Linked Immuno-sorbent Assay (ELISA) kits, Cat No.: RAP002 [17],(<https://www.mybiosource.com>.)

Estimation of lipid profile: Serum levels of total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C) were measured by standardized enzymatic procedures; using kits supplied by Roche Diagnostics (Mannheim, Germany) on the Olympus AU 400 automated clinical chemistry analyzer. Low density lipoprotein cholesterol (LDL-C) was calculated according to formula of **Friedewald et al. [18]** as follows: $LDL - C = Total\ cholesterol - Triglycerides/5 + HDL-C$.

Clinically, a patient is considered to have MetS when three or more of the following five conditions exist, which are (i) waist circumference ≥ 88 cm in women, (ii) blood pressure $\geq 135/85$ mmHg, (iii) triglycerides ≥ 150 mg/dl, (iv) HDL-C < 50 mg/dl in women, and (v) fasting glucose ≥ 100 mg/ dl [19].

- ***Microbiota analysis***

The proportion of Lactobacillus and Bifidobacteria; and Firmicutes/Bacteroidetes ratio strains were assessed in the stool of all participants by using the real time PCR (Polymerase Chain Reaction). Specimen collection and preparation: Stool was collected by defecation in a plain sterilized container allowed to be frozen. Specimen Storage and Preparation: stool was frozen on at -20° . The primers and probes were used to detect Bifidobacterium spp. and Lactobacillus spp; and Firmicutes spp. and Bacteroidetes spp., where based on 16S rRNA gene sequences retrieved from the National Center for Biotechnology Information databases by means of the Entrez program [20].

Reagents provided by kits: DNA extraction Kit. Assay procedure: DNA extraction: The QIAamp DNA Stool Minikit (Qiagen) was used to extract DNA from one gram of fresh or frozen stool sample according to the

manufacturer's instructions. Bacterial quantification by real-time PCR was done.

- **Statistical analysis**

Data were analyzed using the Statistical Package for Social Sciences (SPSS/Windows Version 18, SPSS Inc., Chicago, IL, USA). Normality of data was tested using the Kolmogorov-Smirnov test. The data were normally distributed. So, the parametric tests were used.

The participated women were classified into: 33 with normal BMI ($18- <25 \text{ Kg/m}^2$) and 82 obese with BMI $\geq 30 \text{ Kg/m}^2$. They obese women were classified according to the presence of MetS markers into two groups: 59 obese without MetS (have no or less than 2 markers of MetS), and 23 obese with MetS (have 3 or more markers of MetS).

The parametric data were expressed as mean \pm SE. The various parametric variables of the two groups were analyzed and compared using independent t test. Pearson's correlation test was used to assess the relations between Firmicutes/ Bacteroid ratio and the clinical and metabolic parameters, and between gut microbiota and daily intake of total fat, carbohydrate and fiber among the three groups. $P < 0.05$ was regarded as statistically significant for all tests.

Results

Table (1) showed the mean \pm SE of the age, blood pressure, anthropometric and body composition parameters of the studied sample. Data revealed highly significant difference between the three groups in most of the parameters at $p \leq 0.01$; where the obese women with MetS had the highest values. While the obese women without MetS had the significant highest values regarding WHR, NC and FFM ($p < 0.05$)

Table (2) showed the mean \pm SE of biochemical parameters of the three studied groups. The obese women with MetS had the significant highest values for the liver enzymes [Aspartate aminotransferase (AST) and the Alanine aminotransferase (ALT)], fasting blood glucose, insulin, and serum lipid profile; except the HDL-C which showed the insignificant lowest value compared to both the obese women without MetS and the control. The mean value of the C-reactive protein had the highly significant highest value among the obese women without MetS. The mean serum concentration of leptin hormone in the obese women with MetS was the significantly lowest compared to the other two groups. SCFA was insignificantly the highest among the obese women with MetS.

