Gut Decontamination with Norfloxacin and Ampicillin Enhances Insulin Sensitivity in Mice

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Abstract

Recent data suggest that gut microbiota plays a significant role in fat accumulation. However, it is not clear whether gut microbiota is involved in the pathophysiology of type-2 diabetes. To address this issue, we modulated gut microbiota with two combinations of antibiotics in two different mouse models with insulin resistance. Treatment with norfloxacin and ampicillin for 2 weeks reduced the cecal bacterial DNA below the level of detection in ob/ob, diet-induced obese and insulin resistance (DIO) mice, and significantly improved fasting glycemia and oral glucose tolerance of the treated animals. The enhanced insulin sensitivity was independent of food intake or adiposity because pair-fed ob/ob mice were as glucose intolerant as the untreated ob/ob mice. The reduced liver triglycerides, increased liver glycogen and improved glucose tolerance in the treated mice indicate broad impacts on metabolism by gut decontamination. The treatment with non-absorbable antibiotics polymyxin B and neomycin significantly modified cecal microbiota profile in the DIO mice, and the modified intestinal microbiota was associated with a gradual reduction in glycemia during a washout period. In summary, modulation of gut microbiota ameliorated glucose intolerance in mice and altered the hormonal, inflammatory and metabolic status of the host.

Background

The human digestive tract contains a significant number of microorganisms, bacteria being the most dominant. The composition and functionality of the microbiota in each section of the digestive tract have been an area of active research for many years. Several studies, based on the variations in 16S rRNA gene sequence derived from clone libraries, have made comprehensive surveys on the profile of the microbiota in different parts of the digestive tract. These studies, utilizing culture-independent methods, have revealed
unique microbiota profiles in the periodontal pocket [1], the distal esophagus [2], the stomach [3] and the colon [4]. Especially in the gut, microbial communities have been shown to play a critical role in its maturation [5, 6], development of innate immunity [7], production of essential vitamins [8], and the biotransformation of endogenous and exogenous compounds [9].

Recently gut microbiota has been shown to affect fat storage and energy harvesting, which suggests that intestinal microorganisms may play a role in the development of obesity. Bäckhed et al. [10] demonstrated that germ-free mice had defects in storing fat in white adipose tissue and that this was due to higher amounts of circulating lipoprotein lipase inhibitor Fiaf produced by the gut. In support of this, germ-free Fiaf knockout mice gained more weight than their germ-free wild-type littermates when all mice were fed a Western diet, confirming the protective role of Fiaf on body fat accumulation [11]. Also, the composition of cecal microbiota in obese and insulin-resistant ob/ob mice differed from lean controls with a higher ratio of Firmicutes/Bacteroidetes found in the ob/ob mice [12]. Metagenomic analyses revealed that the cecal microbiota in the ob/ob mice was more capable of producing short-chain fatty acids by fermenting dietary fibers. The increased energy harvesting from dietary fibers contributed partly to the excessive weight gain of the ob/ob mice [13]. In humans, the fecal Firmicutes/Bacteroidetes ratio also decreased after obese individuals consumed different low-calorie weight-loss diets, providing an association between gut microbiota profile and weight management [14]. Thus, available evidence suggests that the microbiota could be a contributing factor to obesity.

In both in vitro and in animal models, an increase in proinflammatory cytokines such as TNFα causes tissue insulin resistance [15, 16]. When the systemic inflammation is suppressed by pharmaceutical interventions, the whole body insulin sensitivity is also improved in both mice and humans [17, 18]. However, the source of the low-grade inflammation has not been clearly defined. In type-2 diabetic patients with periodontal problems, treatments with topical antibiotics lower serum TNFα and improve the markers of insulin sensitivity such as HOMA-IR and HbA-1c levels [19]. This finding suggests that reducing local infection can decrease systemic inflammation and enhance whole body insulin sensitivity. Cani et al. [20] showed that subcutaneous infusion of a low dose of lipopolysaccharide (LPS), a component of the gram-negative bacteria cell wall, leads to excessive weight gain and insulin resistance in mice. In the gut, pattern-recognition Toll-like receptors (TLRs) are important for host defense against bacterial infection and the development of innate immunity [21, 22], and TLR4 is responsible for recognizing bacterial LPS. Upon activation of TLR4, NFkB is translocated to the nucleus where it turns on the expression of inflammatory genes such as TNFα and COX2 [23]. Due to the large number of LPS-containing gram-negative bacteria residing in the gut, chronic stimulation of intestinal TLR4 may exacerbate the low-grade inflammation associated with obesity and insulin resistance. To test this hypothesis, we eliminated most members of the gut microbiota in ob/ob and diet-induced
obese and insulin resistant (DIO) mice using two different combinations of antibiotics. We postulated that insulin resistance can be reversed by removing or reducing the gut microbiota in the two animal models. Our data demonstrate that gut microbiota modulation improves whole body glucose tolerance and reduces hepatic steatosis, suggesting that controlling gut microbiota could be a novel therapeutic strategy in treating or managing type-2 diabetes.

