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# Fecal Bacteria Alteration in Adult Obese Egyptian; *Escherichia coli* and Its Relationship with Body Composition and Blood Lipids

Elham M. Youssef Elabd<sup>1\*</sup>, Wafaa A. Kandeel<sup>2</sup>, Heba A. Elmall<sup>3</sup>,  
Hatim A. El-Baz<sup>1</sup>, K. H. El-Wakeel<sup>2</sup> and Ola M. Abdel Samie<sup>4</sup>

<sup>1</sup>Department of Biochemistry, National Research Centre, 33 Elbuhouth St. Dokki, Cairo,  
Postal Code 12622, Egypt.

<sup>2</sup>Department of Biological Anthropology, National Research Centre, 33 Elbuhouth St. Dokki, Cairo,  
Postal Code 12622, Egypt.

<sup>3</sup>Department of Medical Biochemistry, National Research Centre, 33 Elbuhouth St. Dokki, Cairo,  
Postal Code 12622, Egypt.

<sup>4</sup>Department of Child Health, National Research Centre, 33 Elbuhouth St. Dokki, Cairo,  
Postal Code 12622, Egypt.

### Authors' contributions

This work was carried out in collaboration between all authors. Author EMYE designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors WAK, HAE and HAEB managed the analyses of the study. Authors EMYE and OMAS managed the literature searches. Author KHEW performed the statistical analysis. All authors read and approved the final manuscript.

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## ABSTRACT

**Aims:** To evaluate the differences of culturable gut bacterial flora (aerobes and facultative anaerobes) in fecal samples of obese and normal weight groups of adult Egyptian, and to compare *Escherichia coli* number in both groups to determine whether alterations in blood lipid level, body mass index, fat percentage, and C-reactive protein can be explained by such obesity induced dysbiosis.

\*Corresponding author: E-mail: elhamelabd@yahoo.com;

**Study Design:** Quantitative determination of fecal bacteria and anthropometric measurements were carried out in selected obese and normal weight subjects of adult Egyptian; in addition to the analysis of blood lipid levels and other biochemical parameters.

**Place and Duration of Study:** Department of biochemistry, department of microbiology and immunology, National Research Centre, between May 2016 and May 2017.

**Methodology:** We studied forty-seven female subjects over the age of 20. They were divided into two groups which were identified as obese group (BMI  $\geq 30$  kg m<sup>2</sup>), and control group (BMI 19 -25 kg m<sup>2</sup>). Twelve hour fasting blood samples were collected for biochemical analysis, fecal samples were collected in the morning for bacteria cultivation and *E. coli* colony counting. Anthropometric measurements were evaluated in all subjects.

**Results:** Data analysis revealed variations in gut flora composition (*Clostridium*, *Enterococcus*, *Klebsiella*, and *E. coli*), lipid profile, and C-reactive protein between obese and control groups. Moreover, it showed a significant increase in colonies of *E. coli* species in obese subject when compared with control (p 0.05) and a positive significant correlation between log number of *E. coli* and serum total lipids (r = 0.45; p 0.01), body weight (r = 0.4; p 0.02), chest circumference (r = 0.5; p 0.04), hip circumference (r = 0.4; p 0.03).

**Conclusion:** Our studies suggest that the gut bacteria, *E. coli*, may play an important role in body weight gain and blood lipid levels. Therefore our findings support the potential of therapies altering the gut microbiome to control metabolic disorders.

**Keywords:** Obesity; gut flora; *E. coli*; C-reactive protein; blood lipid.

## 1. INTRODUCTION

It is a well-known fact that the primary individual gut microbiome colonization initially starts through microbial transmission from mother to fetus. Subsequent reshaping of the microbial landscape is then clearly influenced by a series of complicated and dynamic interactions throughout life [1]. Gestational age, methods of delivery (by Caesarean section or natural), diet (breastfeeding or infant formula), hygiene, and antibiotic treatment are factors that are believed to participate in modulating the colonization.

The transient changes in the intestinal ecosystem occur throughout life in some cases can result in the disruption of microbial–host symbiosis and can trigger various metabolic inflammatory disorders [2-5] which have been hypothesized to lead to the development of obesity, insulin resistance and concomitant effects on plasma lipids [3].

