Distribution of Obesity among Male in Aljouf Population, Saudi Arabia

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This is a study of the pattern of distribution of body mass index and prevalence of overweight and obesity among the population of male at Saudi Arabia. Also the principal aim of this study is to investigate and compare the gut microbiota composition in obese and lean persons. It is a cross-sectional population-based national epidemiological randomized survey. There were 300 Saudi subjects, over the age of 15 years. Height and weight were measured for all study population. Body mass index (BMI) was calculated. WHO criteria was used for definition of overweight and obesity. The obesity/overweight (ob/ow) ratio was used to study the relation between prevalence of overweight and obesity. The data on the 300 subjects according to body mass index (BMI) and cholesterol. It shows the number of subjects with a normal body mass index (<25/124), overweight (25- 29.9/ 78) and obesity (>30/98). Faecal samples were collected and subjected to selective plating and quantitative in order to determine the concentrations of bacterial species. In this study, important compositional differences in the faecal gut microbiota of obese and lean were revealed. Underlying factors such as lifestyle, nutritional habits, social and cultural beliefs and habits in different regions need to be studied. There is a need to establish a national control program for combating obesity and related complications.

Keywords: Obesity, Gut Microbiota, Al Jouf Province, KSA.

Obesity is a worldwide serious public health problem. in United States the prevalence of obesity has increased sharply over the past three decades, in 2003-2004, 17.1 % of US children and adolescent were overweight and 32.2 % of adults were obese are overweight and additional 22.5 are obese (Ogden *et al.*, 2006) In the gulf countries reported prevalence of overweight and obesity in adults were 25-50% and 13-50%, respectively. Prevalence appeared higher in women and to hold a non-linear association with age. Current prevalence of impaired glucose tolerance was estimated to be 10-20%. Prevalence appears to have been increasing in recent years. (Alhyas *et al.*, 2011 and AL-Nozha *et al.*, 2005)

Various methods are used to assess body weight status, the most commonly is the Body mass index (BMI) which is the ratio of weight in kilograms to square of height in meters will be used to assess the body weight status of all subjects. According to the National institute of health (NIH), subjects of study will be categorized based on their BMI into underweight (BMI < 18.5), normal (BMI = 18.5-24.9), overweight (BMI = 25-29.5) or obese (BMI e" 30). Many factors contribute to obesity development, such as increased food consumption with reduced physical activity, genetic factors (e.g.

Prader-Willi syndrome), hypothyroidism, stress and certain medications (e.g. atypical antipsychotic) (Schwiertz *et al*., 2009). Recently, however, researchers have discovered another factor that may play a role in obesity, gut microbiota.

The human gut contains trillions of microorganisms (i.e., bacteria, archaea, fungi, protozoa, and viruses) collectively known as gut microbiota (Savage, 1977). Seven bacterial divisions constitute the gut microbiota, ie, Firmicutes, Bacteroides, Proteobacteria, Fusobacteria, Verrucomicrobia, Cyanobacteria, and Actinobacteria, with Firmicutes and Bacteroides being the most abundant species (Backhed *et al*., 2005). The gut microbiota performs essential functions which include pathogen displacement, production of vitamins and amino acids, bile acid biotransformation, and fermentation of non-digestible carbohydrates, dietary fibers and mucus. Fermentation of non-digestible substrates promotes bacterial growth and produces short chain fatty acids and gases (Lefebvre *et al*., 2009 & Wong *et al*., 2006). The major SCFA are acetate, butyrate, and propionate, which can be utilized for lipid or glucose biosynthesis providing additional sources of energy for the body (Wolever *et al*., 1989).

Disruption of gut microbiota is associated with the pathogenesis of many diseases, including inflammatory bowel diseases (Saleh & Elson, 2011), neonatal necrotizing enterocolitis (Siggers, Siggers, Thymann, Boye, &Sangild, 2011), diabetes (Larsen *et al*., 2010), liver disease (Son, Kremer, & Hines, 2010), obesity (Turnbaugh *et al*., 2009), colon cancer (Uronis *et al*., 2009), infectious diseases (Stecher&Hardt, 2008), allergy or asthma (Wen *et al*., 2008). Therefore, the gut microbiota and its interactions with host and diet have long been of interest to researchers. Recently, it has been hypothesised that an increased ratio of Firmicutes to Bacteroidetes may make a significant contribution to the pathophysiology of obesity. Researchers found that two types of bacteria are particularly abundant in the gut and the ratio of these bacteria types corresponds to the expression of either the lean or the obese phenotype. They confirmed this observation by transplanting obese (ob/ob) or lean (+/+) microbiota into germ-free mice

