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Original Article

Tobacco use vs. helminths in Congo basin hunter-gatherers: self-medication in humans?☆

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ABSTRACT

We tested a novel hypothesis that recreational use of neurotoxic plants helps defend against parasites. Specifically, we investigated the relationship between smoking and helminthiasis among the Aka, a remote population of Central African foragers who are avid tobacco smokers, suffer high rates of helminthiasis, and have little-to-no access to commercial anthelmintics. Two hundred and six healthy Aka men provided saliva and stool samples. Saliva samples were assayed for cotinine, a nicotine metabolite; a subsample was genotyped for the CYP2A6 enzyme, which metabolizes nicotine. Stool samples were assayed for intestinal helminth eggs as an index of worm burden. After 1 year, a subsample of participants was located and provided additional saliva and stool samples. We found (1) an exceptionally high prevalence of tobacco use, (2) a significant negative correlation between cotinine (a nicotine metabolite) and worm burden, (3) that treating helminths with albendazole, a commercial anthelmintic, reduced cotinine concentration two weeks later, compared to placebo controls, (4) among treated participants, higher cotinine concentrations in year 1 predicted less reinfection by year 2, and (5) younger and older participants with slow nicotine-metabolizing CYP2A6 alleles had lower worm burdens compared to those with extensive metabolizing alleles. These results provide the first evidence of a link between helminthiasis and smoking. They also suggest that, in populations where intestinal helminths are endemic, tobacco use might protect against helminth infection and reduce worm burden among infected individuals, and that individuals modulate nicotine exposure in response to infection. The results thus support the hypothesis that substance use helps defend against parasites.

1. Introduction

The ancient coevolutionary relationship between vertebrates and helminths (parasitic worms) has potentially profound implications for human health. The fundamental structure and control of the immune system were likely shaped by vertebrate-helminth coevolution, for instance, and the evolutionarily novel *absence* of helminth infections in many populations might therefore be responsible for the increasing prevalence of allergic and autoimmune diseases (the hygiene hypothesis) (Jackson, Friberg, Little, & Bradley, 2009).

It is possible that *behavioral* anti-parasite strategies, also referred to as non-immunological defenses, were also shaped by vertebrate–helminth coevolution. Plants have evolved an enormous variety of toxins to deter herbivores, and herbivores have co-evolved to use plant toxins to defend against their own parasites, a phenomenon referred to as *self-medication*, *zoopharmacognosy*, or *pharmacophagy* (Boppré, 1984; Glander, 1994; Rodriguez & Wrangham, 1993). Woolly bear caterpillars (*Grammia incorrupta*), for example, ingest pyrrolizidine alkaloids to protect themselves from tachinid flies (Singer, Mace, & Bernays, 2009). Tobacco hornworm (*Manduca sexta*) larvae co-opt ingested nicotine to defend against the wolf spider (*Camptocosa parallela*) (Kumar, Pandit, Steppuhn, & Baldwin, 2013) and the endoparasitoid, *Cotesia congregata* (Barbosa, Gross, & Kemper, 1991; Thorpe & Barbosa, 1986). In an interesting use of plant toxins among urban sparrows and finches, cigarette butts containing residual nicotine are placed in nests to defend against nest-dwelling ectoparasites such as mites (Suárez-Rodríguez, López-Rull, & Garcia, 2013). Some species ingest plant toxins to specifically defend against helminths. Domesticated lambs, for instance, respond to gastrointestinal

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parasite infections by increasing their intake of alfalfa tannins (Villalba, Provenza, Hall, & Lisonbee, 2010). Wild chimpanzees (*Pan troglodytes*), which are known to consume a variety of medicinal foods (Huffman, 1997), ingest *Aspilia* (Compositae) and the bitter pith of *Vernonia amygdalina* to treat intestinal helminth infections (Huffman, 2001; Wrangham & Nishida, 1983).

In humans, there is a large literature on the deliberate use of plants for medicinal purposes (ethnomedicine). In addition, there is increasing evidence that the plants that are incorporated into many diets provide protection against parasites (Etkin, 1986; Etkin & Ross, 1991). Indeed, in many cultures, the distinction between plant "foods" and plant "medicines" is not always clear—i.e. many of the plants that are used as medicines are also consumed as foods (Etkin & Ross, 1982, 1991; Moerman, 1996). For example, 90 percent of the plants commonly used by the Hausa of Nigeria to treat malaria are also consumed as foods (Etkin & Ross, 1982, 1991), and cassava (*Manihot esculenta*) and fava bean (*Vicia faba*) contain glycosides that, when consumed as part of the normal diet, protect against *Plasmodium falciparum* malaria among West African and Mediterranean populations, respectively (Jackson, 1990, 1996; Katz & Schall, 1986). Similarly, Billing and Sherman (1998) argue that the addition of spices to food functions to defend against meat-borne pathogens (see also Sherman & Billing, 1999).