Table (3) showed the mean \pm SE of the log number and types of Microbiota among the three studied groups. Bacteroidetes bacteria were the most prevalent type among them, followed by the bad gut microbes Firmicutes, while Lactobacillus and Bifidobacteria were the least prevalent. There were significant differences between the log numbers of the 4 types of studied microbiota in the three groups at $p \leq 0.021$, 0.020, 0.031 respectively. However, insignificant difference was found between the three studied groups, as regard the Firmicutes / Bacteroidetes Ratio (0.72 ± 0.02 , 0.69 ± 0.03 and 0.73 ± 0.02 respectively) and the two beneficial; the Lactobacillus and Bifidobacteria.

Table (4) showed the mean \pm SE & % of the recommended daily allowances (RDAs) of nutrients intake of the studied women. The obese women with MetS consumed the significant highest percentage of calories represented by the high intake of proteins, total fats and carbohydrates with the lowest fiber intake compared to the other twogroups with significant difference at $p \leq 0.05-000$. The intake of vitamin A and D, potassium, calcium, zinc and iron was low in all groups compared to the RDAs, and it was the lowest among the obese women with MetS with significant difference; except for iron and zinc where there were insignificant differences. The intake of sodium was within the limits of the RDAs. For the daily calcium intake, significant difference was found between the obese women with and without Mets and the control at $p \leq 0.021$, while insignificant differences were detected for the other two minerals. Consumption of saturate fatty acid (SFAs) was high compared to the RDAs in all the groups, while the daily intake both the monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFAs) was the lowest among the obese women with MetS but with insignificant differences. Level of cholesterol intake was high among obese with and without Mets compared to the control with significant difference at $p \leq 0.033$. The obese women with and without MetS consumed high fat diet which contributed to 42.29–42.89% of the total caloric intake, significant difference at $p \leq 0.05$ compared to the control was recorded, with insignificant differences in the protein or carbohydrate intake (Table 5).

Table (6) showed the Pearson ρ s correlation coefficient between Firmicutes / Bacteroid Ratio and markers of MetS and other Biochemical parameters of the studied sample. Among the control group, Firmicutes / Bacteroid Ratio had significant positive correlations with SBP, WC, leptin and HDL-C, and significant negative correlations with AST, ALT, insulin, triglycerides and total cholesterol. These correlations became significantly negative with the waist circumference (WC), while significant positive correlation appeared with CRP among the obese group without MetS. Among obese group with MetS, Firmicutes / Bacteroid Ratio had significant positive correlations with total cholesterol and LDL-C and significant negative correlation with SCFA.

Table (7) showed correlation coefficient between gut microbiota and daily intake of total fat, carbohydrate and fiber among the studied groups .Log Bacteroidetes showed significant positive correlation with the daily fat intake and highly significant negative correlations with the carbohydrate and fiber intake in the control group. Log Lactobacillus had significant positive correlations with the carbohydrate and fiber intake among obese women without MetS. While, Log Bifidobacteria, Log Firmicutes and log Firmicutes/ Bacteroid Ratio had insignificant correlations with all the daily intake of total fat, carbohydrate and fiber among the three groups.

Discussion

Obesity has been a motivating force behind the raised medical interest in identification of MetS pathogenesis in western communities and, increasingly, in eastern countries [21].

Current data of this study reported by dietary history revealed that the obese participated women with MetS consumed the highest percentage of calories represented by the high daily intake of total fats and carbohydrates with lower fiber intake compared to the other two groups. This style of feeding was reflected

in the anthropological scales and biochemical parameters, as all of their measures were the highest among the three groups, which is evident in body mass index, waist circumference, as well as all the skin fold thicknesses, in addition to the obvious hyperglycemia, dyslipidemia and higher insulin and CRP concentrations. **Oda, [22] & Battault et al. [23]** reported that MetS involved a set of risk factors including obesity, hypertension, hyperglycemia, dyslipideamia, hyperuricemia and others. Uncontrolled MetS will eventually lead to non-alcoholic fatty liver (NAFLD), obstructive sleep apnea- hypo apnea syndrome (OSAHS), and other diseases. The pathogenesis of MetS is correlated with multiple factors, such as chronic inflammation, insulin resistance, oxidative stress and autonomic dysfunction.