Efficacy of Gut Decontamination

To test the hypothesis, we decided to use one or a combination of several broad range antibiotics to remove the majority of the gut microbiota in ob/ob mice. Since the outcomes of the study depended very much upon the success of gut decontamination, we first checked the efficacy and specificity of several antibiotics in in vitro screening tests. Fecal samples from ob/ob mice were diluted and plated on different selective media for bifidobacteria, lactobacillus, enterobacteria or bacteroides. Our data showed that norfloxacin was the only antibiotic capable of killing fecal enterobacteria, while ampicillin was the most efficient antibiotic in eliminating bacteroides. For lactobacillus and bifidobacteria, both ampicillin and amoxicillin were equally effective. Based on these results, the combination of norfloxacin and ampicillin, both effective against gram-positive and gram-negative bacteria, was selected for the purpose of gut decontamination.

Consuming high doses of antibiotics can cause gastrointestinal irritation with nausea, vomiting and diarrhea. These symptoms can have a significant impact: short-term on food intake, and long-term on body weight and the state of insulin sensitivity of the mice. To minimize these undesirable side effects and to determine the most efficient dose of the antibiotic combination, we performed a dose-response study using different concentrations of the norfloxacin and ampicillin combination in ob/ob mice. At the end of the treatment period, cecal samples were collected and cultured in aerobic and anaerobic conditions. A treatment with norfloxacin and ampicillin at 1 g/l in drinking water achieved the highest level of suppression in the number of cecal aerobic and anaerobic bacteria population in ob/ob mice. With this dose, there was no significant difference in body weight (48.6 ± 1.0 vs. 47.6 ± 1.3 g) but a 17% reduction in food intake did occur (67.1 ± 3.86 g in control vs. 55.4 ± 2.86 g in the 1-g/l group) during the 2-week antibiotic treatment period.

Body Weight and Body Fat Are Affected by Food Intake but Not by Gut Decontamination

Based on the notable reduction in food intake during the dose-response experiment, we designed a pair-feeding study to control for potential impacts
of reduced food intake caused by the antibiotic treatment. As observed in the previous experiment, the treated and the control ob/ob mice had comparable weights. However, both groups tended to weigh more than the pair-fed mice (table 1). The weight of epididymal, retroperitoneal and mesenteric fat pads was similar in the treated and pair-fed mice, and both groups tended to have a lower total fat pad weight than the control group (table 1). This result suggests that the amount of food ingested, rather than gut decontamination, determined the fat mass of the ob/ob mice. Thus, gut decontamination did not affect nutrient digestion and absorption in the ob/ob mice. The reason for the higher body weight and lower fat mass in the treated group was due to the weight of the gut, especially cecum weight. As shown in table 1, the gut weight of treated mice was about 2 g heavier than the control and the pair-fed groups, which is approximately equal to the difference observed in body weight between the treated and the pair-fed animals. In spite of the enlarged cecum, the cecal microbiota was strongly suppressed by gut decontamination. We assessed the profile of the cecal microbiota populations by denatured gradient gel electrophoresis based on the unique DNA sequences of 16S rRNA in bacteria. Unlike the control and pair-fed groups, we were unable to obtain a sufficient amount of DNA to perform denatured gradient gel electrophoresis in cecal samples collected from the treated mice. Despite the 17% food restriction, pair-feeding did not alter the profile of gut microbiota.