It is possible that the human "recreational" use of psychoactive plant drugs is explained, in part, by the antiparasitic properties of these plant compounds. Wyatt (1977), for example, noted the anthelmintic (anti-worm) properties of betel nut, which is widely chewed in Asia and the Pacific. Rodriguez and Cavin (1982) proposed that the origin of tropical hallucinogenic plant use in Amazonia might be explained by the anthelmintic and antimicrobial properties of the plants. Intriguingly, three of the world's most popular recreational drugs are effective treatments against helminths: nicotine (the psychoactive component of tobacco) and arecoline (the psychoactive component of betel-nut) have been used as commercial anthelmintics in animals (Hammond, Fielding, & Bishop, 1997), and cannabis is toxic to plant-parasitic nematodes (Mukhtar, Kayani, & Hussain, 2013). We have proposed that human recreational drug use might involve neurophysiological mechanisms that evolved to regulate the intake of plant neurotoxins to prevent or reduce infections of helminthes and other macroparasites (Hagen, Roulette, & Sullivan, 2013; Hagen et al., 2009). There need be no conscious awareness of the antiparasitic properties of "recreational" drugs, however, just as there is no common awareness of the antiparasitic properties of spices.

1.1. The chemoprophylaxis and chemotherapy hypotheses

Based on an analogy with plant defenses, there are at least two pharmacophagy hypotheses for the human use of psychoactive plant toxins. First, even when they are not under attack, plants maintain a basal level of plant toxins to deter attacks, a form of chemoprophylaxis known as a constitutive defense. Second, when they detect an herbivore attack, plants often up-regulate energetically expensive toxin production, down-regulating production after the attack ceases, a form of chemotherapy (in its non-cancer specific sense) known as an inducible defense (Baldwin, 1999).

Human psychoactive substance use might similarly be explained, at least in part, by both chemoprophylaxis and chemotherapy. Under the chemoprophylaxis hypothesis, the human propensity to consume neurotoxic plants prevents or limits infection by helminths, one hypothesis we test here (populations with virtually no risk of helminth infection, such as the US and other high-income countries, might thus still exhibit considerable use of such substances; if so, this would be an example of an evolutionary mismatch). Under the chemotherapy hypothesis, consumption of psychoactive substances should be up-regulated by infection, serve to limit infection levels (we test this hypothesis here), and be down-regulated if and when the infection is cleared (we test this hypothesis here).

1.2. Nicotine as a model drug

There is an especially solid physiological basis for believing that nicotine could serve as an anthelmintic in humans. Modern anthelmintics such as levamisole and the tetrahydropyrimidines achieve their effects by targeting the same receptors as nicotine – the nicotinic acetylcholine receptors (nAChRs) on somatic muscle cells – which induces spastic paralysis of the parasite, leading to its expulsion (Kohler, 2001). Further, nicotine is an extremely potent neurotoxin and the quantities absorbed via smoking and chewing are surprisingly high. Nausea and vomiting are typically induced by doses of 2–5 mg. The lethal dose for an adult human has been estimated to be 30–60 mg (Gosselin, Smith, & Hodge, 1984; but see Mayer, 2014, who argues that the lethal oral dose of nicotine might be 10× higher). Because smokers typically absorb 1–2 mg per cigarette, and chewers up to 4.5 mg per "wad" (Hukkanen, Jacob, & Benowitz, 2005), tobacco users are regularly absorbing quantities of nicotine just below that which induces acute toxic response.

We are not proposing that humans evolved to consume nicotine specifically. Humans evolved in the Old World, and there is currently little evidence that Old World plants contained high amounts of nicotine (although some Old World foods, such as eggplant, contain low levels of nicotine). Instead, we argue that humans evolved to regulate intake of broad classes of psychoactive plant toxins (Hagen et al., 2009, 2013; Sullivan, Hagen, & Hammerstein, 2008).

Nevertheless, nicotine consumption is not simply an artifact of modern life. *Nicotiana* species were widely used by hunter-gatherers in the Americas (i.e. tobacco) and Australia (i.e. pituri) for thousands of years. *Nicotiana tabacum*, the most well known and widely distributed tobacco species, was domesticated by Andean farmers ca. 1000 YBP (Pickersgill, 2007), but was being consumed by hunter-gatherers in the south-central Andes at least as early as 100 BC (Echeverría & Niemeyer, 2013). By the time of European contact tobacco was being used in a variety of contexts throughout North and South America—it played an important function in many rituals, it was often a medicinal panacea, yet it was also consumed in more mundane, daily, recreational contexts (Tushingham et al., 2013; Wilbert 1987; Winter 2000). Soon after European contact, tobacco was brought to the Old World. Today nearly 20 percent of the global population smokes tobacco (Eriksen, Mackay, & Ross, 2012).

1.3. Population variation in nicotine exposure

1.3.1. Age and sex differences

Cross-nationally, users of popular psychoactive substances, including tobacco, report virtually no use prior to the age of 10. Starting about the age of 12 there is a rapid increase in substance use, so that almost everyone who will ever use a substance has done so by age 20 (Degenhardt et al., 2008). We argue elsewhere that during childhood the risk that plant neurotoxins would disrupt development of the nervous system outweighs any anti-parasitic benefits, so children have an evolved aversion to drug consumption. During adolescence the balance shifts, with increasing benefits outweighing diminishing costs, leading to substantial drug use (Hagen et al., 2013).

Similarly, cross-nationally, men almost always have a higher prevalence of tobacco and other drug use than women (Degenhardt et al., 2008; Hagen et al., 2013). Although proscriptions on women's behavior and lack of access to drugs likely play a role in some of the sex differences in drug use (Kaplan, Carriker, & Waldron, 1990; Mackay & Eriksen, 2002; Waldron et al., 1988), the risk of exposing their fetuses and nursing infants to plant teratogens means that drug use entails a higher fitness cost for women than for men. Therefore, it is possible that the sex difference in substance use is, at least in part, explained by the sex difference in the costs versus antiparasitic or other benefits of toxin consumption (Hagen et al., 2013).

