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Resting energy expenditure and gut microbiota in obese and normal weight subjects

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Abstract. – OBJECTIVES: It is suggested that gut microbiota play a role in the pathogenesis of obesity enhancing energy utilization from digested food. The influence of gut microbiota on resting energy expenditure (REE) has not been evaluated yet.

AIM: The aim of the study is to assess the composition on gut microbiota and its association with REE in obese and normal weight subjects.

SUBJECTS AND METHODS: REE measurement and semi-quantitative analysis of gut microbiota composition in aerobic and anaerobic conditions were performed in 50 obese and 30 normal weight subjects without concomitant diseases.

RESULTS: A count of bacterial colony was greater in obese than in normal weight subjects. However, the proportion of Bacteroides spp. and Firmicutes was similar in both study groups. A positive correlation between REE (kcal/d) and total bacterial count ($r = 0.26$, $p < 0.05$), as well as between REE and the percentage of Firmicutes ($r = -0.24$, $p < 0.05$) was found. The multiple regression analysis did not prove an independent impact of total bacterial as well as Bacteroides spp. and Firmicutes counts on REE.

CONCLUSIONS: The composition of gut microbiota is not associated with the level of resting energy expenditure. The proportion of Bacteroides and Firmicutes in gut microbiota is not related to body mass.

Key Words:
Gut microbiota, Obesity, Resting energy expenditure.

Introduction

Obesity develops as the result of positive energy balance. However, it was suggested that similar daily caloric intake in some subjects results in lower weight gain[1,2]. Therefore, there is a need for a search of factors predisposing to excess energy accumulation. In the recent years numerous studies assessing the contribution of physiological gut microbiota to the excess adipose tissue deposits have been performed[2-5].

Microbiome, the genomes of gut microbiota is a hundred times greater than the human genome[4]. Physiologic gut microbiota contains more than one thousand species, and the number of bacteria estimated at ~10^4 CFU/ml in the small intestine is increasing to 10^{12} CFU/ml in the colon[6,7]. Aerobic Gram-negative bacteria occur mainly in the small intestine, while the anaerobic to aerobic bacteria rate in the colon is 1000:1[8].

Bacteroides and *Firmicutes* (*Ruminococcus*, *Clostridium*, *Peptostreptococcus*, *Lactobacillus*, *Enterococcus*) constitute about 90% of the colon microbiota[9]. The physiological role of gut microbiota included stimulation of the growth and maturation of the gut epithelial cells, gut motility, biodegradation of toxins and carcinogens, synthesis of vitamins, fermentation of the undigested food remnants and modulation of the immune system[10-12].

The results of recently published studies revealed a differences in gut microflora with dominance of *Firmicutes* over *Bacteroides* spp. in both obese animals and humans, whereas low caloric diet and weight reduction resulted in the increase of *Bacteroides* spp[13].

It is suggested that *Firmicutes* may influence energy balance by enhanced fermentation of undigested polysaccharides[14,15], decreased expression of FIAF (*fasting-induced adipocyte factor*) followed by inhibition of intestinal lipoprotein lipase activity in intestinal epithelium[3], and increased secretion of PYY[16]. Additionally, it seems that gut microbiota composition influences on carbon dioxide exhalation and measurement of resting energy expenditure (REE). How-

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ever, no study assessing the gut microbiota composition and REE has been performed so far. Therefore, the aim of the study is to assess the composition of culturable intestinal bacterial microbiota and its association with REE in obese and normal weight subjects.

Subjects and Methods

Eighty subjects without concomitant diseases: 50 obese (39 women and 11 men) – group O, and 30 normal weight (24 women and 6 men) – group S were enrolled. Subjects with acute or chronic diseases, any drug use, including oral contraceptives, weight change exceeding more than 3 kg during preceding 6 months, cigarette smoking, drinking more than 3 drinks per week, endocrine disorders: hyper- and hypothyroidism, Cushing's syndrome, polycystic ovary syndrome were excluded. The characteristics of study group is presented in Table I.