**Gut Decontamination Improved Oral Glucose Tolerance of ob/ob Mice**

Before the overnight fasted ob/ob mice were challenged with an oral glucose tolerance test (OGTT), gut decontaminated ob/ob mice demonstrated completely normalized basal glucose concentrations table 2. The treated mice
also had much improved oral glucose tolerance with a smaller area under the glucose curve (AUC) during the OGTT (fig. 1a, b). Basal plasma insulin concentrations and insulin responses during the OGTT were also reduced in the treated group (fig. 1c). Despite consuming less food and weighing less than the control group, the pair-fed ob/ob mice were as glucose intolerant as the control mice. In contrast to germ-free mice that have a reduced expression of intestinal SGLT-1 [5], the expression of SGLT-1 in the jejunum was not affected by gut decontamination suggesting that the improved oral glucose tolerance was not due to a defect in glucose absorption. In addition, non-fasting blood glucose concentrations in the treated ob/ob mice were also significantly lower than both the control and pair-fed groups (table 2). Together with improved glucose tolerance, lower glucose and insulin concentrations, our data suggest that removing gut microbiota with norfloxacin and ampicillin significantly enhanced insulin sensitivity in ob/ob mice.

### Table 2. Plasma and liver parameters in the ob/ob mice in the fasting and non-fasting states

<table>
<thead>
<tr>
<th></th>
<th>Fasting state</th>
<th>Non-fasting state</th>
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<tbody>
<tr>
<td></td>
<td>control</td>
<td>Nor + Amp</td>
</tr>
<tr>
<td>Blood glucose, mg/dl</td>
<td>156.3 ± 17.9b</td>
<td>97.5 ± 13.2a</td>
</tr>
<tr>
<td>Plasma insulin, ng/ml</td>
<td>3.27 ± 0.39a,b</td>
<td>2.07 ± 0.44a</td>
</tr>
<tr>
<td>Liver glycogen, mg/g liver</td>
<td>3.72 ± 1.42</td>
<td>3.88 ± 1.02</td>
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<tr>
<td>Liver triglycerides, μg/g liver</td>
<td>200.8 ± 15.3b</td>
<td>159.6 ± 14.7a</td>
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Samples were collected either after 15-hour fasting or in the morning. Liver samples were collected at sacrifice. Data are median ± rob SEM (n = 12 for data in the fasting state; n = 6 for data in the non-fasting state). Different letters represent statistical significance: p < 0.05 using Kruskal-Wallis test followed by Wilcoxon test.

Gut Microbiota Modulation Improved Glucose and Lipid Metabolism in the Liver

Liver glycogen is often lower in patients with type-2 diabetes, and restoration of liver glycogen storage is associated with increased hepatic insulin
Indeed, in the non-fasting state, the control ob/ob mice had less liver glycogen than mice treated with antibiotics (table 2). As expected, all groups showed depleted liver glycogen after an overnight fast. In the state of hepatic insulin resistance, insulin is unable to suppress gluconeogenesis in the liver. Thus, we examined whether gut microbiota modulation had a direct effect on the expression of genes involved in gluconeogenesis. In the liver of antibiotic-treated ob/ob mice, the expression of glucose-6-phosphatase was significantly lower than that in the control group, which provides supportive evidence for the normalized blood glucose concentrations and elevated liver glycogen storage observed in ob/ob mice treated with norfloxacin and ampicillin.

The amount of liver triglycerides is also positively associated with insulin resistance. In the untreated ob/ob mice, a high amount of fat can be seen, whereas gut microbiota modulation considerably reduced the amount of micro- and macrovesicular steatosis in hepatocytes. Pair-feeding did not significantly change liver steatosis in ob/ob mice. Table 2 illustrates the quantification of liver triglyceride levels in both the non-fasting and fasting states. In the gut-decontaminated group, there was a significant reduction in hepatic fatty acid synthase and acetyl CoA carboxylase-1 mRNA levels in the non-fasting state, suggesting that reduced lipogenesis possibly contributed to the lower level of liver triglycerides observed in the gut-decontaminated ob/ob mice.
Gut Microbiota Modulation Suppressed Intestinal Immune Responses