Over the last several years, various approaches have been suggested the essential role of gut microbiome in maintaining host physiology through different mechanisms, specifically digestion and degradation of complex nutrient substances, the development and stimulation of the immune system response of the host [6]. They also pointed, that human flora composition does not vary greatly from individual to individual and the degree of identified variation among them is commonly dominated by four major

bacterial phyla (Human Microbiome Project Consortium, 2012) [7]. These phyla are Bacteroidetes, e.g., *Bacteroides* and *Prevotella*, Firmicutes, e.g., *Clostridium*, *Enterococcus*, *Lactobacillus*, *Ruminococcus*, *Actinobacteria*, e.g., *Bifidobacterium*, and *Proteobacteria*, e.g., *Escherichia*, *Enterobacter*, *Proteus*, *Citrobacter*, *Klebsiella*. [8]. *Enterobacteriaceae* are facultative anaerobic, Gram-negative rods that are catalase-positive and oxidase-negative [9]. *Escherichia coli* (*E. coli*) a member of the *Enterobacteriaceae* family, is a common colonizer of the human intestine, with an average of five commensal *E. coli* strains found in a human digestive tract. In healthy individuals *Enterobacteriaceae* constitute only a small fraction (less than 1%) of the gut microbiota. However, Hold, G. L., et al. in 2014 [10] and others reported that *E. coli*, become dominant in the gut microbiota of individuals with IBD and in several animal models of gut inflammation [11]. Moreover, *E. coli* numbers were higher in women with excessive weight gain than in women with normal weight gain during pregnancy [12].

To date the relationship between *E. coli* and obesity is still unclear and needs more investigations. We thought that targeting such gut microbiota might be a promising strategy for the prevention and treatment of some human diseases believed to be affected by the development of dysbiosis. Therefore we undertook this study to identify variations in gut bacteria among some obese and normal weight

adult Egyptian, and find the correlation between *E. coli* and body weight, fat distribution, and lipid profile.

## 2. MATERIALS AND METHODS

Forty seven female subjects over the age of 20 years participated in this study. The study was provided an approval number (17096) by the National Research Centre ethical committee and all subjects were given a written informed consent to participate in our research work. Subjects were excluded from the study if any of the following conditions are present; diabetes, use of any antibiotics within the last 3 months, liver or kidney diseases or major medical or surgical event within 6 months. Two groups were identified as follows; obese group (BMI  $\geq 30$  kg m<sup>2</sup>), and control group (BMI 19 -25 kg m<sup>2</sup>). Fecal and 12- hour fasting blood samples were collected from all subjects.

### 2.1 Bacteria Cultivation and *E. coli* Counting

Bacterial cultivation method is used to investigate the different cultivable gut microbiota in all fecal samples, followed by *E. coli* counting.

Fecal samples were collected in the morning (10 g) followed by homogenization. Serial dilution was carried out in sterile solution in duplicate using selective media within 2 h after collection. Blood agar plates, MacConkey agar plates as well as mannitol salt agar plates were used. All samples were streaked in duplicate plates and incubated aerobically and anaerobically for isolation of aerobic, as well as anaerobic bacteria found in samples. Aerobic plates were incubated at 37°C for 24 hrs. However, anaerobic plates were incubated anaerobically in an anaerobic jar and were incubated at 37°C for 3-5 days. Identification of bacterial isolates was carried out microscopically [13], as well as biochemically [14]. For Gram positive coccobacilli the method used was according to Quinn et al. 2002 [15] and for Gram negative bacteria the method used was according to Cruickshank et al. 1975 [13]. *E. coli* was then counted and the number was converted to log value [16].

### 2.2 Blood Samples Collection and Biochemical Analysis

Blood samples were collected from all subjects after 10-12 hours fasting. Serum total cholesterol was measured by CHOD-PAP-enzymatic colorimetric method [17], triglyceride and high

density lipoprotein cholesterol (HDLc) were determined by auto analyzer Olympus 400 [18, 19], low density lipoprotein cholesterol (LDLc) was calculated according to the equation, as follows:

$$\text{LDLc} = \text{Total cholesterol} - \text{HDLc} - (\text{Triglyceride}/5) \text{ [20].}$$

C - reactive protein concentration (CRP) was determined by High Sensitivity Enzyme Immunoassay kit purchased from BIOS Company.