Recently, the disturbance of gut microbiota has been discovered as a risk factor for MetS development [1]. The gut microbiota has developed a symbiotic relationship with the host involving the control of gene expression, gut barrier function, metabolism, nutrition and the general immunological function of the host [24]. Obesity is connected with changes in the relative abundance of the two dominant bacterial divisions; the Bacteroidetes and the Firmicutes; according to **Xiao & Kang, [25]**.

The results of this study are in agreement with what was previously reported where significant increase in Bacteroidetes and the Firmicutes bacteria over the other types (*Lactobacillus* and *Bifido* bacteria) was detected. However, interestingly, the data in the current study detected insignificant difference between the three groups for each type of the studied of microbiota. It is likely that the eating pattern of the control lean group was relatively high in fibers which are important for the growth of the beneficial microbiota. **Holscher [26]** stated that dietary fibers promote a healthy gut microbiome, and that the consumption of dietary fibers and probiotic can modulate the microbiota in the gastrointestinal tract.

Obese animals and humans have been found by research to possess a higher Firmicutes/Bacteroidetes ratio in their gut microbiota than normal-weight people, proposing that this ratio could be used as an obesity biomarker. As a result, the Firmicutes/Bacteroidetes ratio is recently acknowledged as a hallmark of obesity in the scientific literature [27]. Furthermore, the F/B ratio has been proposed as an important sign of the gut microbiota health [28]. This ratio has been connected to multiple clinical conditions, including those related to ageing [29] and others associated with obesity and metabolic syndrome [30]. However, there are some controversies regarding the composition of the gut microbial communities in obese individuals in different populations where several studies have shown contradictory results (for example, reduced F/B ratios in obese individuals [31, 32]. There are various reasons for these inconsistent results. Perhaps the association between F / B ratios and obesity depends on specific population, age group, gender, environmental and genetic factors[32], and as already mentioned, other phyla (Proteobacteria, etc.) has an important role. In addition, the number of specific bacterial species from the Firmicutes and Bacteroides phylums associated with obesity is limited. Firmicutes *R. bromii* is associated with obesity, utilizes and degrades more resistant starch than *Eubacteriumrectale*, *B. taiotaomicron*, and *Bifidobacterium adolescentis* [33]. Interestingly, certain *Bacteroides* species also carry various genes for carbohydrate-degrading enzymes [34].

In this context, the current study showed low Firmicutes / Bacteroidetes Ratio with only numerical difference between the three studied groups, the detected values were 0.72 and 0.69 in the obese without

and with MetS respectively compared to the control, 0.73. The lack to detect modest differences between healthy and obese subjects was in agreement with **Magne et al [27]** who suggested that the Firmicutes/Bacteroidetes ratio is not a robust marker of micro-biomedysbiosis associated with obesity. However, data revealed significant correlation between the F/B ratio and some markers of the metabolic syndrome represented by negative correlation with the waist circumference and positive correlation with C-reactive protein in the obese women without MetS, while significant positive correlations were detected with LDL-C and total cholesterol among obese women with MetS. Among the control group, F/B ratio had significant positive correlations with SBP, WC, leptin and HDL-C, and significant negative correlations with AST, ALT, insulin, triglycerides and total cholesterol. This may clarified the presence of changes in the relations between F/B ratio and the different markers of MetS among the obese and normal weight women. This has been shown in results that revealed the difference in the significant response between the biochemical parameters and the Firmicutes / Bacteroid Ratio in the control group. In the same time only few significant relations were detected among obese and MetS women. On this basis, this study indicates the importance of this ratio among Egyptian obese women with and without MetS.

The Present study showed significant positive correlation between Log Bacteroidetes with the daily fat intake, and highly significant negative correlations with the carbohydrate and fiber intake in the control group. However, Log Lactobacillus had significant positive correlations with the carbohydrate and fiber intake among obese women without MetS. On the other hand, Log Bifidobacteria, Log Firmicutes and log Firmicutes/ Bacteroidetes ratio had insignificant correlations with the entire daily intake of total fat, carbohydrate and fiber among the three groups.