Some of the interactions between gut microbiota and host tissues rely on the activation of TLRs. For example, bacterial lipopeptide in gram-positive bacteria and LPS in gram-negative bacteria can be recognized by TLR2 and TLR4, respectively [25]. Activation of TLR4 particularly leads to the activation of NF\textkappa B and inflammatory pathways including expression of TNF\alpha [26]. Since a significant reduction in total cecal bacteria and Enterobacteria was observed after treatment with norfloxacin and ampicillin, the question was asked whether inflammatory responses in the gut were also reduced. First, the expression of genes involved in the TLR4-signaling pathway in the jejunum was examined. Gut microbiota modulation did not alter the expression of TLR4, CD14, or MyD88. As predicted, the TNF\alpha mRNA concentration in the jejunum was reduced in the ob/ob mice treated with norfloxacin and ampicillin.

Gut Microbiota Modulation Reduced Plasma Endotoxemia

LPSs from gram-negative bacteria in the gut have been shown to play an important role in the development of insulin resistance [20] and non-alcoholic fatty liver disease [27]. To examine whether the plasma endotoxin level was associated with improved insulin sensitivity in the gut microbiota-modulation group, we measured plasma LPS concentrations in all groups, and found that plasma LPS levels were reduced by 32\% in the antibiotic-treated ob/ob mice (table 3). The counts of cecal Enterobacteria, one of the sources of LPS, were also reduced by 5 logs after antibiotic treatment. In addition, plasma adiponectin concentrations were 14\% higher in the treated ob/ob mice than the control mice (table 3). Reduced plasma LPS and increased adiponectin levels

<table>
<thead>
<tr>
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<th>Control</th>
<th>Nor + Amp</th>
<th>Pair-fed</th>
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<tbody>
<tr>
<td>LPS, EU/ml</td>
<td>24.74 ± 2.67\textsuperscript{b}</td>
<td>16.93 ± 1.73\textsuperscript{a}</td>
<td>28.20 ± 4.25\textsuperscript{b}</td>
</tr>
<tr>
<td>Adiponectin, (\mu\text{g/ml})</td>
<td>15.79 ± 0.57\textsuperscript{b}</td>
<td>18.23 ± 0.78\textsuperscript{a}</td>
<td>15.93 ± 1.12\textsuperscript{a, b}</td>
</tr>
<tr>
<td>ALT activity, U/l</td>
<td>194.2 ± 36.3\textsuperscript{b}</td>
<td>116.6 ± 16.9\textsuperscript{a}</td>
<td>163.5 ± 23.6\textsuperscript{a, b}</td>
</tr>
</tbody>
</table>

Plasma samples were collected after 15-hour fasting. Data are median ± rob SEM (n = 12). Different letters represent statistical significance: \(p < 0.05\) using Kruskal-Wallis test followed by Wilcoxon test.
support the results of improved oral glucose tolerance and liver metabolism in ob/ob mice that received gut decontamination. To rule out the possibility that antibiotics caused toxic side effects to the liver, we measured plasma alanine transaminase (ALT) activity. As shown in table 3, plasma ALT activity was markedly reduced after gut microbiota modulation suggesting that it improved liver functions in ob/ob mice.