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1.3.2. Differences in drug metabolism

Drug exposure (i.e. the level of a psychoactive substance in the body) is related to both intake and elimination (e.g. drug metabolism). Most drugs, including nicotine, are metabolized by a superfamily of detoxification enzymes known as cytochrome P450 (CYP) hemoproteins. Mammalian CYP enzymes are concentrated in the liver, where the majority of them function to metabolize endogenous fatty acids and perform steroidogenesis. However, a subset of CYPs evolved to also detoxify xenobiotics (e.g. dietary phytochemicals) (Lewis, 2001; Nelson, 1999). About a dozen of the 57 human P450 enzymes are primarily responsible for xenobiotic metabolism. Compared to other CYP enzymes, the xenobiotic metabolizing enzymes are highly polymorphic (Nelson, 1999; Nelson et al., 2004). These polymorphisms, some of which occur at high frequencies in some populations, can have a profound influence on drug elimination rates.

Nicotine is primarily metabolized by CYP2A6, which has over 30 currently recognized alleles (Nelson et al., 2008). Compared to the wild type, some 2A6 alleles increase nicotine metabolism and some reduce metabolism, or are non-functional. Most of the alleles have low frequencies, but a few have high frequencies in some populations. For example, approximately 20% of Japanese have a slow or non-functioning 2A6 allele (Sullivan et al., 2008).

We are proposing that, compared to extensive metabolizers, slow metabolizers might have increased protection against infection because serum nicotine concentrations remain higher for a longer period of time, possibly increasing the prophylactic and/or therapeutic effects.

2. Study

The impact of tobacco use on human helminthiasis has never been investigated, so far as we know, yet about one billion adults are tobacco users (Guindon & Boisclair, 2003), smoking about 15 billion cigarettes every day, and approximately one billion people in developing regions of sub-Saharan Africa, Asia, and the Americas are infected with one or more helminths (Hotez et al., 2008). Hence, the regular intake of an anthelmintic – nicotine – by a broad swath of the global population could be critical to, both, the epidemiology of helminth diseases, which comprise a substantial fraction of disease burden (Hotez et al., 2008), as well as understanding the alarming increase in smoking in the developing world, where tobacco-attributable deaths are expected to double in the next 20 years (Mathers & Loncar, 2006).

To test the pharmacophagy hypothesis for the human 'recreational' use of tobacco (Hagen et al., 2009; Sullivan et al., 2008) we required a population with a high prevalence of helminthiasis yet with little-to-no access to commercial anthelmintics, whose members might therefore be motivated (consciously or unconsciously) to seek out and consume locally available substances, like nicotine, that are potentially effective against helminths. We also needed a population with ready access to tobacco.

We chose a remote population of African hunter-gatherers, the Aka of the Central African Republic (CAR), which met each requirement. Aka "pygmies" number between 15,000 and 30,000. (The term pygmy is now viewed as derogatory in some contexts, but no suitable replacement has yet emerged.) The Aka share several traits with other foragers across the Central African rain forest, such as a strong identity with (and preference for) forest life, relative egalitarianism, polyphonic music, and an association with farmer populations. The Aka spend part of the year camped near villages working in the fields for these farmers, and the rest of the year in the forests hunting and gathering. Aka camps are dispersed along trails that radiate out from farming villages into the forest, with about 6–12 adult Aka per camp (Hewlett, 1996). Previous studies found a high rate of helminthiasis in Aka and other Congo basin hunter-gatherers (Froment, 2014; Lilly, Mehlman, & Doran, 2002).

2.1. Aka recreational drug use

Like other central African foragers, Aka smoke tobacco, marijuana, and the leaves of a native tree they call *motunga* (*Polyalthia suaveolens*, Annonaceae, a.k.a. *Greenwayodendron suaveolens* Verdc., Annonaceae; Engl. & Diels) (Hewlett, 1977; Oishi & Hayashi, 2014). The Aka gather motunga leaves from the trees in the forest, dry them, and roll them in a leaf to smoke. The psychoactive compounds in *P. suaveolens* have not yet been identified. Motunga smoking dates back at least three or four generations, based on our interviews, and the Aka refer to it as the "tobacco of the ancestors," as do the Baka foragers of Cameroon (Oishi & Hayashi, 2014). Given the Aka's rich ethnobotanical knowledge, they probably used motunga, and perhaps other psychoactive plant drugs, long before cannabis and tobacco were introduced into the region. Alternatively, because motunga is typically used when Aka do not have tobacco, such as when they are in the forest, they might have only begun to smoke it as a replacement for tobacco and cannabis after those drugs were introduced.

Cannabis, which contains the psychoactive compound THC, is native to Asia. The approximate date that cannabis was introduced to Africa is disputed, although it was grown in Egypt for as many as a thousand years (Du Toit, 1975). Cannabis spread down the east coast with the Arab caravan trade and might have reached both southern Africa and the Congo Basin before European colonization in the 1500's. The Aka and their trading partners do not smoke cannabis as frequently as they smoke tobacco, perhaps because it is currently illegal in the Central African Republic, which might limit availability.

