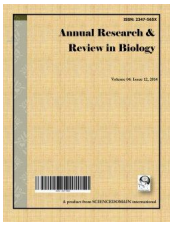

Annual Research & Review in Biology

17(6): 1-9, 2017; Article no.ARRB.36386
ISSN: 2347-565X, NLM ID: 101632869

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

Elham M. Youssef Elabd[1*], Wafaa A. Kandeel[2], Heba A. Elmalt[3], Hatim A. El-Baz[1], K. H. El-Wakeel[2] and Ola M. Abdel Samie[4]

[1]Department of Biochemistry, National Research Centre, 33 Elbuhouth St. Dokki, Cairo, Postal Code 12622, Egypt.
[2]Department of Biological Anthropology, National Research Centre, 33 Elbuhouth St. Dokki, Cairo, Postal Code 12622, Egypt.
[3]Department of Medical Biochemistry, National Research Centre, 33 Elbuhouth St. Dokki, Cairo, Postal Code 12622, Egypt.
[4]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.

Article Information

DOI: 10.9734/ARRB/2017/36386
Editor(s):
(1) Eshrat Halim, University of Toronto,Canadian Diabetes Association, Canada and Department of Laboratory Medicine, All India Institute of Medical Sciences, New Delhi, India.
(2) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Shorena Khetsuriani, Tbilisi State Medical University, Georgia.
(2) Gabor Ternak, University of Pécs, Hungary.
(3) Wagih Mommtaz Ghannam, Mansoura University,Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/21193

Original Research Article

Received 26th August 2017
Accepted 19th September 2017
Published 2nd October 2017

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 ≥30 kg m^2), and control group (BMI 19 -25 kg m^2). 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, Klespsiella. [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 ≥30 kg m^2), and control group (BMI 19 -25 kg m^2).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:

LDLc = Total cholesterol - HDLc - (Triglyceride/5) [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 ± S.E.M. Microbial counts were expressed as

log10 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 ±12, body mass index: 33.5±2.6, and fat mass 32.7±4, along with significant increases in fat measurements in some depots such as chest, waist circumferences: 116.7±11, 109.4±8.3, hip: 127.6±8.4, trunk fat % 34.6±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 ± 58 mg/dl, total cholesterol (Tc): 220 ± 40 mg/dl; triglycerides (TG): 171 ± 40 mg/dl, LDLc 183 ± 4.4 mg/dl, CRP: 686 ± 30, and *E. coli* log number/g feces 6.4±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 ±14	157.8±9.7
Weight(kg)	98.1±12*	60.1±9.6
Body mass index	33.5±2.6*	28.3±3.2
Mid upper arm circumference(cm)	102.3±7	88.6±7.7
Chest circumference(cm)	116.7±11*	88.8±10.8
Waist circumference(cm)	109.4±8.3*	80.1±8.1
Hip(cm)	127.6±8.4*	85.5±4.3
Thigh(cm)	57.04±3.5	40.8±2.6
Leg(cm)	30.5±7.5	24.8±7.7
Triceps skin fold thickness(mm)	23±8.3	18.3±3.6
Biceps skin fold thickness(mm)	32.2±6.9	24.5±5.2
Subscapular skin fold thickness(mm)	29±5	19.6±5.1
Suprailiac skin fold thickness(mm)	37.2±8	24±7.0
Total fat %	47.7±9.6*	24.1±5.0
Total body water(Kg)	37.1±5.7	32.9±6.4
Visceral fat rating	14±3.6	10.5±10
Trunk fat %	34.6±1.5*	20.7±2
Fat mass(Kg)	32.7±4*	15.7± 0.3
Fat Free Mass(Kg)	40.5±4.1	54.6±4.9