The study protocol was approved by the Bioethics Committee of Medical University of Silesia (KNW/0022/KB1/41/10). The study was conducted after obtaining informed consent from each participant.

Anthropometric measurements (weight, height, waist circumference) were performed in the morning between 8 and 9 after 16 hour overnight fast. BMI was calculated according to standard formula. Body composition was measured using the bioimpedance method (Bodystat 1500, Douglas, Isle of Man). Resting energy expenditure (REE) was assessed after 60 minute rest on the basis of 30 minute examination by indirect calorimetry method (MedGraphics, St, Paul, MN, USA). Calibration of the device has been performed before each examination[17].

Analysis of Fecal Microflora

All 80 fecal samples obtained from study subjects were processed according to scheme presented in Figure 1. Each fecal sample was diluted (1:100) in PBS, cultured using the appropriate media (no. 1-9) in aerobic (no. 1-5) and anaerobic (no. 6-9) conditions, respectively:

1. CBA – Columbia blood agar × 2 (bioMerieux, Marcy L'Etoile, France),
2. MC – Mac Conkey agar (Becton Dickinson and Company, Rihône Alpes, France),
3. Ch – Chapman agar with mannitol (Becton Dickinson and Company, Franklin Lakes, NJ, USA),
4. Sab – Sabouraud agar (bioMerieux, Marcy L'Etoile, France),
5. DCO – D-Coccosel agar (bioMerieux, Marcy L'Etoile, France),
6. MRS – MRS agar (Becton Dickinson and Company, Rihône Alpes, France),
7. RC – Reinforced Clostridial agar (Oxoid, Hampshire RG24 8PW, England, UK),

Table I. Anthropometric and metabolic parameters in obese and normal weight groups (mean values and 95% confidential intervals – 95% CI).

	Obese [n = 50]	Normal weight [n = 30]	Statistical significance
Age [years]	51.9 (48.1-55.7)	42.6 (38.1-47.1)	$p < 0.01$
Body mass [kg]	97.0 (92.8-101.1)	64.8 (61.0-68.6)	$p < 0.001$
BMI [kg/m^2]	35.7 (34.3-37.1)	23.2 (22.5-23.9)	$p < 0.001$
Fat free mass [kg]	44.3 (41.2-47.4)	21.2 (19.4-23.0)	$p < 0.001$
Fat mass [kg]	52.7 (49.7-55.6)	43.6 (39.6-47.6)	$p < 0.001$
The percentage of fat mass [%]	45.6 (43.4-47.7)	33.2 (30.3-36.1)	$p < 0.001$
FIO_2 [%]	20.7 (20.7-20.7)	20.8 (20.7-20.8)	$p < 0.05$
REE [kcal/d]	1620 (1523-1716)	1258 (1161-1356)	$p < 0.001$
REE [kcal/kg/h]	0.56 (0.5-0.76)	0.80 (0.7-0.89)	$p < 0.001$
REE [kcal/m^2/h]	32.8 (28.2-37.3)	29.3 (26.2-32.4)	NS
RQ	0.85 (0.83-0.88)	0.83 (0.81-0.86)	NS
VCO_2 [ml/min]	198 (184-212)	151 (138-163)	$p < 0.001$
VO_2 [ml/min]	230 (216-243)	182 (168-197)	$p < 0.001$

FIO_2: fraction of inspired oxygen; REE: resting energy expenditure; RQ: respiratory quotient; VO_2: volume of oxygen; VCO_2: volume of carbon dioxide.