(Turnbaugh *et al*., 2006). Researchers found that the divisions of bacteria present in the host recipient were characteristic of the host's naturally occurring bacterial community (Rawls *et al*., 2006). Schwiertz *et al*., 2009, examine the differences within the human intestinal microbiota and fecal SCFA concentration of lean and obese subjects and he found that SCFA concentration was higher in obese group than in lean group and the ration of Firmicutes to Bacteroidetes changed in favor of the later in overweight and obese subjects. Some studies have demonstrated that gut microbiota have a substantial correlation with obesity through regulation of metabolism (Ley *et al* 2006;. Backhed *et al* 2004; Vijay-Kumar *et al* 2010; Ley *et al* 2006 ; Turnbaugh *et al* 2006; Round and Mazmanian 2009).

The aim of the present study is to determine overweight and obesity prevalence among inhabitants of Sakaka city, Al-Jouf region, Saudi Arabia and to correlate their body weight with their gut microbiota composition.

MATERIALS AND METHODS

Patients

A total number of 300 volunteers of the aging groups will be selected for this study. The volunteers will be informed about the study and will be asked to sign a written Helsinki declaration based consent and, the body mass index (BMI) which is the ratio of weight in kilograms to square of height in meters will be determined and used to assess the body weight status of all subjects. According to the National institute of health (NIH), subjects of study will be categorized based on their BMI into underweight (BMI < 18.5), normal (BMI = 18.5-24.9), overweight (BMI = 25-29.5) or obese (BMI e" 30).

Lipids and lipoproteins profile of study subjects

Blood will be collected after an overnight fast from obese and normal subjects. Plasma will be separated from erythrocytes by centrifugation at 3,000 rpm for 10 min at 4° C immediately after collection. Plasma samples will be stored frozen at -80 ^{o}C until assayed. Plasma Triacylglyceride, cholesterol, LDL and HDL concentrations will be quantified using a commercially available enzymatic kit.

Monitoring bacterial community in gut of obese and normal participants

Analysis of cultivable fecal bacteria using plate culture media

Fecal samples from both study groups will be collected and immediately keep at 4ºC. The subjects in the study must not be treated with antibiotics for at least one month before the sampling time. Fecal samples (2 g wet weight) will be 10-fold diluted in phosphate-buffered saline, and homogenized in a Lab Blender. Appropriate serial decimal dilutions were made in PBS and aliquots were plated on each agar selective media in duplicated.

Identification of bacterial isolates
Conventional methods

The appearance of cultures, cell morphological characteristics and physiological characteristics of the purified selected identified isolates were studied. Media and reagent were prepared according to standard and procedures. Identification was carried out by traditional characters and biochemical tests for isolates and confirmed out by aid of appropriate API Kits, automation VITEK II.

Statistical Analysis

Statistical analysis was done using the Statpack Gold statistical analysis package. Data were presented as median, mean (SD), percentiles and percentage. Student's t-test was used for comparison of continuous variables. A P-value of 0.05 was considered significant.

RESULTS AND DISCUSSION

Table 1 shows the data on the 300 subjects according to body mass index (BMI) and cholesterol. It shows the number of subjects with a normal body mass index (<25kg=124), overweight (25 – 29.9kg=78) and obesity (>30kg=98). The bacterial count showed variation in counts. Generally the bacterial counts large in numbers in obese than normal persons. The bacterial identified by conventional and molecular methods and belonged to *E. coli*, *Bacillus* sp., *Clostridium difficile*, *Enterococcus feacalis* and *Staphylococcus aureus* (Fig. 1).
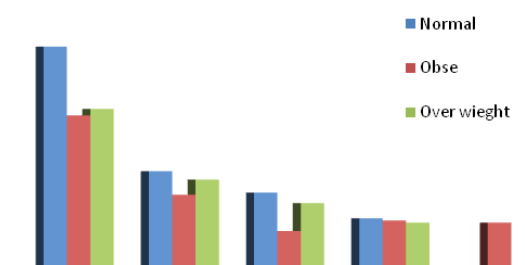

Fig. 1. Bacterial Counts in obesity population.