### 2.3 Anthropometric Measurements

Anthropometric measurements including height, weight, mid upper arm circumference, waist circumference, and hip circumference were taken by practitioners. Height was measured to the nearest 0.1 cm in the standing position and head in the Frankfort plane using fixed stadiometer (Seca, Japan). Body weight was measured in light clothing to the nearest 0.1 kg. Mid upper arm circumference was measured at the midpoint between the acromion process and the olecranon. Waist circumference was measured at the midpoint between the lower rib margin and the iliac crest, with the participant standing at the end of normal expiration. Hip circumference was measured at the level of the greater trochanters with the individual wearing minimum clothing. Non-stretchable tape was used for measurement of the three circumferences. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. The WHR was calculated. The mean of three consecutive measurements of each anthropometric measure was evaluated using standardized equipment and following the recommendations of the International Biological Program [21]. Skin fold thickness as: Triceps, Biceps, Subscapular and Suprailiac skin folds were measured to the nearest 0.1 mm using skinfold caliper (Holtain, Crymych, UK). Body composition was measured using the Tanita BC-418 MA Segmental Body Composition Analyzer (Tanita, Japan). It prints out a complete body composition profile including weight, BF%, body fat mass and BMI, and visceral fat rating, in which the rate from 1 to 12 indicates a healthy level of visceral fat and that from 13 to 59 indicating an excess level of visceral fat [22,23].

### 2.4 Statistical Analysis

Quantitative data were expressed as the mean  $\pm$  S.E.M. Microbial counts were expressed as

log<sub>10</sub> microorganisms per gram of wet weight feces. Statistical analyses were performed with (SPSS) statistical software using student's t-tests. All tests were two-tailed paired, and the level used to establish significance was  $P < 0.05$ .

### 3. RESULTS AND DISCUSSION

#### 3.1 Results

In the present work, we investigated the fecal gut flora of 47 subjects, 29 of them were obese and 18 were normal control. Our data showed microbial composition changes in Firmicutes and Proteobacteria phyla in the studied fecal samples. Clostridium and Enterococcus represented Firmicutes were detected in 60% of obese group while Klebsiella and *E. coli* represented proteobacteria phylum were detected in 65% and 80%.

As expected, our obese subjects with such gut flora alteration showed significant ( $P=0.05$ ) increases in weight:  $98.1 \pm 12$ , body mass index:  $33.5 \pm 2.6$ , and fat mass  $32.7 \pm 4$ , along with significant increases in fat measurements in some depots such as chest, waist circumferences:  $116.7 \pm 11$ ,  $109.4 \pm 8.3$ , hip:  $127.6 \pm 8.4$ , trunk fat %  $34.6 \pm 1.5$ , compared with control group ( Table 1, Fig. 1.).

Biochemical analysis of fasting venous blood sample in both groups showed significant ( $P=0.05$ ) increase in total lipids:  $593 \pm 58$  mg/dl,

total cholesterol (Tc):  $220 \pm 40$  mg/dl; triglycerides (TG):  $171 \pm 40$  mg/dl, LDLc  $183 \pm 4.4$  mg/dl, CRP:  $686 \pm 30$ , and *E. coli* log number/g feces  $6.4 \pm 0.4$  in obese group compared with control group, while there was significant ( $P=0.05$ ) decrease in HDLc. (Table 2, Fig. 2 & Fig. 3).

Positive correlation between *E. coli* log number and total lipids  $p 0.01$   $r = 0.45$ , body weight  $p 0.04$   $r = 0.32$ , and some fat depots represented by chest circumference  $p 0.028$   $r = 0.44$ , hip circumference  $p 0.03$   $r = 0.21$  (Figs. 4, 5, 6, 7).

#### 3.2 Discussion

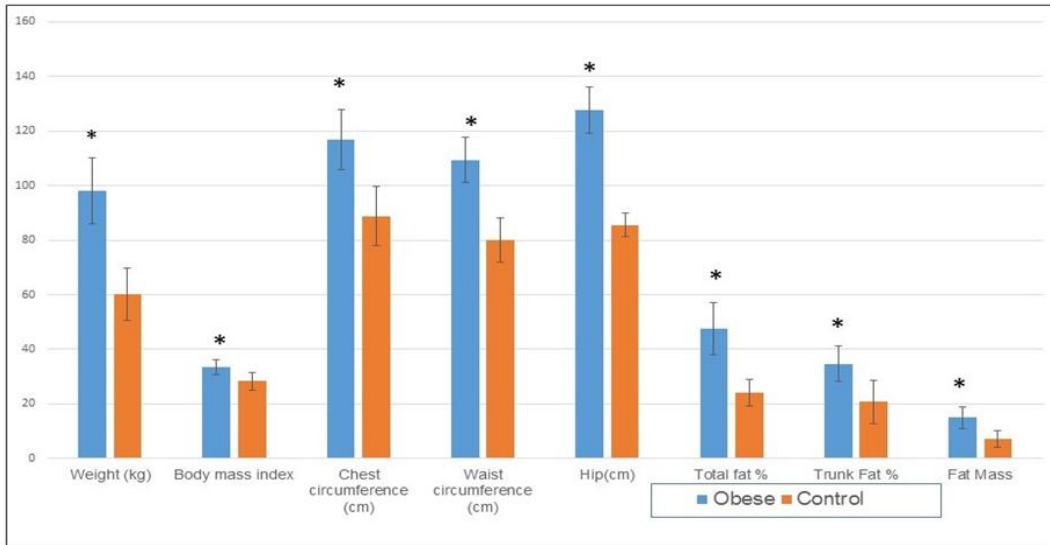
In our study the changes in Clostridium, Enterococcus, *Klebsiella*, and *E. coli* represented Firmicutes and Proteobacteria phyla were consistent with previous studies carried out on dogs in 2015 [24] and are in line with Mujico et al.'s and Possemiers et al.'s 2009 who found clear alterations associated with switching to the high-fat diet, including a decrease in Bacteroidetes and an increase in both Firmicutes and Proteobacteria in mice [25,26].