Tobacco is native to the Americas and was introduced to the African west coast by Europeans as early as the 1500's (Jeffreys, 1963). It quickly spread throughout the continent and into the interior. The Aka smoke two forms of tobacco. The first is called *bangaya*, and is grown locally by neighboring Ngandu farmers. The second are manufactured cigarettes, sold by the Ngandu and other villager merchants in the market. Aka provide labor or forest products to their village-trading partner in return for cannabis and tobacco.

Aka perceive smoking these plant drugs to be both beneficial and harmful. On the one hand, tobacco is believed to increase strength and warmth, making one a better worker, hunter, and dancer. The Aka also highlight *ndjala*, or a "desire," as a main reason why they continue to use tobacco. On the other hand, most Aka recognize that long term use can lead to negative health consequences, such as prolonged coughing and chest pain or birth defects (unpublished data, Roulette, C.J., Hagen, E.H., and Hewlett, B.S.).

Although the Ngandu farmers control access to most of the psychoactive compounds used by the Aka (i.e. tobacco, cannabis, and alcohol) we currently have little data on Ngandu use of plant drugs. There has also been surprisingly little systematic study of the recreational use of tobacco and other drugs in extant hunter-gatherers or similar small-scale societies (Black, 1984). Damon (1973) presents results from a survey of smoking in seven "preliterate" societies, including the !Kung. Although the sample sizes were small (e.g., 14 ! Kung), he found that adults smoked as much as possible, unless forbidden by religion, and that, compared to the other groups, the ! Kung had more favorable attitudes toward smoking, which served an important social function, similar to what we observed among the Aka.

2.2. Aka medicinal uses of recreational drugs

The Aka do not associate smoking tobacco, cannabis or motunga with any antiparasite or medicinal benefits. It is intriguing, however, that they make a tea with the roots and bark of *motunga* to treat helminths (unpublished data, Roulette et al.). According to laboratory studies, *P. suaveolens* contains numerous pharmacological compounds (Abad, Ansuategui, & Bermejo, 2007; Lamidi et al., 2005), including the antiparasitic compound polycarpol (Nyasse, Ngantchou, Nono,

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& Schneider, 2006), and the antibacterial compound suaveolindole (Yoo et al., 2005). Local peoples throughout Africa have taken advantage of these compounds by incorporating the plant into their pharmacopeia. In some parts of Africa, for example, it is used for rheumatism and toothache, and even as an aphrodisiac (Okorie, 1980). The Baka, a closely related group of forest foragers (Bahuchet, 1992, 1993), use *P. suaveolens* (which they term *botunga*) for a variety of medicinal purposes including treating headaches, snake-bites and malaria (Betti, 2004; Hattori, 2010). Virtually every Aka we interviewed used *motunga* as a treatment for parasites. However, the Aka also have numerous other medicinal applications of *motunga*, most of which involve making a tea with the roots and bark of the plant.

Although Cannabis has anti-parasitic activity (Mukhtar et al., 2013), the Aka do not use it to treat or prevent helminth infection. The only medicinal use of cannabis mentioned by the Aka was as a treatment for yellow fever (consumed as a tea).

Of the three recreational plants drugs used by the Aka, tobacco is the only known source of nicotine; it also contains numerous other bioactive compounds, such as nornicotine and anabasine (Hukkanen et al., 2005). Tobacco is included in traditional pharmacopeias throughout Africa, including as a treatment for epilepsy and the Guinea worm (Neuwinger, 1996). In the southwest of C.A.R. a tobacco tea is consumed to treat hemorrhoids. The only medicinal use of tobacco mentioned by the Aka we interviewed was an epicutaneous treatment of an unidentified skin infection, called *dombo*. Although Aka use tea infusions to treat numerous ailments, we found no evidence of them using tobacco tea. The fact that there are many medicinal uses of *motunga*, including as an anthelmintic, but few medicinal applications of cannabis and tobacco, might suggest a long history of *motunga* use, but a relatively recent use of cannabis and tobacco.

The Aka are one of the most egalitarian societies known to anthropology. Nevertheless, 95% of Aka men self-report tobacco use compared to only about 15–30% of Aka women, depending on the population surveyed. The high male prevalence might be partially explained by a pro-tobacco enculturation of young Aka males—i.e. children grow up with adult males who smoke tobacco, they learn that tobacco is associated with success in male subsistence activities, and women prefer male partners who use tobacco because it is associated with success in subsistence activities. The low female use is consistent with fetal protection. Aka men and women mention that tobacco causes harm to a developing fetus and/or breastfed child. While women are not proscribed from using tobacco, the fear of harming their children is a strong motivation preventing many reproductive-aged women from using tobacco (in comparison, a significantly greater proportion of post-reproductive aged women smoke tobacco than do reproductive aged women) (unpublished data, Roulette et al.).

2.3. Predictions

Although the hypothesis we propose applies to all recreational plant drugs, here we only investigate the use of tobacco. We tested four predictions, the first three derived from the chemotherapy hypothesis and one from the chemoprophylaxis hypothesis. First, in a population with endemic helminth infections, worm burden should be inversely correlated with nicotine exposure from tobacco use (i.e., nicotine treats helminth infections). Second, treating helminth infections with a commercial anthelmintic should reduce smoking (i.e., drug intake is down-regulated when it is no longer needed) relative to placebo controls, with the biggest reduction in those with the highest worm burden. Third, individuals with CYP2A6 alleles that reduce nicotine metabolism (slow metabolizers) should have higher nicotine levels for a given level of smoking, and thus lower worm burdens than individuals with normal or fast metabolizing alleles. Fourth, among individuals whose helminth infections have been treated with a commercial anthelmintic, higher smoking levels should limit reinfection with helminths (chemoprophylaxis).