Diet is one of the provocative factors in progression of obesity and is greatly linked to gut microbiota composition [35]. Nutrient intake and eating habits directly impact the composition, diversity, and metabolism of gut microbiota [35, 36]. As previously mentioned, dietary intake of both obese groups revealed high caloric intake with high fat intake and low fiber content. Many dietary patterns such as Western diet, Mediterranean diet, vegetarian diet, and the gluten-free diet have been shown to affect the discrete diversity of the gut microbiota which can disturb host metabolism [36]. The Western diet involves high intake of saturated fats, sugar, salt, refined grains and high fructose corn syrup with a low intake of fibers; it is highly related to obesity and metabolic diseases. The Western diet was found to promote inflammation and changes the profile of the gut microbiota from healthy to the obese pattern [37]. It also has been shown to decrease the total bacteria amount as well as the beneficial Lactobacillus species (sp.) and Bifidobacterium sp. in the gut [38].

The gut microbiota is involved in the development of obesity by direct interactions with proximal organs or indirect interactions with distant organs through metabolic products (mainly SCFAs) including communication with the liver, adipose tissue, and brain [39]. SCFAs can play a crucial role in the pathogenesis of obesity. They interact with adipose tissue via two G-protein-coupled receptors expressed in adipocytes (Gpr41 and Gpr43); this promotes adipocytes formation and inhibits lipolysis [40]. Furthermore, SCFAs down regulate the synthesis of the hunger-suppressing hormones leptin, peptide YY, and glucagon-like peptide 1[41]. SCFA influence lipid and glucose metabolism in order to provide energy for the host, proposing that they may have an impact on the occurrence of metabolic risk factors [42] .

Data of this study showed that the serum concentration of SCFA was mostly elevated among the obese women with MetS and in the same time showed negative association with the Firmicutes/ Bacteroidetes ratio. Also it was evident that the levels of liver enzymes and the serum insulin concentration were also the highest among obese MetS participants, while the level of the leptin hormone was the lowest, which agree with what was mentioned in the previous research.

Conclusion

It was concluded from this study that both Bacteroidetes and Firmicutes bacteria are the most abundant bacteria in the gut among the studied sample, whether in the obese with and without MetS or normal weights women. In addition the results confirm the low Firmicutes/ Bacteroid Ratio among all groups. No clear relations were found between this ratio and the fat, carbohydrate, fiber content of the diets. It was one of the most important findings of this research: the links between the short chain fatty acids which is the metabolic product of the gut microbiota and the promotion of obesity and the ensuing metabolic disorders.

Declarations

Conflict of Interest:

The authors declare that there are no financial and personal relationships with other people or organizations that could inappropriately influence (bias) the present work

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Author contribution

N.E.: conceived and designed the study; she is the PI of the project from which this data was derived. S.M.: wrote the draft of the article, S.A. and N.H.: statistical analysis and interpretation of the data, M.I.: responsible about the laboratory investigations, M.S., M.A.: supervision on collection of data and references. A.N., A.I., Kh.M.: collected the data. All authors contributed to the collection of references, drafting of the article and final approval of the version to be submitted. All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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Tables

Table (1): Characteristic anthropometric parameters and blood pressure of the studied women ANOVA