The increase in blood lipid levels and body weight in our obese subjects was significant ( $p=0.05$ ) and related to the microbial composition changes [5,6,23]. The most probable explanations for that are the increased glucose absorption in the intestines, energy extracted from non-digestible consumed food and

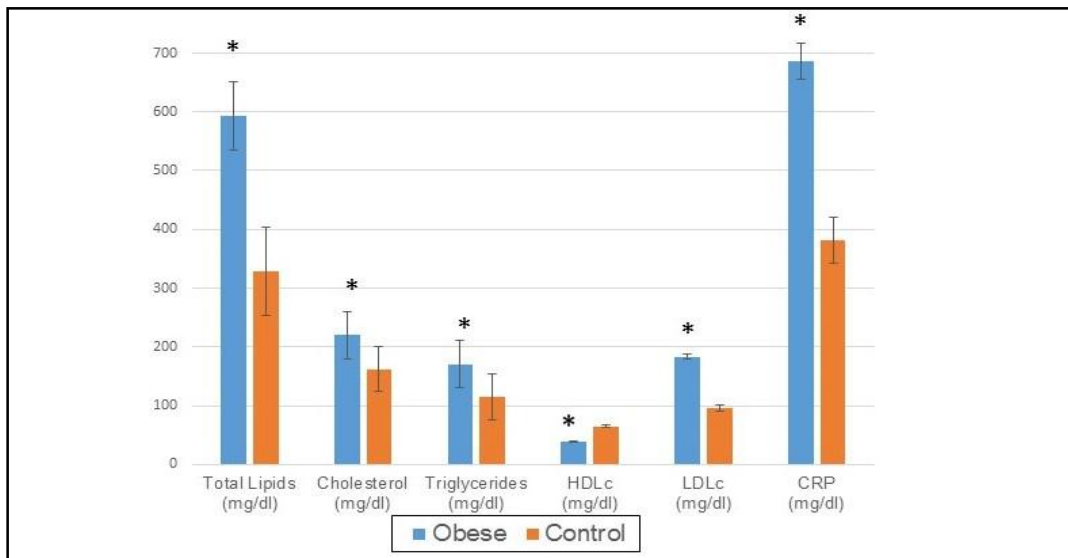
**Table 1. The mean and S.E.M of anthropometric measurements of obese and control groups**

| Parameter                           | Obese             | Control         |
|-------------------------------------|-------------------|-----------------|
| Height(cm)                          | $158 \pm 14$      | $157.8 \pm 9.7$ |
| Weight(kg)                          | $98.1 \pm 12^*$   | $60.1 \pm 9.6$  |
| Body mass index                     | $33.5 \pm 2.6^*$  | $28.3 \pm 3.2$  |
| Mid upper arm circumference(cm)     | $102.3 \pm 7$     | $88.6 \pm 7.7$  |
| Chest circumference(cm)             | $116.7 \pm 11^*$  | $88.8 \pm 10.8$ |
| Waist circumference(cm)             | $109.4 \pm 8.3^*$ | $80.1 \pm 8.1$  |
| Hip(cm)                             | $127.6 \pm 8.4^*$ | $85.5 \pm 4.3$  |
| Thigh(cm)                           | $57.04 \pm 3.5$   | $40.8 \pm 2.6$  |
| Leg(cm)                             | $30.5 \pm 7.5$    | $24.8 \pm 7.7$  |
| Triceps skin fold thickness(mm)     | $23 \pm 8.3$      | $18.3 \pm 3.6$  |
| Biceps skin fold thickness(mm)      | $32.2 \pm 6.9$    | $24.5 \pm 5.2$  |
| Subscapular skin fold thickness(mm) | $29 \pm 5$        | $19.6 \pm 5.1$  |
| Suprailiac skin fold thickness(mm ) | $37.2 \pm 8$      | $24 \pm 7.0$    |
| Total fat %                         | $47.7 \pm 9.6^*$  | $24.1 \pm 5.0$  |
| Total body water(Kg)                | $37.1 \pm 5.7$    | $32.9 \pm 6.4$  |
| Visceral fat rating                 | $14 \pm 3.6$      | $10.5 \pm 10$   |
| Trunk fat %                         | $34.6 \pm 1.5^*$  | $20.7 \pm 2$    |
| Fat mass(Kg)                        | $32.7 \pm 4^*$    | $15.7 \pm 0.3$  |
| Fat Free Mass(Kg)                   | $40.5 \pm 4.1$    | $54.6 \pm 4.9$  |