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

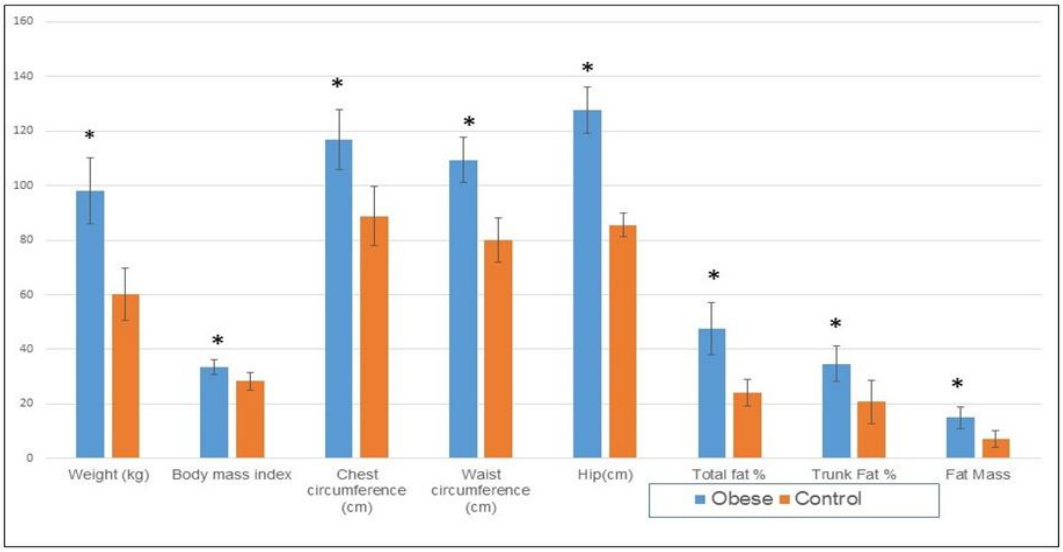

Fig. 1. Anthropometric measurements of obese and control groups
*Measurements: significant from normal control, * P < 0.05*
Mean ± S.E.M = Mean values ± Standard error of means.

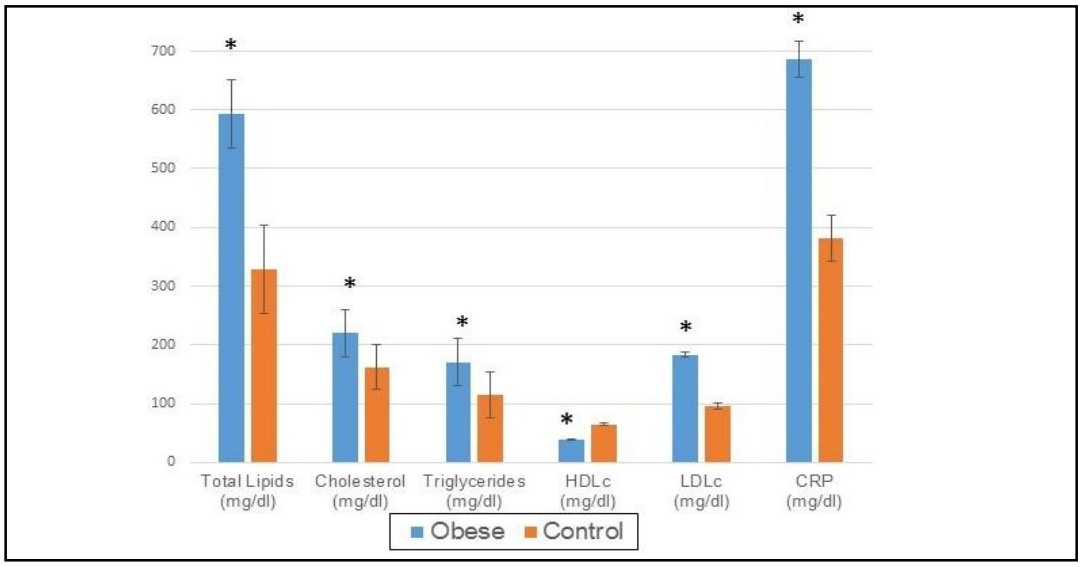

Fig. 2. Mean and S.E.M of biochemical parameters of obese and control groups
*Measurements: significant from normal control, * P < 0.05*
Mean ± S.E.M = Mean values ± Standard error of means.

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.