Parameters	Obese		P
	Obese without MetS N=59	Obese with Mets N=23	
	Control N=33		
Mean± SEM			
Age (year)	48.65±0.85	43.47±0.59	31.70±0.61 ^{b, c} 0.000***
Blood pressure			
SBP (mmHg)	115.38±0.45	137.50±0.53	105.00±0.39 ^{b, c} 0.000***
DBP (mmHg)	72.92±0.36	85.68±0.40	63.75±0.31 ^{b, c} 0.000***
Anthropometry			
Height (cm)	158.12±0.81	159.11±0.60	157.59±0.45 0.542
Weight (Kg)	91.82±0.63	100.10±0.94	49.04±0.83 ^{b, c} 0.000***
BMI (Kg/m ²)	36.19±0.48	40.01±0.93	19.55±0.53 ^{b, c} 0.000***
MWC (cm)	102.66±0.31	113.96±0.35	73.01±0.33 ^{b, c} 0.000***
Hip C (cm)	123.17±0.83	119.19±0.54	85.30±0.32 ^{b, c} 0.000***
WHR (cm/cm)	0.66±0.02	0.653±0.03	0.57±0.07 ^{b, c} 0.045*
Neck cir. (cm)	39.59±0.79	38.01±0.67	32.62±0.53 ^{b, c} 0.011**
Skin fold thickness			
TSF (mm)	31.71±0.12	33.00±0.17	17.30±0.15 ^{b, c} 0.000***
BSF (mm)	26.24±0.13 ^a	30.39±0.17	13.72±0.10 ^{b, c} 0.000***
Subscap.(mm)	31.41±0.16	34.32±0.19	14.40±0.11 ^{b, c} 0.000***
Supra-iliac (mm)	27.71±0.26 ^a	32.57±0.35	15.80±0.23 ^{b, c} 0.000***
Abd. SF (mm)	31.40±0.15	37.67±0.16	21.10±0.13 ^{b, c} 0.000***
Body composition:			
Body fat (%)	44.27±0.41	45.96±0.43	23.52 ±0.33 ^{b, c} 0.000***
FFM (kg)	53.67±0.49	49.93±0.52	37.43±0.37 ^{b, c} 0.000***
Body water (%)	36.55±0.61	39.29±0.66	27.41±0.72 ^{b, c} 0.000**

BMR (KJ)	6538.21±0.18	7034.13±0.17	48014.31±0.14 ^{b,c}	0.000**
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BMI: Body Mass Index, BMR: Basal Metabolic Rate, MWC: Minimal Waist Circumference, TSF: Triceps skin fold, BSF: Biceps skin fold, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure. a : Obese without MetS V Obese with Mets, b : Obese without MetS V Control, c : Obese with Mets V Control.

*Significant at $p \leq 0.05$, **highly Significant at $p \leq 0.01$

Table (2): Mean ±SE of Biochemical parameters of the three studied groups

Parameters	Obese No:59	Obese with Mets No:23	Control No:33	P
Mean ±SE				
ALT (U/L)	18.58±0.62	23.26±0.93	15.480.79 ^c	0.005**
AST(U/L)	21.03±0.44	25.69±0.35	19.12±0.87 ^c	0.001***
Leptin	216.82±0.42	191.18±0.31 ^a	253.30±0.47 ^{b,c}	0.017*
SCFA	14.84±0.04	17.47±0.06	12.21±0.01 ^c	0.063
CRP	723.42±0.65	693.35±0.74 ^a	390.85±0.79 ^{b,c}	0.000***
FBG (mg/dL)	114.66±0.03	130.13±0.34 ^a	86.33±.27 ^{b,c}	0.000***
Insulin (mIU/L)	12.21±0.07	16.73±0.04	7.93±0.03 ^{b,c}	0.001***
Lipid profile:				
T C (mg/dL)	195.57±0.45	208.52±0.39 ^a	177.82±0.41 ^{b,c}	0.020*
TG (mg/dL)	98.50±0.42	154.39±0.91 ^a	73.12±0.71 ^{b,c}	0.000***
HDL-C (mg/dL)	57.01±0.40	54.34±0.64	59.12±0.36	0.332
LDL-C (mg/dL)	117.55±0.51	123.26±0.43	100.73±0.31 ^{b,c}	0.033*

FBG: Fasting blood glucose, TC: Total Cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein – Cholesterol LDL-C: Low density lipoprotein-Cholesterol, ALT:Alanine aminotransferase , AST: Aspartate aminotransferase, SCFA: Short chain fatty acid, CRP: C-reactive protein. Significant between groups* $p \leq 0.05$, ** 0.01 . *** 0.001 - a : Obese without MetS V Obese with Mets, b : Obese without MetS V Control, c : Obese with Mets V Control. a : Obese without MetS V Obese with Mets, b : Obese without MetS V Control, c : Obese with Mets V Control.