\* Significance in the anthropometric measurements of obese and control groups .



**Fig. 1. Anthropometric measurements of obese and control groups**  
 Measurements: significant from normal control, \*  $P < 0.05$   
 Mean  $\pm$  S.E.M = Mean values  $\pm$  Standard error of means.

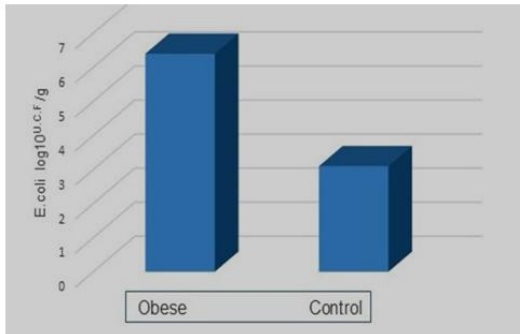


**Fig. 2. Mean and S.E.M of biochemical parameters of obese and control groups**  
 Measurements: significant from normal control, \*  $P < 0.05$   
 Mean  $\pm$  S.E.M = Mean values  $\pm$  Standard error of means.

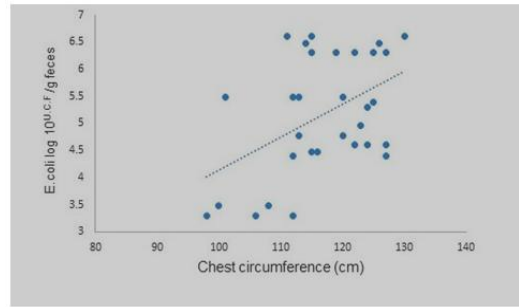
activation of certain enzyme activity enhancing denovo lipogenesis [26]. These data together with the associated significant ( $P=0.05$ ) increases in anthropometric measurement such as ,body mass index, and fat mass, chest ,waist circumferences, and trunk fat (Table 1, Fig. 1.) support the role of gut microorganisms in the regulation of energy homeostasis and the impact

of their imbalance on the amount of accumulated body fat and metabolic phenotype [26-30].

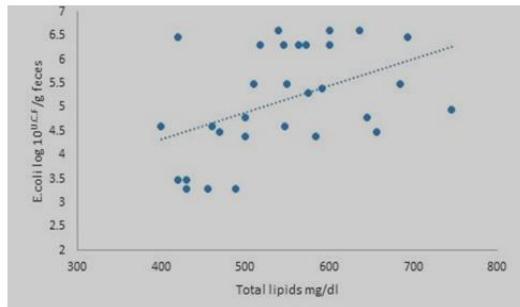
As *E. coli* found in the majority of the obese individuals in our study, we investigated whether they related to adiposity changes, body composition, blood lipid levels, and inflammation among individuals.



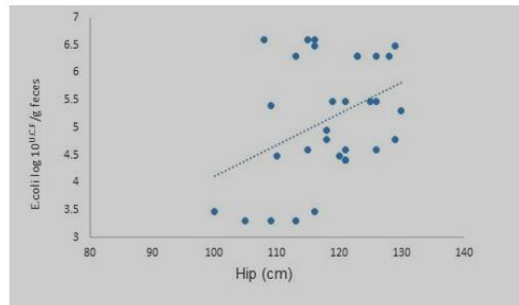
**Fig. 3. *E. coli* log $10^{U.C.F}$  /g feces of obese and control groups.**  
*E. coli* log  $10^{U.C.F}$  significant from normal control, \*  
 $P = 0.05$