**Gut Microbiota Modulation Improved Glucose Tolerance in Diet-Induced Obese Mice**

To further demonstrate the beneficial effect of gut microbiota removal on insulin sensitivity in mice, we treated DIO mice with two combinations of antibiotics: (1) polymyxin B and neomycin, and (2) norfloxacin and ampicillin. Polymyxin B and neomycin were selected because of their low oral bioavailability, which allowed us to directly examine the role of gut microbiota in the regulation of glucose tolerance in animals. In contrast to the previous observations using the combination of norfloxacin and ampicillin, the combination of polymyxin B (0.5 g/l) and neomycin (1.0 g/l) only resulted in gut microbiota modulation. We treated DIO mice with polymyxin B and neomycin and examined whether the alternative profile of gut microbiota by a ‘milder’ antibiotic combination can improve the regulation of blood glucose. In addition, using the DIO rather than ob/ob mouse model allowed us to validate the beneficial effects of gut decontamination by norfloxacin and ampicillin. After 10 weeks of high-fat diet feeding, DIO mice were treated with a placebo control, the combination of polymyxin B and neomycin, or the combination of norfloxacin and ampicillin for 2 weeks. At the end of the antibiotic treatment period, half of the mice in each group were sacrificed, and the remaining mice entered a 4-week washout by removing antibiotics from their drinking water. Similar to the results found in the previous experiment with ob/ob mice, blood glucose concentrations in the norfloxacin- and ampicillin-treated DIO mice were markedly reduced (table 4), indicating the robust effect of gut decontamination. In contrast, the blood glucose concentrations in DIO mice treated with polymyxin B and neomycin were not different from the mice in the control group. Surprisingly, during the washout period, mice previously treated with polymyxin B and neomycin showed a continuous reduction in blood glucose concentration whereas the blood glucose concentrations of the norfloxacin- and ampicillin-treated mice remained at the same level as at the end of antibiotic treatment (table 4). The effect of washout on the blood glucose concentration was not due to food intake or body weight as mice in all groups consumed a similar amount of food and gained a similar amount of weight. The microbiota profile, however, was very different among all groups. At the end of the 2-week antibiotic treatment period, polymyxin B and neomycin significantly altered the cecal microbiota profile, and norfloxacin
and ampicillin drastically reduced the cecal bacterial DNA concentrations below the limit of detection. After the 4-week washout period, the pattern of cecal microbiota was similar to that observed at the end of antibiotic treatments, regardless of the combination of antibiotics. Our data demonstrate that a drastic reduction in gut decontamination by norfloxacin and ampicillin rapidly reduced the blood glucose concentrations in DIO mice, and a milder gut microbiota modulation by polymyxin B and neomycin gradually ameliorated the hyperglycemia of DIO mice.

### Conclusions

Gut decontamination with norfloxacin and ampicillin reversed the insulin resistance characteristic of ob/ob mice via multiple pathways (fig. 2). Gut microbiota modulation with polymyxin B and neomycin altered the gut microbiota composition and then reduced fasting glycemia further, supporting the importance of interactions between intestinal bacteria and the host in the regulation of glycemic control in mice. Although the mechanisms of how gut microbiota influence host physiology are still unknown, it is plausible that bacterial-induced inflammatory responses in the gut play a significant role. Since low-grade systemic inflammation is observed in the insulin-resistant state, the presence of certain gut bacteria might exacerbate the inflammatory responses and insulin resistance. Our data indicate that gut microbiota affected insulin resistance independent of obesity, since the mice treated with antibiotics were more insulin-sensitive and yet had similar adiposity to those of the pair-fed mice. Our results support the idea that modulating gut microbiota plays a vital role in whole body insulin sensitivity. However, more work has to be done in order to prove that gut microbiota modulation is an effective therapeutic strategy to treat or manage type-2 diabetes.
Fig. 2. Gut microbiota modulation improved whole body glucose tolerance by reducing the population of gut microbiota and suppressing gut microbiota originated factors including TNFα and LPS. The liver responded to the changes by increasing glycogen storage and decreasing triglyceride accumulation. Elevated adiponectin levels further enhanced insulin sensitivity. Together, a reduced low-grade systemic inflammation was likely the cause of enhanced insulin sensitivity by gut microbiota modulation.

References

Gut Decontamination with Norfloxacin and Ampicillin Enhances Insulin


Discussion

Dr. Bier: I would just like to view the persistence of the gut bacteria in a somewhat different way, and it goes back to discussions we have had about the fetal origins of disease for some years, that is how does the adult organ or cell know what it ate as a fetus or a young child. We have talked about changes in developmental programming, now we have hardwiring, we have talked about clonal selection, etc. Another way of maintaining memory is the introduction and persistence of bacteria that are sending signals and talking. So this is just perhaps another memory phenomenon working to the gut bacteria and I think it is very exciting.

Dr. Chou: Of course this could be true. In our study after we modulate the flora and do the washout, the flora maintains the same pattern in support of your hypothesis. But the question is, after we change the pattern of the flora, can the body relearn new things? So we refresh the memory by exposing it to new environmental stimulation.
Dr. Bier: Of course that is another question: can we change something that has happened. At least some of the implications of the work in humans is that the gut microbiota patterns are different among people but remarkably stable within the people. If that is the case, in a sense this is personalized gut memory being carried in the microflora. The issue was always that cells turnover in a matter of days and a person lives 50 years, so how is this memory being maintained? The gut microbiota can be turning over all the time, but if they are remaining approximately the same species over time, they are carrying the same memory. I just think we are opening up areas of really interesting kinds of research.