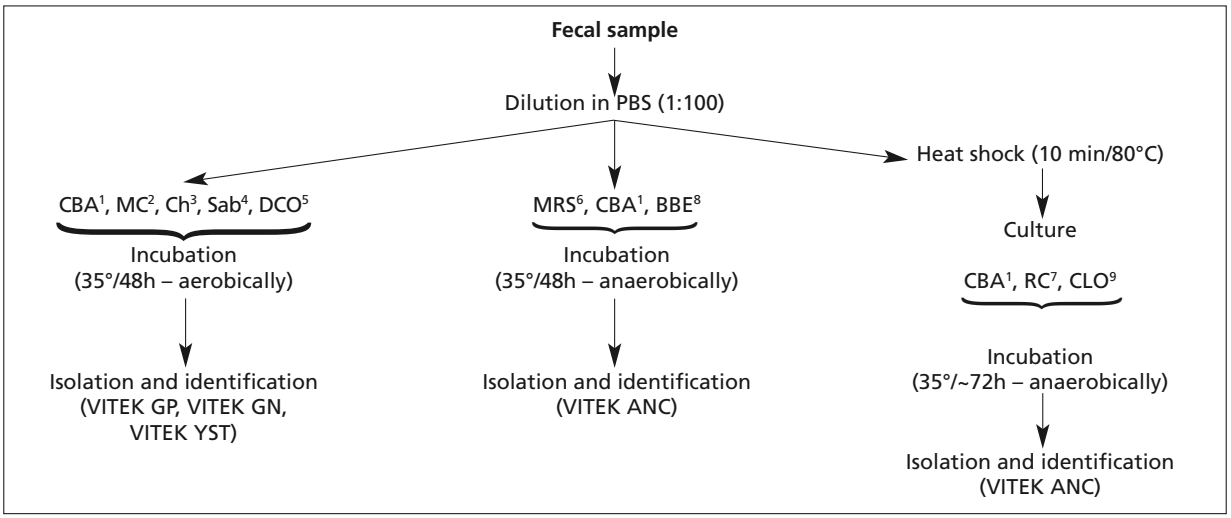

Figure 1. Fecal samples processing scheme. [1]CBA: Columbia blond agar; [2]MC: Mac Conkey' agar; [3]Ch: Chapman mannitol agar; [4]Sab: Sabouraud agar; [5]DCO: D-Coccosel agar; [6]MRS: MRS agar; [7]RC: Reinforced Clostridial agar; [8]BBE: Bacteroides Bile Esculin agar with Amikacin; [9]CLO: Clostridium difficile agar.

8. BBE – Bacteroides Bile Esculin Agar with Amikacin (BD BBL, Amtsgericht Mannheim, Germany),
9. CLO – Clostridium difficile Agar (bio-Merieux, Marcy L'Etoile, France)

About 1 g of each fecal sample was subjected to 10 min heat shock (80°C, Termoblock RED-HOT 35) and cultured onto Columbia blood (no. 1) and Reinforced Clostridial (no. 7) agars for 3-5 days in anaerobic conditions (Whitley A-35 Anaerobic Workstation, Shipley, West Yorkshire BD17 7SE, UK). After incubation all plates were evaluated, bacterial colonies were encountered, Gram stained and identified using appropriate cards (GP, GN, YST, ANC) for automatic identification system of microorganisms – VITEK 2 compact (bioMerieux, Marcy L'Etoile, France). As a reference strains *Bacteroides ovatus* ATCC BAA-1296, *Clostridium septicum* ATCC 12464, *Clostridium perfringens* ATCC 13124, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 from ATCC collection were used.

Statistical Analysis

Statistical analysis was performed using the STATISTICA 8.0 PL software (StatSoft Polska, Cracow, Poland). The results are presented as mean values with 95% confidence interval (CI) or median values with inter-quartile ranges, when appropriate. Chi-square test was used for comparison of the frequency of qualitative variables in studied groups, student's *t*-test or Mann-Whitney U-test were used for comparison of quantitative variables between groups. The univariate correlation coefficients were calculated according to Spearman. Three models of multiple regression analyses for REE as independent variable, fat free mass and alternatively the total bacterial count, total *Bacteroides* and *Firmicutes* counts as potential explanatory variables were performed. The results were considered as statistically significant with a p value of less than 0.05.

Results

As expected, body mass, BMI and the percentage of fat mass were higher in the obese than in normal weight subjects. The obese group were older by 9 years in average than the normal weight group (Table I).

REE expressed as kcal/day was significantly higher in obese than in normal weight subjects. However, REE expressed on body surface was similar in both groups. While, REE expressed per kilogram was lower in the obese group, as the consequence of higher percentage of fat mass (Table I).

