Research Article

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Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity

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Background & Aims: Obesity has been associated with changes in the composition and function of the intestinal microbiota. Modulation of the microbiota by antibiotics also alters bile acid and glucose metabolism in mice. Hence, we hypothesized that short term administration of oral antibiotics in humans would affect fecal microbiota composition and subsequently bile acid and glucose metabolism.

Methods: In this single blinded randomized controlled trial, 20 male obese subjects with metabolic syndrome were randomized to 7 days of amoxicillin 500 mg t.i.d. or 7 days of vancomycin 500 mg t.i.d. At baseline and after 1 week of therapy, fecal microbiota composition (Human Intestinal Tract Chip phylogenetic microarray), fecal and plasma bile acid concentrations as well as insulin sensitivity (hyperinsulinemic euglycemic clamp using [6,6-$^2H_2$]-glucose tracer) were measured.

Results: Vancomycin reduced fecal microbial diversity with a decrease of gram-positive bacteria (mainly *Firmicutes*) and a compensatory increase in gram-negative bacteria (mainly *Proteobacteria*). Concomitantly, vancomycin decreased fecal secondary bile acids with a simultaneous postprandial increase in primary bile acids in plasma (p <0.05). Moreover, changes in fecal bile acid concentrations were predominantly associated with altered *Firmicutes*. Finally, administration of vancomycin decreased peripheral insulin sensitivity (p <0.05). Amoxicillin did not affect any of these parameters.

Conclusions: Oral administration of vancomycin significantly impacts host physiology by decreasing intestinal microbiota diversity, bile acid dehydroxylation and peripheral insulin sensitivity in subjects with metabolic syndrome. These data show that intestinal microbiota, particularly of the *Firmicutes* phylum contributes to bile acid and glucose metabolism in humans. This trial is registered at the Dutch Trial Register (NTR2566).

Introduction

The intestinal microbiota consists of several thousands of species, which collectively maintain gut physiology and homeostasis [1], including metabolic energy balance and immune responses [2,3]. Recent studies in both mice and humans revealed a novel concept in which, depending on genotype and lifestyle, the changes in intestinal microbiota are thought to actively contribute to the development of obesity and systemic insulin resistance [4–7]. In line, obesity has been found to be associated with significant alterations in the composition of the intestinal microbiota [7–10]. Landmark experiments by Gordon and colleagues elegantly provided evidence for a regulating function of the gut microbiota in energy homeostasis [7,9]. Germ-free mice were shown to be protected from obesity and insulin resistance when consuming a high fat diet [11,12], whereas colonization led to increased body fat content with concomitant insulin resistance [13]. The fact that colonization of germ-free mice

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with microbiota obtained from obese mice induced an even greater increase in (visceral) adipose tissue lends further support to a causal relation between gut microbiota, systemic fat and insulin homeostasis [9,14]. Recently, we have extrapolated these experimental findings towards human pathophysiology as we demonstrated that transfer of lean donor fecal microbiota to obese subjects with the metabolic syndrome significantly altered the composition of the intestinal microbiota, which was associated with an improvement of peripheral insulin sensitivity [15].

Oral antibiotic treatment results in short- and long-term changes of the intestinal microbiota in both mice and humans [16–19]. Long-term intravenous vancomycin administration was also reported to correlate with the development of obesity in a retrospective study [20]. Recently, animal and human studies implicated that prolonged antibiotic treatment early after birth is associated with an increased risk of overweight [21,22]. In line, it has been long recognized that antibiotic treatment can induce profound changes in bile acid metabolism [23]. Primary bile acids (i.e., cholate and chenodeoxycholate) are produced in the liver from cholesterol by the enzyme cholesterol 7α-hydroxylase (CYP7A1). Prior to their secretion into the small intestine, bile acids are conjugated with either taurine or glycine. Subsequently, intestinal microbiota further modify the bile acids within the intestine [24]. Several gram-positive bacterial species, such as *Lactobacilli* deconjugate primary bile acids [24], which is in contrast to most gram-negative intestinal bacteria, with the exception of two strains of *Bacteroides* [25]. After deconjugation, additional microbial modifications occur, including oxidation and dehydroxylation, giving rise to the formation of secondary bile acids [26], which is only carried out by a minor population of gram-positive anaerobic *Clostridium* species [27–30]. Interestingly, bile acids have recently emerged as potential regulators of systemic energy homeostasis. For example, binding of particularly secondary bile acids (i.e., deoxycholate and lithocholate) to the G protein-coupled receptor TGR5 in the intestine strongly induces secretion of the incretin GLP-1, thereby affecting glucose homeostasis [31–33].