Dr. Chou: It is an interesting idea to find associations between the colonization of gut microbiota in an infant and certain physiological outcomes in adult life. Another thing that I would like to emphasize: not only is the diversity of gut microbiota important, the functionality of gut microbiota is as well. New technology, such as metagenomic analysis, would help us to gain more insight into the metabolic pathways of gut microbiota. The activity of gut microbiota could contribute to the tissue memory of humans.

Dr. Walker: I have a question about microbiota in the context of before and after the ob/ob mice developed excessive weight and fat distribution. Is the flora different? Theoretically once flora is formed because of genetic implications and environmental factors, it remains fairly stable during the entire lifetime. The question is does the development of obesity and excessive deposition of fat influence a change in the existing flora or does that flora exist prior to their developing the obese state?

Dr. Chou: That is a great question, and I have the same question regarding the data published by Turnbaugh et al. [1]. I think in that study the different composition of the gut microbiota found in ob/ob mice was due to excessive food intake rather than obesity. Ob/ob mice eat about 6 g/day in our study, which is twice as much as a regular wild-type animal consumes daily. When imaging gut microbiota in ob/ob mice, you also have to deal with an excessive amount of dietary fiber and other nutrients. So is the response of the gut microbiota in ob/ob mice a result of genetic predisposition (leptin deficiency) or just excessive food intake?

Dr. Salminen: In your mouse experiment you showed that 40 days after withdrawal from antibiotic treatment the blood glucose levels were still quite significantly low. Do you also have gut microbiota data for that point? How quickly does this change return to the baseline you started from? In germ-free mice or in mice treated with antibiotics cecal enlargement is always observed. Is it perhaps an adaptive physiological mechanism, or is it actually the consequence of the changes in nutrient absorption?

Dr. Chou: Regarding your first question whether the difference in gut microbiota profile remained after 4 weeks of washout, the answer is yes. To be honest we were surprised by the results because we thought the composition of the gut microbiota would quickly return to baseline. And to answer your second question about cecal enlargement, I think some data have shown that if some dietary ingredients such as chlorides are introduced to germ-free mice, the size of the cecum is reduced [2]. So the size of the cecum is a result of physiological adaptation to the germ-free state.

Dr. Saavedra: This is a great and probably the most provocative presentation yet in terms of all the things we probably need to learn regarding those relationships between bacteria and humans. First just a comment: I think this may have something to do with the concept that Peter Gluckman was mentioning earlier yesterday with regard to a relative mismatch. Yesterday we were talking of this mismatch relative to an energy rich world: Can this mismatch be relative to the hypobacterial world that we live in today compared to what we were before? The reason for this question is that the secular trends in increasing autoimmune disease and obesity are so similar that they could almost be superimposed one over the other. Could the bacterial changes in our diet and environment be related to both autoimmune disease and obesity? My
question goes to the other concept that we were talking about yesterday relative to the differences between flora in the cecum and colon versus flora in the small bowel. There are a number of very nice experiments showing that the cytokine response pattern of the same bacteria orally ingested in the small bowel is very different from the cytokine response when you expose the colon to those bacteria. Can you comment on that?

Dr. Chou: We looked at the gene expressions in the inflammatory pathway in the small intestine. The TLR2 TLR4 expressions were not changed in the jejunum of the antibiotic-treated ob/ob mice. The intestinal TNFα mRNA level was slightly reduced in the treated mice. Also the flora in the small intestine could not only trigger different inflammatory responses but could also directly compete with the nutrient absorption in the host. The result published by Dumas et al. [3] supports the argument that gut microbiota is able to alter nutrient bioavailability and eventually affect the metabolic phenotypes of mice. So there are quite a few hypotheses ongoing that we are very interested in but we don’t have any results.

Dr. Berry: Concerning the lipotoxicity hypothesis in type-2 diabetes or specifically the toxicity of free fatty acids; did you actually measure the plasma concentration of short-chain fatty acids? Did they change during the course of antibiotic exposure?