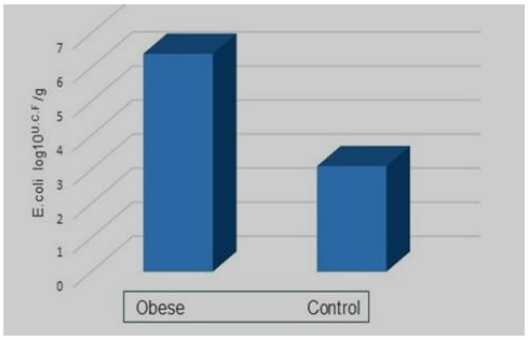

Fig. 3. *E. coli* log10$^{U.C.F}$ /g feces of obese and control groups.

*E. coli log 10$^{U.C.F}$ significant from normal control, * P = 0.05*

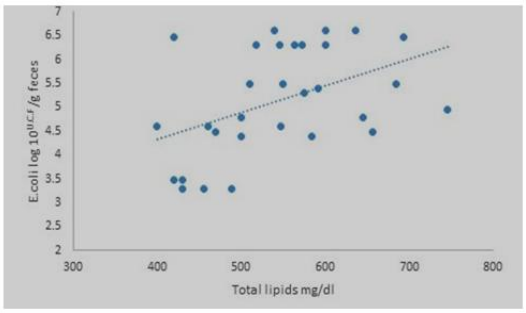

Fig. 4. Positive correlation between *E. coli* log10$^{U.C.F}$ /g and serum total lipids mg\dl in obese group

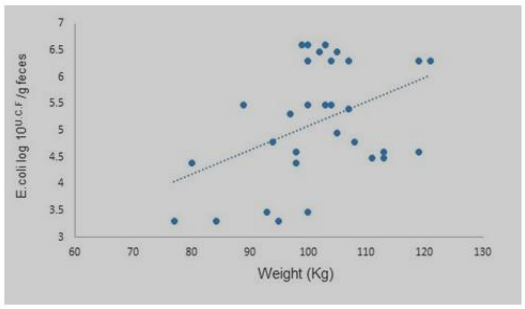

Fig. 5. Positive correlation between *E. coli* log10$^{U.C.F}$ /g and body weight (Kg) in obese group

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 consistent with those by Santacruz, A et al. 2010; Qiao et al. 2013, and Zhu, L et al. 2013 [32,33] .

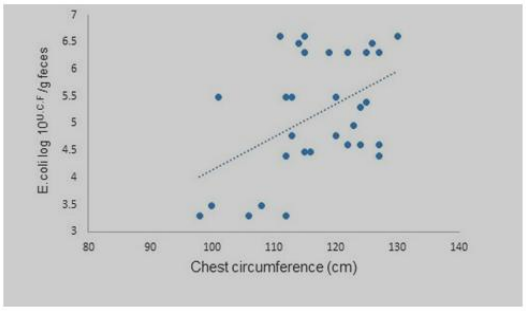

Fig. 6. Positive correlation between *E. coli* log10$^{U.C.F}$ /g and chest circumference (cm) in obese group

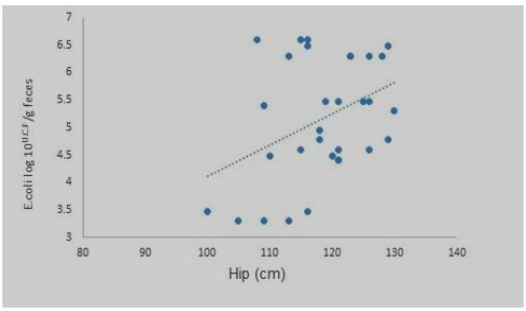

Fig. 7. Positive correlation between *E. coli* log10$^{U.C.F}$ /g and hip circumference (cm) in obese group