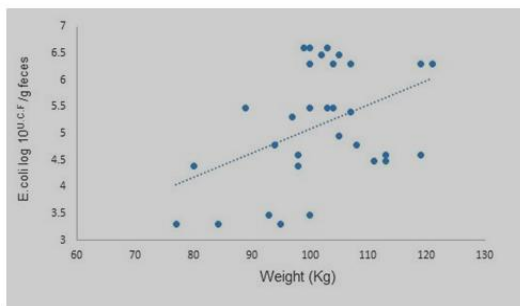
**Fig. 6. Positive correlation between *E. coli* log $10^{U.C.F}$  /g and chest circumference (cm) in obese group**



**Fig. 4. Positive correlation between *E. coli* log $10^{U.C.F}$  /g and serum total lipids mg/dl in obese group**



**Fig. 7. Positive correlation between *E. coli* log $10^{U.C.F}$  /g and hip circumference (cm) in obese group**



**Fig. 5. Positive correlation between *E. coli* log $10^{U.C.F}$  /g and body weight (Kg) in obese group**

The significant increase of *E. coli* number associated with obese group subjects in our experiment, even though differs from some published in 2013 by Million et al. [31], it is consistent with those by Santacruz, A et al. 2010; Qiao et al. 2013, and Zhu, L et al. 2013 [32,33].

**Table 2. The mean and S.E.M of biochemical parameters and *E. coli* log number of obese and control groups**

| Parameter                              | Obese      | Control   |
|--|------------|-----------|
| Total Lipids                           | 593±58**   | 329±75    |
| Cholesterol                            | 220±40*    | 162±58    |
| Triglycerides                          | 171±40*    | 115±4.0   |
| HDL                                    | 38±1.2*    | 65±1.9    |
| LDL                                    | 183±4.4*   | 96±5      |
| VLDL                                   | 28±2       | 26±1      |
| CRP                                    | 686 ± 30*  | 381 ± 39  |
| Log. number of <i>E. coli</i> /g feces | 6.4 ± 0.4* | 3.1 ± 0.2 |

\* Significance in the biochemical parameters of obese and control groups.

However, our results point to the potential importance of *E. coli* in weight gain through increasing of the capacity of energy harvest from food, as we could also show a correlation between its log number and total lipids  $p 0.01$   $r = 0.45$ , body weight  $p 0.04$   $r = 0.32$ , and some fat depots represented by chest circumference  $p 0.028$   $r = 0.44$ , hip circumference  $p 0.03$   $r = 0.21$  (Figs. 4, 5, 6, 7).

The significant increase of C –reactive protein levels in obese group fairly well with Dev, N., & Marcus in 2012 [34] and further support the relationship between obesity and inflammation induced by LPS in gut microbes. The increase in CRP levels indicates a state of low-grade inflammation and can be explained by different mechanisms: 1- during consumption of a high-fat diet, the gut microbiota is modified, which leads to increases in gut permeability and in the systemic levels of bacterial products such as LPS. Additionally, excess fat intake triggers an increase in chylomicrons in the intestine during the postprandial period (following a meal), which favors LPS infiltration into the circulation. Once they reach the systemic circulation, LPS infiltrate tissues such as liver and adipose tissue, triggering an innate immune response and low-grade inflammation through endotoxin toll like receptor 4 axis [26,35]. Another possible mechanism is that *E. coli*, produces indol that have been suggested to interact with host signaling pathways and thus affect host immunity through interacting with inflammation-related processes in the human host [36].

In accordance with our findings some researches confirmed the positive correlation between weight gain and fattening on one side and the increase in *E. coli* on another side emphasizing the inflammatory response that appears, in part, to be mediated by adipose tissue [37-38].

#### 4. CONCLUSION

In conclusion we have provided further evidence about the important role of gut microbiota in metabolism and metabolic inflammation. Moreover findings of this study have highlighted the correlation of altered gut bacteria with blood lipid levels and the accumulation of body fat. Our research have stressed the correlation between *E. coli* specifically and adiposity, supporting the idea of its potential impact on fat mass and weight gain. Despite the limited sample size and the bacteria counting method used in this study, results so far have been very promising for future work on gut microbiota as a new strategy of obesity treatment and prevention.

#### CONSENT

All authors hereby declare that all experiments have been examined and approved by the ethics committee of the National Research Centre /Egypt with number (17096) and have therefore been performed in accordance with the ethical

standards laid down in the 1964 Declaration of Helsinki.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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