Dr. Chou: We did not measure the concentration of short-chain fatty acids but we did measure the typical long-chain fatty acids which were not different in the treated ob/ob mice.

Dr. Berry: They are not reduced. You probably have to use a different technique to measure the head space chromatography. Is that something you are planning to do?

Dr. Chou: Yes, we are working with another group in NRC, and we are trying to use metabonomic technology, a proton NMR, to find out the profile of lipids in the liver and plasma.

Dr. Berry: Do you think that the amount of short-chain fatty acids that is being produced could significantly impact plasma levels? I am interested in the short-chain fatty acids as a fuel versus the signaling agents that could produce insulin desensitivitat.

Dr. Chou: I talked to a colleague who did a stable isotope tracer study in humans. Short-chain fatty acids, like acetate, contributed about 7% of the whole body energy supply, and 40% of those short-chain fatty acids come from fermentation in the gut [4]. When the calculation is done, about 2.8% of the whole body energy source comes from the acetate produced in the gut. Assuming that an adult consumes 2,400 kcal/day, the contribution of energy from the fermentation in the gut is less than 70 kcal. Given a generous reduction of short-chain fatty acid production by 50% due to the different gut microbiota composition, we are talking about a less than 40 cal/day difference. Does that really mean it will cause obesity? That is questionable.

Dr. Berry: Has anyone infused the short-chain fatty acids to try to induce a state of glucose intolerance in these experimental mice?

Dr. Chou: In the past I did long-chain fatty acid infusion causing insulin resistance, but I have never done a short-chain fatty acid infusion because it is known that long-chain fatty acids induce insulin resistance. But I am not sure about short-chain fatty acid.

Dr. Bier: I don’t think acetate is 40% of the energy of the organism. We don’t work on acetate, we work on acetyl-CoA. There may be some acetate coming into the gut bacteria, I am just not sure of that. It is a little bit awkward to know what it means when you say acetate is responsible for 40% of the energy because the acetyl moiety comes through oxidation of fat, but it is not really acetate.

Dr. Chou: Actually the study looked at acetate and acetyl-CoA together as a total pool level of acetate. Also the energy contribution of total acetate is 7%, not 40%. 40% of the total acetate pool comes from the gut. So it is a very small amount.
Dr. Bier: Given that this is almost certainly an exploding field, and entirely new methods are being introduced into a field where there are a lot of old methods, and where there are people reporting things in different ways, etc., is there any effort through, for example, Nestlé or Danone or some international organizations to set the rules about how we are going to describe the organisms, the amounts, etc., so that we are all comparing the same things when the studies start being published?

Dr. Chou: I had the same problem when I tried to summarize the literature results; a lot of them cannot be used because of the way the data were presented. I think it requires collaborative efforts from everybody here to promote this idea.

Dr. Bier: The issue of probiotic becomes a problem in a journal unless it is described. I think that the field needs a set of rules and standardization. If it were done with some of the international microbiology societies and the industry or whatever, and a set of rules and descriptions were produced of what actually enhances the publication issues, then I think several journals might be interested in publishing that kind of document or using it as the standard for reporting of things that have to go into those journals. So it seems to me that the time has come to think about these things.

Dr. Salminen: We have discussed a lot about the strain-specific properties of probiotics. It is really our duty and that of any respectable scientific journal to clearly identify the strains used, and use the international culture collection reference numbers and have the strains accessible to researchers. This is the only way that we can actually promote reliable science in probiotics. The same applies in a different way to prebiotics, to actually describe and identify clearly what kind of component is being used.

Dr. Bier: I agree with that, but it seems to me that more is involved: the method, for example, which one, what are the quantities involved, etc. We heard percent colonization yesterday but what fraction of the total of the bacteria; what are the numbers that we need to know to compare studies? I honestly don’t know all of them, but having a group who is knowledgeable to set the rules would be very helpful. This is going to grow extraordinarily in the next decades.

Dr. Chou: There is also the issue of how to assess the gut microbiota. Currently several techniques for the assessment of gut microbiota are being developed, such as microarray-based analyses, cloned library sequencing and quantitative PCR. Each technique has its pros and cons. The results will be much clearer after a comparative study using different technologies has been done. Then we can decide on the best technology to go forward.

References