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 *E. coli* /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.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Ehab Ali Fouad: Department of Microbiology and Immunology, National Research Centre, Egypt for his contribution in the microbiology part of this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rodríguez JM, Murphy K, Stanton C, Ross RP, Kober OI, Juge N, Avershina E, Rudi K, Narbad A, Jenmalm MC, Marchesi JR. The composition of the gut microbiota throughout life, with an emphasis on early life. Microbial Ecology in Health and Disease. 2015;26(1):26050.
2. Hildebrandt MA, Hoffmann C, Sherrill–Mix SA, Keilbaugh SA, Hamady M, Chen YY, Knight R, Ahima RS, Bushman F, Wu GD. High-fat diet determines the composition of the murine gut microbiome independently of obesity. Gastroenterology. 2009; 137(5):1716-24.
3. Wu X, Ma C, Han L, Nawaz M, Gao F, Zhang X, Yu P, Zhao CA, Li L, Zhou A, Wang J. Molecular characterisation of the faecal microbiota in patients with type II diabetes. Current Microbiology. 2010; 61(1):69-78.
4. Vijay-Kumar M, Aitken JD, Carvalho FA, Cullender TC, Mwangi S, Srinivasan S, Sitaraman SV, Knight R, Ley RE, Gewirtz AT. Metabolic syndrome and altered gut microbiota in mice lacking toll-like receptor 5. Science. 2010;328(5975):228-31.
5. Marchesi JR, Adams DH, Fava F, Hermes GD, Hirschfield GM, Hold G, Quraishi MN, Kinross J, Smidt H, Tuohy KM, Thomas LV. The gut microbiota and host health: A new clinical frontier. Gut. 2015;gutjnl-2015.
6. Rajilić-Stojanović M, de Vos WM. The first 1000 cultured species of the human gastrointestinal microbiota. FEMS microbiology reviews. 2014;38(5):996-1047.
7. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature. 2012; 486(7402):207.

8. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science. 2005;307(5717):1915-20.
9. Brenner DJ. Facultatively anaerobic gram-negative rods. Family I. Enterobacteriaceae. In Bergey's Manual of Systematic Bacteriology. 1984;1:408–420. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
10. Hold GL, Smith M, Grange C, Watt ER, El-Omar EM, Mukhopadhya I. Role of the gut microbiota in inflammatory bowel disease pathogenesis: what have we learnt in the past 10 years? World journal of gastroenterology: WJG. 2014;20(5):1192.
11. Jostins L, Ripke S, Weersma RK, Duerr RH, McGovern DP, Hui KY, Lee JC, Schumm LP, Sharma Y, Anderson CA, Essers J. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature. 2012; 491(7422):119.
12. Collado MC, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. The American Journal of Clinical Nutrition. 2008; 88(4):894-9.
13. Cruickshank R, Duguid JP, Marmion BP, Swain RH. Tests for identification of bacteria. Medical Microbiology. 1975; 2:170-89.
14. Funk JA, Davies PR, Nichols MG. Evaluation of sample weight for the isolation of *Salmonella* spp. From Swine Feces.
15. Quinn PJ, Markey BK, Leonard FC, Hartigan P, Fanning S, FitzPatrick ES. Veterinary microbiology and microbial disease. John Wiley & Sons; 2011.
16. Miles AA, Misra SS, Irwin JO. The estimation of the bactericidal power of the blood. Epidemiology & Infection. 1938; 38(6):732-49.
17. Allain CC, Poon LS, Chan CS, Richmond WF, Fu PC. Enzymatic determination of total serum cholesterol. Clinical Chemistry. 1974;20(4):470-5.
18. Wahlefeld AW, Bergmeyer HU. Methods of enzymatic analysis. New York, NY, USA: Academic. 1974;1831.
19. Warnick GR, Benderson JM, Albers JJ. Quantitation of high-density-lipoprotein subclasses after separation by dextran sulfate and MG-2+ precipitation. In Clinical Chemistry. 1982;28(7):1574-1574. 2101 L Street NW, Suite 202, washington, DC 20037-1526: Amer Assoc Clinical Chemistry.
20. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical Chemistry. 1972; 18(6):499-502.
21. Hiernaux J, Tanner JM. Growth and physique: Anthropometry. Human biology, a guide to field methods. Philadelphia, FA Davis Company. 1969;2-42.
22. Bosy-Westphal A, Geisler C, Onur S, Korth O, Selberg O, Schrezenmeir J, Müller MJ. Value of body fat mass vs anthropometric obesity indices in the assessment of metabolic risk factors. International Journal of Obesity. 2006;30(3):475.
23. Romero-Corral A, Somers VK, Sierra-Johnson J, Thomas RJ, Bailey KR, Collazo-Clavell ML, Allison TG, Korinek J, Batsis JA, Lopez-Jimenez F. Accuracy of body mass index to diagnose obesity in the US adult population. International Journal of Obesity. 2005;2008:32(6):959.
24. Park HJ, Lee SE, Kim HB, Isaacson RE, Seo KW, Song KH. Association of obesity with serum leptin, adiponectin, and serotonin and gut microflora in beagle dogs. Journal of Veterinary Internal Medicine. 2015;29(1):43-50.
25. Mujico JR, Baccan GC, Gheorghe A, Díaz LE, Marcos A. Changes in gut microbiota due to supplemented fatty acids in diet-induced obese mice. British Journal of Nutrition. 2013;110(4):711-20.
26. Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, Geurts L, Naslain D, Neyrinck A, Lambert DM, Muccioli GG. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut. 2009;58(8):1091-103.
27. Velagapudi VR, Hezaveh R, Reigstad CS, Gopalacharyulu P, Yetukuri L, Islam S, Felin J, Perkins R, Borén J, Orešič M, Bäckhed F. The gut microbiota modulates host energy and lipid metabolism in mice. Journal of Lipid Research. 2010; 51(5):1101-12.
28. Frank DN, Zhu W, Sartor RB, Li E. Investigating the biological and clinical significance of human dysbioses. Trends in Microbiology. 2011;19(9):427-34.

