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COMMENTARY

Metabolic Syndrome in Insects Triggered by Gut Microbes

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Abstract

Emerging research suggests that intestinal microbiome composition is an important factor in the development of obesity. However, little is known about the mechanistic details of this relationship. A recent insect study demonstrated for the first time that metabolic syndrome and symptoms such as obesity and insulin resistance are not restricted to mammals and can be induced by means of a protozoan intestinal infection. This article describes the findings of this study and integrates them with findings from studies relating obesity to the gut microbiota of mammals.

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Root causes of obesity and metabolic syndrome remain unclear. This article highlights a potentially important, yet largely neglected factor in the development of these diseases that involves signals emanating from microbes in the gut. Studies have shown that (1) composition of the gut microbiome affects intestinal nutrient absorption and nutrient deposition in mice[1,2] and (2) lean and obese human subjects differ consistently in the relative proportion of two major bacterial taxa (i.e., Bacteroides and Firmicutes[3]). What causes this variation among individuals remains unknown, but it suggests that disturbances of "normal" mammalian gut microbial community structure may affect metabolism and constitute an environmental factor that triggers metabolic disease such as obesity. Manipulation of the gut microbiome could therefore provide a new approach to the treatment of such diseases.[3] However, there is little basic science knowledge of gut microbial composition and dynamics, and how gut microbes affect metabolism. Thus, there is a clear need for further characterization of the causes and effects of gut microbiome variation on host metabolism.

Most animal taxa have symbiotic relationships with intestinal microbes, and nonmammalian species have often provided experimental models that greatly broaden basic knowledge that is relevant to biomedicine. However, few (if any) nonmammalian studies thus far have either addressed symptoms analogous to mammalian metabolic disease or related such symptoms to changes in the intestinal microbe community. This commentary summarizes findings from an insect study that addresses these matters.

We have found that metabolic disturbances similar to mammalian obesity and metabolic syndrome occur in *Libellula pulchella* dragonflies infected with an intestinal protozoan common in natural populations of a wide variety of invertebrates.[4] Gregarine (Apicomplexa:

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Abbreviations: (AMPK) AMP-activated protein kinase, (MAPK) mitogen-activated protein kinase, (TNF-α) tumor necrosis factor-α

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Eugregarinorida) intestinal parasites are found commonly in insects, both in natural settings and in artificially reared stocks. These parasitic infections are generally considered benign, unless their numbers become large enough to impede passage of food through the insect midgut.

We performed the first study examining physiological impacts of gregarines on invertebrate hosts and found that infected dragonflies suffer negative effects from this infection, regardless of the number of gregarines present in their gut. The most apparent symptom consists of a decrease in overall flight performance, which ultimately results in lower mating success in the field.[5] As a result, infected males tend to adopt a more sedentary "satellite" lifestyle, in which they engage only infrequently in territorial male-to-male flight contests (which require high flight performance). In our attempts to understand this potentially important ecological effect of parasitic infection from a physiological and mechanistic perspective, we found that the flight muscles of infected males generate less mechanical power[4] and they lose the normal positive relationship between lipid reserves and muscle power output.[5] These findings were the starting point of our investigation of the physiological underpinnings of the infection-associated symptoms described later.

In experiments measuring gas exchange by working flight muscles, we found that gregarine infection is associated with an inability of flight muscles to oxidize fatty acids. This was manifested as an increase in respiratory quotient (respiratory exchange ratio) from approximately 0.8 (a stoichiometry indicative of oxidation of a mixture of fatty acids and carbohydrates) in healthy dragonflies to 1.0 (oxidation of carbohydrates only) for infected ones. Moreover, infected dragonflies had 26% more fat deposited around their flight muscles, which we interpret as excess lipid buildup due to the loss of lipid oxidation ability in the muscles. Fatty acid oxidation by insect flight muscles is considered to be required for prolonged flight.[6] The behavioral change from "territorial" to "satellite" by infected males is therefore likely caused by their inability to oxidize lipids.

In addition to poor muscle fatty acid oxidation, infected dragonflies also display an insulin resistant-like state. Blood carbohydrate concentrations (mostly trehalose, the main blood sugar in insects) are double that of healthy dragonflies. When we injected insulin, infected dragonflies showed no drop in blood carbohydrate concentration, whereas healthy dragonflies showed a significant decrease in hemolymph carbohydrate concentration within 30 minutes. These findings regarding loss of fat oxidation by muscles, elevated carbohydrates in the blood, and lack of responsiveness to insulin led us to propose for the first time that metabolic syndrome-like disease states are not restricted to mammals, but can occur in invertebrates as well.

How does this disease state arise? One possibility is through the induction of a (chronic) inflammatory state. Inflammation is now recognized as a common factor to many constituents of the mammalian metabolic syndrome, and inflammatory cytokines produced by adipose tissue especially are considered important to the development of insulin resistance.[7] Moreover, systemic inflammation caused by oral bacterial infections has long been implicated as a contributing or exacerbating factor to obese or diabetic conditions in humans.[8–10] Our results support the hypothesis that not only adipose tissue but also microbes can be the trigger of inflammatory cytokines capable of inducing an insulin-resistant state.

We simulated the presence of gregarine parasites in healthy dragonflies by feeding them water containing trace amounts of excretory–secretory products obtained from live gregarines. A 2-day feeding regime resulted in a doubling of hemolymph carbohydrate concentration in healthy dragonflies. This result shows that even in the absence of gregarines, their antigens can quickly cause symptoms associated with insulin resistance in healthy dragonflies, prior to substantial fat deposition around the flight muscles.