Table (3) Mean± SE of the number and types of Microbiota among the three groups

Type of Microbiota	Obese	Obese with Mets No:23	Control	P
	No:59		No:33	
Mean± SE				
Log Lactobacillus	6.1096±0.096	5.8462±0.174	5.9163±0.068	0.207
Log Bifidobacterium	6.1352±0.091	6.1200±0.121	5.9403±0.088	0.337
Log Bacteroidetes	13.2459±0.196	13.0548±0.205	12.7845±0.167	0.256
Log Firmicutes	9.4530±0.194	8.9127±0.298	9.2845±0.185	0.281
P	0.021*	0.020*	0.031*	
Firmicutes/ Bacteroid Ratio	0.7197±0.016	0.6890±0.028	0.7313±0.018	0.456

*Significant at p < 0.05,

Table (4): Mean ± SE & %of the RDA of nutrients intake of the studied women.

Nutrient intake	Obese NO:59	Obese with Mets No:23	Control No:33	RDAs	P
	Mean± SEM&%of RDAs				
Energy (Cal)	2253.45±9.41 102.43	2520.13±8.32 114.55	c1906.89±6.74 86.68	2200	0.001***
Protein (g)	74.76±3.69 149.52	86.04±3.52 ^a 172.08	62.26±4.33 ^{b,c} 124.52	50	0.000***
Fat (g)	105.89±6.39 137.52	120.09±5.73 ^a 155.96	82.21±8.27 ^{b,c} 106.77	77	0.000***
Carbohydrate(g)	250.35±4.51 83.45	273.79±6.10 91.26	229.49±8.60 ^c 76.50	300	0.026*
Dietary fiber (g)	17.59±0.34 70.36	15.86±0.41 63.44	21.37±0.58 ^c 85.48	25	0.042*
Vit. A (µg)	498.65±0.07 62.33	476.92±0.13 59.62	638.25±0.02 ^c 79.78	800	0.031*
Vit. D (µg)	2.89±0.09 57.80	2.11±0.05 42.20	3.62±0.07 ^c 72.40	5	0.037*
Sodium (mg)	1506.12±21.11 100.41	1510.30±24.01 100.69	1101.26±31.04 ^{b,c} 73.42	1500	0.024*
Potassium (mg)	1520.80±16.25 76.04	1357.19±12.30 67.86	1610.69±11.73 ^c 80.53	2000	0.031*
Calcium (mg)	381.06±8.17 47.63	338.14±7.11 42.27	565.04±9.10 ^{b,c} 70.63	800	0.021*
Iron (mg)	5.22±0.50 65.25	4.87±0.30 60.88	5.93±0.20 ^c 74.13	8	0.051
Zinc (mg)	5.43±1.07 45.25	5.10±1.03 42.50	6.29±1.01 ^c 52.42	12	0.067

Sat. FA (g)	33.71±1.13 13.46	37.89±1.09 13.53	24.55±1.20 c 11.59	No more than 7% of Total Calories intake	0.065
MUFs (g)	29.02±0.04 11.59	24.07±0.05 8.60	29.88±1.40 c 14.10	12%-14% of Total Calories intake	0.079
PUFAs (g)	18.04±0.05 7.20	16.11±0.03 5.75	20.15±0.08 c 9.51	6%-8% of Total Calories intake	0.063
Cholesterol (mg)	238.30±3.17 119.15	264.73±6.72 132.37	192.42±9.15 b, c 96.21	200	0.033*

Significant between groups* $p \leq 0.05$, p**0.01.p***0.001- a : Obese without MetS V Obese with Mets, b : Obese without MetS V Control, c : Obese with Mets V Control.

Table (5): The percent of Calories delivered from fat, protein, and carbohydrates of total daily calories intake of the studied women.