3. Materials and methods

This study was approved by the Washington State University Institutional Review Board (IRB) and the IRB of the Medical School of the University of Bangui, CAR. Informed consent was obtained from all participants. Consent was oral, and not written, because the Aka are non-literate. Both IRBs approved oral consent, and consent was documented by a local translator. Permission to conduct this research was obtained from the Ministere de l'Education Nationale, de l'Enseignement superieur et de la Recherche, CAR.

Because so few women self-report as smokers, and because many variables in the study vary by sex, we only tested our predictions in male participants. However, to confirm the low prevalence of female smoking, we also recruited a small sample of women.

Participants were recruited from three regional subpopulations along the Lobaye river, CAR, with a combined Aka population of about 300 adult men and 300 adult women. We visited all camps within 1 km of a logging road and asked all healthy men present to participate. The sample comprises the 206 men from 69 camps on 23 trails who provided informed consent and a self-report of tobacco use, and for whom we had an age estimate. Nearly all remaining men were absent on extended hunting trips (see the CONSORT diagram in supplemental materials). We did not pre-screen participants for tobacco use, health complaints, or on any other criteria. We recruited 44 adult women from the largest of the 3 regional subpopulations.

3.1. Sample collection

Using provided kits, participants supplied 2–5 ml of saliva and one stool sample every other day for about six days, for a total of three baseline samples each (s1–s3). We instructed participants to provide saliva immediately upon wakening and before smoking their first cigarette (after first rinsing their mouths with water). Stool was collected using ParaPak vials with formalin. Saliva was stored at −20 °C in a solar-powered freezer. Three post-intervention samples (s4–s6) and three one-year samples (s7–s9) were collected similarly. Participants received the equivalent of 1 day's wage for every 3 samples returned (see Fig. 5).

3.2. Cotinine assay

Nicotine plasma half-life is 2–3 hours and cotinine half-life approximately 17 hours. Cotinine is thus a superior biomarker of recent nicotine exposure (Benowitz, 1996). Saliva samples were assayed in the Bioanthropology Laboratory at WSU Vancouver using a Salimetrics enzyme immunoassay kit according to the manufacturer's protocol.

3.3. Semi-quantification of worm burden

Stool samples were analyzed by The Institut Pasteur in Bangui, CAR. A total of three species of geohelminths were assessed for the worm burden score—*Trichuris trichiura* (round worm); *Ascaris lumbricoides* (giant roundworm); and hookworm (including both *Ancylostoma duodenale* and *Necator americanus* because their eggs cannot be distinguished). Worm burden was estimated per-species using (1) a wet smear that was microscopically examined for helminth eggs; (2) the merthiolate iodine formaldehyde (MIF) concentration technique (Blagg, Schloegel, Mansour, & Khalaf, 1955); and (3) the Kato method (Komiya & Kobayashi, 1966). Worm burden comprised the average number of eggs on each ×40 microscope field. There were thus 3 measurements for each of the 3 helminth species (hookworm, whipworm, Ascaris), for a total of 9 measurements per stool sample,

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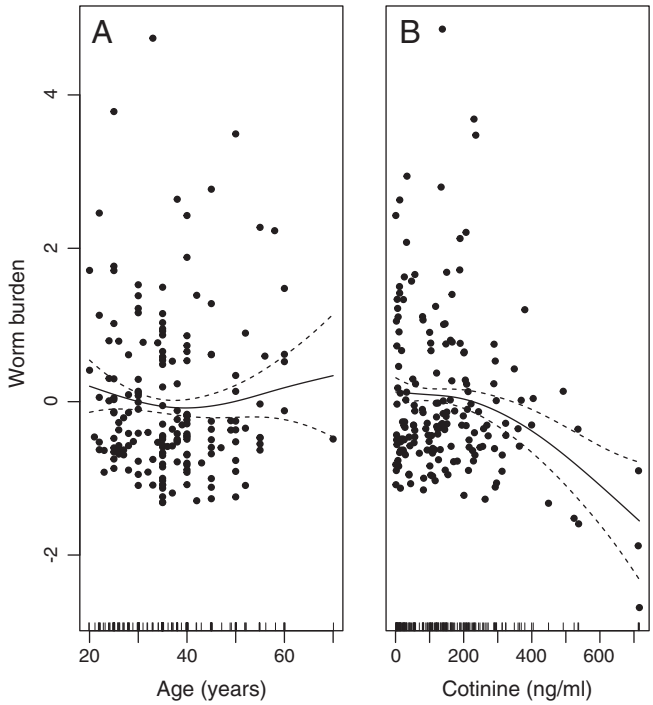

Fig. 1. Plots of male worm burden as smooth functions of age (A) and cotinine concentration (B), controlling for linear functions of village, acculturation, and wealth. Lines fit by a negative binomial GAM with log-link function (the y-axis is thus in units of log worm burden score). For model parameters and p-values, see Table S1. Dashed lines represent ±2 SE.

which were summed to produce the sample worm burden score. The MIF technique is the most sensitive, so this count was weighted by a factor of 2. The sum was then adjusted for the volume of stool examined.