Oxygen consumption and carbon dioxide exhalation were higher in obese; however, respiratory quotient (RQ) was similar in both study groups (Table I).

The total bacterial count was higher in obese compared with normal weight subjects. There was no difference in bacterial species count between studied groups, with the exception of

Table II. The composition of gut microbiota in obese and normal weight groups (median values and interquartile ranges).

	Obese [n = 50]	Normal weight [n = 30]	Statistical significance
The total bacterial count [CFU/μl]	2962 (2345-3598)	2675 (2325-2950)	$p < 0.05$
Bacteroides [CFU/μl]	800 (500-1000)	700 (400-1000)	NS
Firmicutes spp. [CFU/μl]	1016 (610-1253)	823 (511-1052)	NS
The percentage of Bacteroides [%]	26 (18-30)	27 (18-38)	NS
The percentage of Firmicutes spp. [%]	31 (20-42)	32 (23-42)	NS
The rate of Bacteroides/Firmicutes spp.	0.78 (0.5-1.25)	0.75 (0.42-1.70)	NS
Escherichia coli [CFU/μl]	200 (50-300)	100 (40-225)	NS
Cylindrical Gram-positive (+) [CFU/μl]	1000 (500-1000)	500 (500-1000)	NS
Cylindrical Gram-negative (-) [CFU/μl]	500 (500-500)	500 (500-500)	NS
Cylindrical Gram-variable [CFU/μl]	500 (500-500)	500 (2-500)	NS
Cocci Gram-positive (+) [CFU/μl]	500 (500-500)	–	–
Cocci Gram-variable [CFU/μl]	500 (500-500)	–	–
Cocci-cylindrical Gram-variable [CFU/μl]	500 (500-500)	–	–

Gram-positive and Gram-variable *cocci*; only cultured from the fecal samples of obese subjects. *Bacteroides* to *Firmicutes* rate was similar in both study groups (Table II).

Correlation Analysis

The correlation coefficients were calculated for all study subjects. A significant positive correlation between REE and body mass ($r = 0.43$, $p < 0.05$) and the percentage of fat free mass ($r = 0.48$, $p < 0.01$) was found. There was also a negative correlation between REE and fat mass ($r = –0.39$; $p < 0.05$) or the fat mass percentage ($r = –0.48$; $p < 0.01$).

Additionally, a positive correlation between REE (kcal/day) and total bacterial count ($r = 0.26$, $p < 0.05$) and negative with the percentage of *Firmicutes* ($r = –0.24$, $p < 0.05$) was shown. REE expressed on the body surface (kcal/m^2/h) was positively correlated with the total bacterial count ($r = 0.25$, $p < 0.05$), *Bacteroides* count ($r = 0.24$, $p < 0.05$) and *Bacteroides* to *Firmicutes* rate ($r = 0.26$, $p < 0.05$), while negatively with the percentage of *Firmicutes* colonies ($r = –0.24$, $p < 0.05$). REE expressed as kcal/kg/h correlated negatively with the percentage of *Firmicutes* colonies ($r = –0.48$, $p < 0.01$) only.

There was also a positive correlation between *Firmicutes* count and the percentage of fat mass ($r = 0.29$, $p < 0.01$).

The volume of consumed oxygen (VO_2) correlated positively with the total bacterial count ($r = 0.24$, $p < 0.05$) and negatively with the percentage of *Firmicutes* ($r = –0.23$, $p < 0.05$). The volume of exhalated carbon dioxide (VCO_2) correlated negatively with the total bacterial count ($r = –0.24$, $p < 0.05$).

Multiple Regression Analysis

The multiple regression analysis did not prove an independent from fat free mass contribution of total bacterial count as well as *Bateroides* and *Firmicutes* counts on REE (kcal/day).

Discussion

This is the first study assessing the relation between the composition of gut microbiota and REE in obese and normal weight subjects.