Many infectious microbes induce the production of inflammatory cytokines, which in turn can affect downstream stress signaling pathways such as c-Jun-N-terminal kinase and p38 mitogen-activated protein kinase (MAPK).[11] Basal p38 MAPK activation is increased in muscles of type 2 diabetic humans.[12] In dragonflies, we have found that infection caused chronic activation of p38 MAPK in the flight muscles (i.e., a systemic inflammatory state). We also found that we could induce a similar activation in muscles from healthy individuals within 45 minutes by exposing them to the excretory–secretory products obtained from gregarines.

The question remains whether the excretory–secretory products from gregarines (or co-occurring bacteria or viruses) residing in the dragonfly midgut pass into the body and directly affect p38 MAPK activation in flight muscle or if the effect is caused by intermediate signaling steps, perhaps initiated by gut tissue. To illustrate how disturbances by foreign microbes such as gregarines may induce such a response, consider a study[13] showing that colonization of germ-free (gnotobiotic) mice by one of

two Bacteriodes species induces the expression of tumor necrosis factor-α (TNF-α), a well-known inflammatory cytokine produced by immune system-specific cells (and also a known activator of p38 MAPK). Moreover, it has been suggested that the known increase in TNF-α (among other cytokines) in obesity can suppress the activity of AMP-activated protein kinase (AMPK) indirectly, and AMPK is a critical regulator of fatty acid oxidation.[14] Mice that have been colonized with bacteria have higher activity levels of AMPK and mitochondrial fatty acid oxidation compared to germ-free mice.[15] Indeed, with regard to the demonstrated loss of the ability to oxidize fatty acids, it would be interesting to examine AMPK activity levels in the muscles of infected dragonflies. This line of research is new in both dragonflies and mice, but it seems apparent that there are opportunities for cross-fertilization of results and ideas from studies in different kinds of animals harboring different mixtures of intestinal microbes. Utilization of evolutionarily conserved aspects of cellular and molecular biology have greatly benefited biomedicine in general; we suggest that a similar broad-based approach might benefit the study of metabolic disease as well.

In summary, we have found a system in dragonflies that shows many parallels to what members of Dr. Jeffrey Gordon's laboratory (http://gordonlab.wustl.edu/[1–3,9]) have revealed in mice, i.e., there are mechanistic and causal links between changes in gut microbial composition and overall host metabolism. Our dragonfly work identifies lipid metabolism in muscle as one critical downstream effect of a gut microbial disturbance. Interestingly, we do not know if gregarine protozoans themselves trigger the observed metabolic syndrome or if the presence of gregarines may have introduced different bacteria or viruses or perhaps changed the normal community of gut microbes. Experiments that introduced excretory–secretory products of gregarines suggest a direct effect of these protozoans, but there was nothing in the experimental protocol to prevent the introduction of other gregarine-associated microbes or viruses. Thus, much remains to be learned regarding how variation in the gut microbial community composition affects host metabolic physiology. This is true not just for insects, but for mammals as well.

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References:

1. Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A. 2004 Nov 2;101(44):15718-23.
2. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006 Dec 21;444(7122):1027-31.
3. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature. 2006 Dec 21;444(7122):1022-3.
4. Schilder RJ, Marden JH. Metabolic syndrome and obesity in an insect. Proc Natl Acad Sci U S A. 2006 Dec 5;103(49):18805-9.
5. Marden JH, Cobb JR. Territorial and mating success of dragonflies that vary in muscle power output and presence of gregarine gut parasites. Anim Behav. 2004;68:857-65.
6. Beenakkers AMT, Van der Horst DJ, et al. Role of lipids in energy metabolism. In: Downer RGH, editor. Energy metabolism in insects. New York: Plenum; 1981.
7. Wisse BE. The inflammatory syndrome: the role of adipose tissue cytokines in metabolic disorders linked to obesity. J Am Soc Nephrol. 2004 Nov;15(11):2792-800.
8. Pischon N, Heng N, Bernimoulin JP, Kleber BM, Willich SN, Pischon T. Obesity, inflammation, and periodontal disease. J Dent Res. 2007 May;86(5):400-9.
9. Iacopino AM. Periodontitis and diabetes interrelationships: role of inflammation. Ann Periodontol. 2001 Dec;6(1):125-37.
10. Mealey BL, Oates TW; American Academy of Periodontology. Diabetes mellitus and periodontal diseases. J Periodontol. 2006 Aug;77(8):1289-303.
11. Dirgahayu P, Fukumoto S, Miura K, Hirai K. Excretory/secretory products from plerocercoids of Spirometra erinaceieuropaei suppress the TNF-alpha gene expression by reducing phosphorylation of ERK1/2 and p38 MAPK in macrophages. Int J Parasitol. 2002 Aug;32(9):1155-62.
12. Koistinen HA, Chibalin AV, Zierath JR. Aberrant p38 mitogen-activated protein kinase signalling in skeletal muscle from Type 2 diabetic patients. Diabetologia. 2003 Oct;46(10):1324-8.
13. Sonnenburg JL, Chen CT, Gordon JI. Genomic and metabolic studies of the impact of probiotics on a model gut symbiont and host. PLoS Biol. 2006 Nov;4(12):e413.
14. Steinberg GR. Inflammation in obesity is the common link between defects in fatty acid metabolism and insulin resistance. Cell Cycle. 2007 Apr 15;6(8):888-94.
15. Backhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci U S A. 2007 Jan 16;104(3):979-84.