3.4. Control variables

The negative relationship between cotinine levels and worm burden predicted by the pharmacophagy hypothesis could instead result if, e.g., wealth, acculturation, location, other substances, or age confounded tobacco use and access to commercial anthelmintics, health care, exposure to helminths, or immunity. To help rule out some of these alternative hypotheses, we measured 13 control variables in 5 domains: wealth, acculturation, use of commercial anthelmintics, subpopulation, and age. Material wealth was the self-reported number of clothes, watches, flashlights, radios, and batteries owned, with the 'wealth score' equaling the total sum. Acculturation was self-reported attendance in school as a child and as an adolescent, church attendance, and preference for living in the village vs. the forest (yes = 1, no = 0), with the 'acculturation score' equaling the total sum. Participants provided a self-report of use of worm pills or traditional treatment for worms in the last year. The worm pills

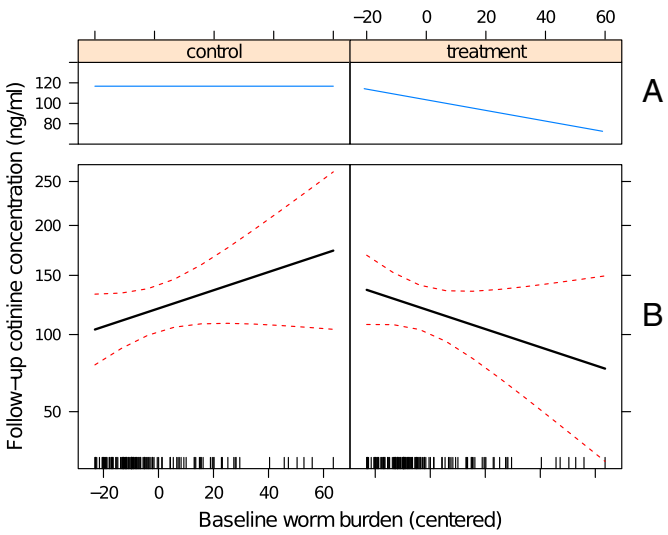

Fig. 2. A. Predicted effect of the intervention * baseline worm burden interaction on follow-up cotinine concentration. B. Study results. Baseline worm burden is centered at the mean. Plotted at mean. Dashed lines represent ±SE. For model parameters and p-values, see table 2.

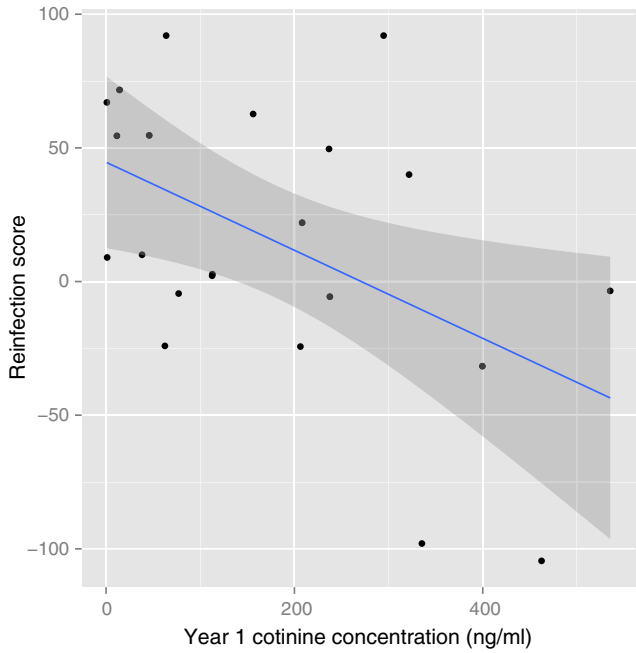

Fig. 3. Scatterplot of reinfection scores vs. year 1 cotinine concentration. Reinfection score = mean year 2 worm burden (s7–s9) minus mean year 1 worm burden (s4–s6). Line fit by linear regression. Shading represents ±SE.

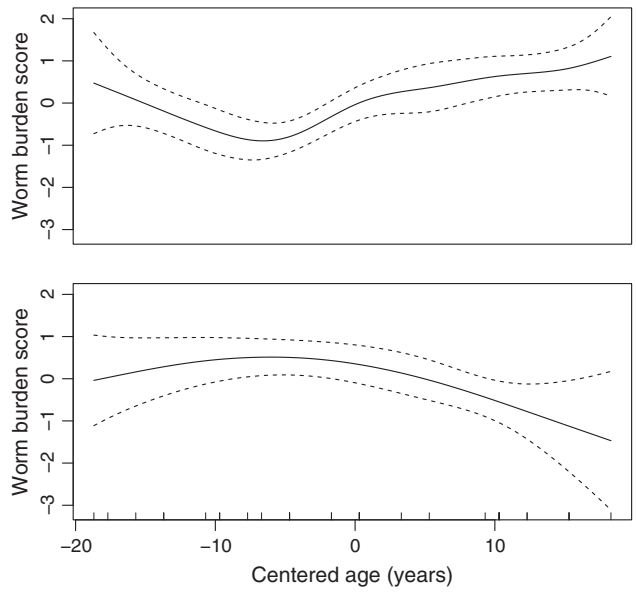

Fig. 4. The effect of CYP2A6 polymorphisms on worm burden. Plots of male worm burden as a smooth function of age, controlling for cotinine concentration. Top: Extensive metabolizers. Bottom: Slow metabolizers. Lines fit by a negative binomial GAM with log-link function; y-axis in log units; dotted lines represent ±2 SE. For model parameters and p-values, see Table S4.