To date, it is poorly understood whether and to what extent intestinal bacteria are involved in the regulation of human bile acid and energy homeostasis. In view of the different role of gram-positive and -negative bacteria in intestinal bile acid metabolism, modification of either of these bacteria may have distinct effects on bile acid homeostasis. We thus hypothesize that the intestinal microbiota composition can affect bile acid composition with subsequently altered FGF-19 signaling thereby affecting glucose metabolism. In the present study, we thus set out to evaluate the impact of two different antibiotic regimens known to affect murine bile acid metabolism (amoxicillin and vancomycin orally administered) on intestinal microbiota composition, bile acid homeostasis and systemic insulin sensitivity in humans. We show that reduction of the gram-positive intestinal microbiota by vancomycin is associated with a decrease in peripheral insulin sensitivity in obese subjects and that modulation of the bile acid pool may be instrumental in mediating this effect.

Patients and methods

Subjects

Male Caucasian obese subjects were recruited via local advertisements and screened for characteristics of the metabolic syndrome, including waist circumference >102 cm and fasting plasma glucose >5.6 mmol/L [34]. Subjects with a history of cholecystectomy, as well as subjects who used any medication (including probiotics and/or antibiotics in the past 3 months) were excluded. All subjects had a stable weight for 3 months prior to inclusion. Written informed consent was obtained from all subjects. The study was approved by the Institutional Review Board and conducted at the Academic Medical Center Amsterdam, The Netherlands, in accordance with the Declaration of Helsinki (updated version 2008). The study was registered at the Dutch Trial Register (NTR 2566). All authors had access to the study data, reviewed and approved the final manuscript.

Study design

A randomized, controlled single (physician) blinded intervention study was performed. Following assessment of eligibility and baseline measurements, participants were randomized to oral intake of 500 mg three times a day (t.i.d) of either amoxicillin or vancomycin, for a period of 7 days with daily dosages based on clinical used treatment regiments for systemic infection and enterocolitis, respectively. Two days before the antibiotic treatment and 2 days after cessation (to ensure proper wash-out of antibiotics), weight and height were measured, a standardized mixed meal test (MMT) was performed (day 2 and day 9) and glucose kinetics were measured in the basal state and during a two-step hyperinsulinemic euglycemic clamp using [6,6-$^2H_2$]-glucose as an isotopic tracer (day 1 and day 10). All measurements were conducted following a 12 h overnight fast (Supplementary Fig. 1). Participants were allowed to continue their usual diet, but were asked to complete an online nutritional diary (www.dieetinzicht.nl) to monitor caloric intake including the amount of dietary carbohydrates, fat, protein, and fibers during the study. Compliance was tested by counted amount of pills returned after one week treatment. The complete methods are included in the Supplementary data.

Statistical analyses

Statistical analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, USA). Depending on the distribution of the data, data are expressed as mean ± SEM or median with range. The primary endpoint was change in intestinal microbiota composition, whereas secondary endpoints were changes in insulin sensitivity and bile acid metabolism after antibiotic treatment. For significant changes between and within treatment groups, clinical parameters were tested with (paired) Student's *t* test or Mann Whitney test (differences between groups) and Wilcoxon Signed (differences within treatment group) depending on distribution of the data. Also, correlations were calculated using Pearson (parametric) or Spearman (nonparametric) coefficients. With respect to intestinal microbiota analyses, the microarray data were normalized and further analyzed using a set of R-based scripts (http://www.r-project.org/) in combination with a custom designed relational database, which runs under the MySQL database management system (http://www.mysql.com). Hierarchical clustering of probe profiles was carried out using Pearson correlation-based distance and complete linkage method. False discovery rate *q* values were calculated to account for multiple testing. Significance level are expressed as $p < 0.05$ or *q* value <0.05. Gut microbial diversity was determined by Simpson reciprocal index as previously described [35].

Results

Baseline characteristics

Twenty male subjects fulfilling the criteria of the metabolic syndrome were randomized to either amoxicillin (500 mg t.i.d.) or vancomycin (500 mg t.i.d.) (Supplementary Fig. 1). Table 1 shows the baseline characteristics for both groups. Almost half of the volunteers in each treatment group reported diarrhea during antibiotic treatment, and no difference was found in the reported stool frequency or compliance between the two treatment groups. No other side effects were noted and there was no change in body weight or daily caloric intake after either intervention. Moreover, there were no significant changes in both plasma lipopolysaccharide binding protein (LBP) levels and (postprandial) lipid profiles during the mixed meal test following antibiotic use in both groups (Table 1).