The results of the previously published studies revealed the differences between obese and normal weight subjects in the gut microbiota composition. The predominance of *Firminutes* over *Bacteroides* in gut microflora of obese has been observed. It was suggested that it may result in enhancing energy harvesting from food, the change of lipid metabolism and escalating obesity-related systemic microinflammation[13,18].

In the present work we observed higher total bacterial count in obese compared with normal weight subjects. This may be explained by lower fibre but higher fat consumption and a greater prevalence of constipation in obese. No difference in *Bacteroide*s and *Firmicutes* counts was found between study groups, that is in accordance with more recent publications[15,19]. The reasons of inconsistence in the published re-

ports assessing the gut microbiota composition in obese and normal weight subjects are unclear. It seems that the diet composition, the methodology of material collection, and processing may explain it[15,19]. The hypothesis concerning the impact of diet on gut microflora composition is supported by the study performed on animal model showing that gut microbiota is related to the dietary fat content[20]. Moreover, some recent papers showed that *Bacteroides* count depends on daily caloric intake, and that implementation of low-fat and low-carbohydrate diet result in changes in the gut microbiota composition in obese, that becomes similar to those in normal weight subjects[21].

It is suggested that not only *Bacteroides* to *Firmicutes* rate, but also the enhancement of intestinal short-chain fatty acids production contribute to the pathogenesis of obesity. Increased amount of propionic acid enhances gluconeogenesis and lypogenesis[22], whereas acetic acid becomes a substrate for cholesterol synthesis in the liver[23]. Increasing number of Gram-negative bacteria induced by high-fat diet may enhance systemic microinflammation and participate in type 2 diabetes development[16].

Contrary to the findings of these studies[13,18], the predominance of *Bacteroides* over *Firmicutes* in pregnant women with rapid weight gain was revealed[24]. These discrepancies pointed to a necessity for a detailed study on the relations between the gut microbiota and diet composition. However, the collecting of data on long-lasting eating habits has limited accuracy.

More abandoned bacterial growth from fecal samples of obese observed in the present study may partially result in higher REE, which was proved by the result of the correlation analysis. Additionally, REE values were proportional to the percentage of *Bacteroides* and inversely related to the percentage of *Firmicutes*. The finding is in accordance with previous published works showing the predominance of *Firmicutes* over *Bacteroides* spp. in gut microflora facilitate the obesity development[13,18]. However, no difference between obese and normal weight subjects in *Bacteroides* to *Firmicutes* rate was found. Therefore, it seems that the association between the decrease of REE and the increase of the percentage of *Firmicutes* is indirect, and may be the result of the negative correlation between *Firmicutes* count and free fat mass.

The result of our study does not allow for a precise assessment of the influence of gut microbiota composition on REE and its contribution to the obesity development. The hypothesis concerning the colonization of the intestine by bacterial strain favorable for the obesity development is very attractive, generating a possibility of intervention, such as the use of antibiotics for eradication of detrimental bacterial strain or probiotics, which could improve the gut microbiota composition. However, our data as well as the results of numerous other studies suggest that the differences in gut microbiota composition are secondary to the lifestyle (diet composition and physical activity affecting intestinal motility), than constitute an important link in the obesity pathogenesis.

The criticism to of the study is the cross-sectional design and the semi-quantitative analysis of gut microbiota. The amount and composition of gut microflora is changing in small intestine, and from ceacum to rectum. Thus, analysis of fecal samples does not adequately reflect the human gut microbiom. However, the aim of this study was not the analysis of the whole microbiome and demonstration of thousands of culturable bacteria, nor demonstrating the ecosystem differences between stool and mucosa communizing differences composition as described by Eckburg et al[25]. We tried to assess the composition of culturable intestinal bacterial flora and its association with REE in obese and normal weight subjects as a first step of the study.

We did not analyze the diet composition and physical activity, thus we are unable to prove that gut microbiota composition and physical activity are related.

Conclusions

The composition of gut microbiota does not exert the influence on the resting energy expenditure. The *Bacteroides* to *Firmicutes* rate in obese subjects is independent from body mass.

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Declaration of Interest

None of the Authors declares conflict of interest. None of the Authors declares any personal interest in the results of the study.

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