Table 1. Data on the obesity population sample studied in Al Jouf Province, KSA

BMI (body mass index)(Kg/m^2)	< 25	25-29.9	< 30
n	124	78	98
Age	22.52	24.64	23.5
Total cholesterol (mg/dl)	139.88	165.18	201.22
Triglyceride (mg/dl)	88.81	104.91	142.66
HDL cholesterol (mg/dl)	30.35	35.41	52.90
LDL cholesterol (mg/dl)	209.93	273.18	319.22
Visceral fats	3.56	6.14	8.39
Body fats %	25.49	36.72	45.85
Muscles %	32.82	28.77	24.69
Bacterial counts (cfux10^3)			
Enterococci	103	71	74
E. coli	45	34	41
Staphylococci	35	17	30
Bacilli	23	22	21
Clostridial	0	21	0

This population-based epidemiological study addressed the issue of regional variation with respect to the pattern of BMI distribution and the prevalence of overweight and obesity among Al Jouf Province of Saudi Arabia population. The study population sample was representative of normal population distribution, as it was derived through a national household randomized survey. The administrative divisions of Saudi Arabia were adopted, as they reflect the nature of lifestyle of the populations residing in these region. All measures were taken to ensure accuracy of weight measurement, whether through instructing the subjects on wearing standardized light clothes or through using clinical scales with periodic calibration. Northern regions have a higher prevalence of overweight and obesity. Factors affecting the regional variation with respect to the prevalence of obesity and the relationship between the prevalence of overweight and obesity are open for speculation. Social, cultural, economical and nutritional factors are important contributing elements. Northern region, with its strong tribal attitude, may consider obesity, especially among subjects, a sign of affluence and beauty. These may be important contributing factors to the surge of obesity in these two groups (Al Shammari et al., 1994).

At birth, the gut microbiota of an infant is sterile but rapidly assembles over days or months. In this cross-sectional study, the obese gut microbiota composition was compared with that of a lean one. We focused on E. coli, Clostridium, Staphylococcus and Bacillus. Different bacterial groups were selected according to the frequency to which they have been described in relevant literature (Ferrer et al., 2013) and the ease of detection by the techniques used. On the one hand, quantitative plating was used as the 'gold standard' technique to isolate and characterise the selected bacterial groups. However, only 10 to 50% of all bacteria associated with the human body can be cultivated successfully (Ferrer et al., 2013). Subsequently, high-throughput culture-independent techniques, which use DNA sequences encoding the 16S ribosomal RNA subunit, were applied in order to assign an organism to a phylogenetic classification more accurately (Zoetendal et al., 2008). Selective media have been used successfully to identify and enumerate

bacteria from human faeces. In agreement with the findings of previous studies, we describe higher concentrations of Bacillus spp. in the obese gut microbiota. However, the use of quantitative plating did not permit the detection of a significantly higher concentration of Bacillus spp. In faeces of obese person, which we did see using PCR. These findings thus suggest a possible role of Bacillus spp. at species level in body weight and obesity. Previous investigators also showed significant associations between this ratio and obesity in mice and humans (Ley et al., 2006).

One major limitation of the current study is the small sample size and therefore these results should be interpreted with caution. Further longitudinal research on the cause-effect relationship between gut microbiota and obesity is highly justified, since different bacterial species could play a significant role in the human energy harvest and weight regulation. Moreover, consideration of lifestyle factors in gut microbiota studies is highly recommended, since changes in dietary pattern and physical activity could influence gut microbiota composition and the development of obesity. Finally, we suggest to focus future research not only on the elucidation of gut microbiota composition in obese subjects, but also on the study of gut metabolites. The magnitude of the problem is expected to increase in the near future, with a larger segment of the population advancing in age. There is a need to establish a national control program for prevention and treatment of obesity and related complications. All age groups and segments of the society should be targeted. Such a program should be monitored for its effectiveness over the years.

CONCLUSION

Existing evidence definitely warrants further exploration and assessment of the human gastrointestinal microbial ecology. Research is directing action towards modifying a person's gut microbiota to treat people who are overweight or obese. Having more accurate assessment tools to identify the environmental and genetic factors that determine individual characteristics of one's microbiota is warranted. More research is also needed to determine what actual dietary changes and supplements healthcare practitioners could

recommend to their patients and clients to implement into their lifestyle for weight loss success. In this study, important compositional differences in the faecal gut microbiota of obese and lean were revealed. This was generally reflected by an elevated the presence of bacteria in the obese gut microbiota.

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