29. Qiao Y, Sun J, Ding Y, Le G, Shi Y. Alterations of the gut microbiota in high-fat diet mice is strongly linked to oxidative stress. Applied Microbiology and Biotechnology. 2013;97(4):1689-97.
30. Fu J, Bonder MJ, Cenit MC, Tigchelaar E, Maatman A, Dekens JA, Brandsma E, Marczynska J, Imhann F, Weersma RK, Franke L. The gut microbiome contributes to a substantial proportion of the variation in blood lipids. Circulation Research. 2015; CIRCRESAHA-115.
31. Million M, Angelakis E, Maraninchi M, Henry M, Giorgi R, Valero R, Vialettes B, Raoult D. Correlation between body mass index and gut concentrations of *Lactobacillus reuteri, Bifidobacterium animalis, Methanobrevibact er smithii* and *Escherichia coli*. International Journal of Obesity. 2005;2013:37(11):1460.
32. Santacruz A, Collado MD, Garcia-Valdes L, Segura MT, Martin-Lagos JA, Anjos T, Marti-Romero M, Lopez RM, Florido J, Campoy C, Sanz Y. Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. British Journal of Nutrition. 2010;104(1):83-92.
33. Zhu L, Baker SS, Gill C, Liu W, Alkhouri R, Baker RD, Gill SR. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: A connection between endogenous alcohol and NASH. Hepatology. 2013;57(2):601-9.
34. Dev N, Marcus SR. High sensitive C-reactive protein, an independent and early novel inflammatory marker in healthy obese women. Biomedical Research; 2012.
35. Verdam FJ, Fuentes S, de Jonge C, Zoetendal EG, Erbil R, Greve JW, Buurman WA, de Vos WM, Rensen SS. Human intestinal microbiota composition is associated with local and systemic inflammation in obesity. Obesity. 2013; 21(12).
36. Venkatesh M, Mukherjee S, Wang H, Li H, Sun K, Benechet AP, Qiu Z, Maher L, Redinbo MR, Phillips RS, Fleet JC. Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and Toll-like receptor 4. Immunity. 2014;41(2):296-310.
37. Karlsson CL, Molin G, Fåk F, Hagslätt ML, Jakesevic M, Håkansson Å, Jeppsson B, Weström B, Ahrné S. Effects on weight gain and gut microbiota in rats given bacterial supplements and a high-energy-dense diet from fetal life through to 6 months of age. British Journal of Nutrition. 2011;106(6):887-95.
38. Piya MK, Harte AL, McTernan PG. Metabolic endotoxaemia: Is it more than just a gut feeling? Current Opinion in Lipidology. 2013;24(1):78-85.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21193