Parameter	Obese without MetS	Obese with MetS	No:23	Control	P
	No:59	Percent	No:33		
Fat	42.29	42.89		38.80	0.047
Protein	13.27	13.65		13.06	0.218
Carbohydrates	44.44	43.46		48.14	0.054

Significance difference $p \leq 0.05$

Table (6): Pearson ρ s Correlation Coefficient between Firmicutes/ Bacteroidetes Ratio and markers of MetS and other Biochemical parameters of the studied sample

Parameters	Firmicutes/ Bacteroid Ratio					
	Obese without MetS		Obese with MetS		Control	
	No:59		No:23		No:33	
	R	p	r	p	r	p
SBP	- 0.151	0.284	-0.391	0.072	0.511	0.011*
DBP	- 0.079	0.579	-0.103	0.647	0.213	0.318
BMI	0.002	0.990	-0.231	0.289	0.101	0.595
MWC	- 0.272	0.037*	-0.145	0.51	0.443	0.014*
% Fat	0.009	0.948	-0.158	0.471	-0.295	0.114
AST	- 0.038	0.788	-0.005	0.981	-0.606	0.000**
ALT	- 0.028	0.840	-0.296	0.17	-0.619	0.000**
Leptin	- 0.061	0.663	-0.088	0.691	0.530**	0.001**
SCFA	0.029	0.837	-0.411	0.050*	-0.253	0.155
BCAA	0.027	0.851	-0.103	0.64	0.002	0.993
CRP	0.273	0.048*	-0.301	0.163	-0.055	0.759
FBG	- 0.218	0.116	-0.097	0.658	-0.326	0.064
Insulin	- 0.019-	0.892	-0.374	0.079	-0.439	0.011*
TG	0.007	0.963	0.3	0.164	-0.362	0.039*
HDL-C	0.233	0.093	-0.222	0.308	0.461	0.007**
LDL-C	- 0.048	0.734	0.485	0.019*	-0.342	0.051
TC	- 0.071	0.614	0.562	0.005**	-0.426	0.014*

FBG: Fasting blood glucose, TC: Total Cholesterol, TG: Triglyceride, HDL-C: High density lipoprotein – Cholesterol LDL-C: Low density lipoprotein-Cholesterol, ALT: Alanine aminotransferase , AST: Aspartate aminotransferase, SCFA: Short chain fatty acid, CRP: C-reactive protein

Significance difference * $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.000$

Table (7): Pearson ρ s Correlation Coefficient between gut microbiota and daily intake of total fat, carbohydrate and fiber of the studied groups

	Log Lactobacillus		Log Bifidobacterium		Log Bacteroidetes		Log Firmicutes		Log Firmicutes/ Bacteroid Ratio	
	r	p	r	p	R	p	r	p	r	p
Obese without Mets										
Fat	0.064	0.631	- 0.002	0.990	0.090	0.499	- 0.100	0.449	- 0.163	0.217
Carb	0.263	0.045*	- 0.080	0.547	0.098	0.461	0.002	0.991	- 0.072	0.585
Fiber	0.282	0.031*	- 0.029	0.826	0.099	0.454	0.081	0.540	- 0.008	0.951
Obese with Mets										
Fat	0.025	0.908	- 0.234	0.282	- 0.095	0.666	0.004	0.987	0.037	0.887
Carb	0.112	0.609	- 0.235	0.279	0.015	0.945	0.093	0.672	0.088	0.680
Fiber	0.140	0.525	- 0.148	0.500	0.088	0.756	- 0.168	0.442	- 0.167	0.447
Control										
Fat	0.045	0.402	- 0.279	0.058	0.312	0.038*	- 0.132	0.231	- 0.265	0.068
Carb	- 0.108	0.275	- 0.192	0.142	- 0.462	0.003**	- 0.138	0.223	0.097	0.295
Fiber	- 0.228	0.101	- 0.179	0.159	- 0.480	0.002**	- 0.180	0.158	0.072	0.345

Significance difference *p≤0.05 **p≤0.01