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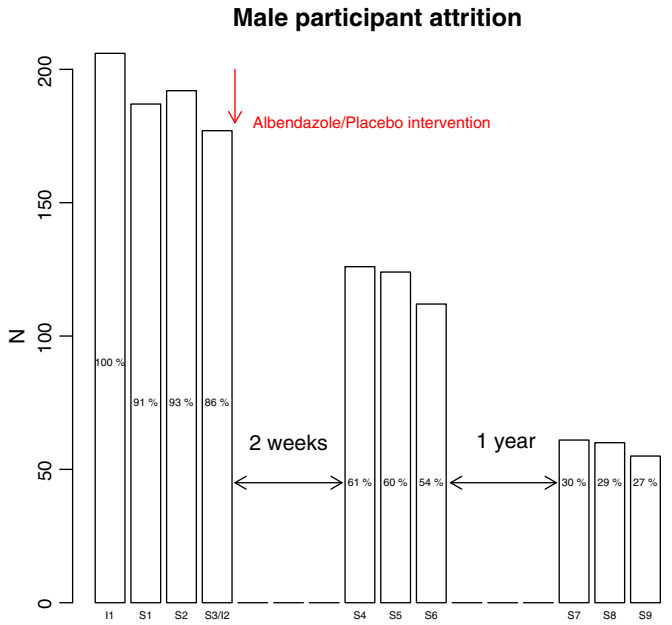

Fig. 5. Saliva and stool sample collection schedule and male participant attrition. 'I1' = initial interview. Wealth and acculturation data were collected during a second interview (I2) upon receipt of sample s3. There is an increase in s2 relative to s1 because some men joined the study after collection of s1.

are available in the market and are widely distributed commercial anthelmintics like albendazole and mebendazole. The traditional medicine was often *motunga* tea. All individuals were coded as belonging to one of the 3 regional subpopulations. Aka do not keep track of age, so age was estimated by Ngandu research assistants who had lifelong associations with these Aka populations.

3.5. Randomized control trial (RCT)

Eligibility for the RCT required male sex, returning at least one saliva and one stool sample, providing a tobacco use self-report, having an age estimate, and being camped near the road when participants were randomized into equal-sized treatment and control groups (double-blind).

Packets containing two pills were prepared by an individual not otherwise involved in the research. In treatment packets the first pill contained 400 mg albendazole (a full course) and the second was a placebo of identical appearance, whereas in control packets the first pill was placebo and the second albendazole. Packets were code-labeled and the list kept secret. Researchers blind to packet contents assigned them to participants and then observed participants take pill #1 (along with a fatty pastry to improve absorption). The immediate psycho-physiological effects of albendazole are difficult to distinguish from baseline symptom rates (Horton, 2000), which should have minimized study participants' ability to distinguish albendazole from placebo. About two weeks later, participants provided 3 follow-up saliva and stool samples (s4–s6), and then took pill #2, concluding the RCT (all participants thus ultimately received albendazole treatment).

3.6. Cotinine vs. reinfection

Aka shift residence every few weeks or months. After 1 year, 22 men in the treatment group were located; these men provided saliva and stool samples (s7–s9). A reinfection score was computed by subtracting mean post-treatment year 1 worm burden scores (s4–s6) from mean year 2 worm burden scores (s7–s9).

3.7. CYP2A6 genotyping

Due to limited funds, we could only genotype a random subsample of 39 males for common CYP2A6 polymorphisms. Compared to the wild type, CYP2A6*1B is associated with faster in vivo nicotine metabolism, CYP2A6*9 and CYP2A6*17 with slower metabolism, and CYP2A6*20 lacks enzymatic activity (Mwenifumbo & Tyndale, 2009). We obtained DNA samples from cheek swabs and Whatman FTA cards or dried blood spots stored at −20 °C.

We extracted DNA using Qiagen reagents and blood kit materials. CYP2A6*1B was tested using a two-step allele-specific PCR method; CYP2A6*17 by a PCR-RFLP method; and CYP2A6*9 and CYP2A6*20 by PCR amplification followed by sequencing. Unclassified alleles were assigned to the CYP2A6*1A wild type allele category (Nakajima et al., 2006).

A predicted metabolic phenotype was computed by assigning fast, normal, and slow alleles scores of 1, 0, and −1, respectively, and then summing the two scores for each individual. Individuals with a predicted phenotype ≥ 0 were assigned to the 'Extensive' category (n = 27) and individuals with a phenotype <0 were assigned to the 'Slow' category (n = 12).

3.8. Data analysis

Statistics were computed with R version 2.15.0 (2012-03-30). The worm burden score was a sum of egg counts, and was overdispersed, and was therefore modeled by a negative binomial distribution. Cotinine concentration was square-root transformed to better approximate a normal distribution when used as a response variable. To assess the effects of population structure (camps and trails), models with and without random effects for these groups were compared by AIC when technically feasible. ΔAIC was <1 in each case, so only models without random effects are reported. All data are available at doi:10.7910//DVN/25824.

4. Results

Table 1 presents summary statistics of the sample at baseline (samples s1–s3; see also Fig. 5). The sample size for cotinine concentration and worm burden is reduced because some participants did not return any saliva or stool samples. Sample size for wealth and acculturation is reduced because these data were collected during a second interview upon the receipt of sample s3, and some attrition had occurred (Fig. 5).

Table 1
Baseline sample characteristics based on interview data and samples s1–s3.

Males (206)	N	Range	Median	Mean	SD
Age	206	18–70	35	36.3	9.8
Cotinine (ng/ml)	199	0.8–715.9	120.2	148.5	139.5
Worm burden score	199	0–95.3	16.3	23.1	19.3
Material wealth score	191	1–8	2	2	1.26
Acculturation score	191	0–4	2	2.2	0.85
Females (44)					
Age	44	20–55	30	32.2	9.4
Cotinine (ng/ml)	42	0.1–332	0.9	10.0	51.3
Worm burden score	42	0–128	19.5	29.3	28.5

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4.1. Helminth infection patterns

Helminth infections were widespread, with 97.9% of the total sample (male and female) testing positive for at least 1 species of helminth. Hookworm was found in 95.4% of the sample, whipworm in 52.7%, and *Ascaris* in 54.8%.

Polyparasitism was common in our sample, with 47.7% infected with two helminth species, and 28.6% infected with three. Infection patterns were similar by sex, although there were 7.1% uninfected females and 1% uninfected males, a difference that was marginally significant ($\chi^2 = 3.8$, $p = 0.052$).

4.2. Smoking patterns

By self-report, 95% of the men were tobacco smokers, and 70% were cannabis smokers, whereas only 16% of the women were tobacco smokers and 5% were cannabis smokers. Using a 5 ng/ml threshold concentration of salivary cotinine to distinguish regular smokers from nonsmokers exposed to environmental tobacco smoke (ETS) (see Supporting information), 94% men were recent tobacco smokers, which accords well with their self-reported use of tobacco, but only 5% of women had recently smoked, a marked discrepancy with their 16% self-reported smoking rate (we did not collect urine samples necessary to assay THC). The geometric mean cotinine concentration among recent Aka male smokers was 100.1 ng/ml. In comparison, the geometric mean cotinine concentration in a large, nationally representative sample of US male smokers was 122.1 ng/ml (Benowitz, Bernert, Caraballo, Holiday, & Wang, 2009).

4.3. Cross-sectional relationship between smoking and worm burden

Missing interview data for some cases yielded a final sample size of 177 men for the analysis of worm burden as a function of cotinine concentration, controlling for age, acculturation, wealth, and subpopulation. Worm burden was higher in the subpopulation located near a large farming village, and lower in the other two, so subpopulation was re-coded as 'village' vs. 'not village.' Participants who had been treated for worms at the local health clinic ($n = 7$; 3%) had significantly higher worm burden scores than those who had not ($W = 293$, $p = 0.01$). However, the hypothesis applies only to individuals without access to commercial anthelmintics. These participants were therefore excluded from this particular analysis only (including them with a dummy variable yielded similar results).

A generalized additive model (GAM) (Wood, 2006) of worm burden as a smooth function of cotinine, controlling for a smooth function of age and linear functions of village, acculturation, and wealth, found that cotinine was a significant predictor ($p = 0.00042$; see Fig. 1; for model parameters, see Table S1).

Examination of Fig. 1B shows that, at high levels, cotinine concentration is strongly negatively correlated with worm burden, as predicted, but at low levels the relationship is essentially flat.

4.4. Randomized controlled trial

Of the original sample of 206 men, 179 (86.9%) participated in the RCT. See Fig. 5. The number of days between the intervention and the start of follow-up (between collection of sample s3 and s4) ranged from 4 to 30, with a mean of 13.6 (SD = 3.9).

4.4.1. Randomization check

To confirm that the sample was successfully randomized, treatment and control groups were compared on size, age, baseline cotinine concentrations, baseline worm burden scores, and distribution among the regional subpopulations. Mean values were compared with t-tests (transforming variables, if necessary), the nonparametric Wilcoxon rank-sum test, permutation tests, and the Kolmogorov–Smirnov test, which is sensitive to both location and shape. No significant differences were found between the treatment and control groups (results not reported).

4.4.2. Attrition

Attrition in an RCT reduces statistical power, can bias estimates of the treatment effect, and can undermine the generalizability of the study. In this study, attrition was primarily caused by participants leaving for extended trips to forest to hunt and gather after receiving the first pill in their packets, and not returning prior to the departure of the research team from the field (such trips are typical for Aka). The primary concern is that dropout differed between the two arms of the RCT, and was related to the outcome measure, nicotine exposure.

There was some evidence of biased attrition: t-tests and two-way ANOVA's of age and baseline cotinine and worm burden values as functions of intervention group and lost status, and their interaction, found that, compared to the remaining participants, participants lost to follow-up were significantly younger ($M = 32.7$ vs. $M = 38.1$, $t = 3.5$, $p = .0006$) and had higher mean worm burden scores ($M = 28.1$ vs. $M = 21.2$, $t = -1.98$, $p = .051$), but did not have significantly different mean root cotinine concentrations ($M = 10.2$ vs. $M = 11.2$, $t = 1.13$, $p = .26$). However, there were no significant interactions of intervention group with lost status, i.e., no significant differences between treatment and control groups in the lost vs. remaining groups for age ($F = 0.77$, $p = 0.38$), cotinine ($F = 0.14$, $p = 0.71$), or worm burden ($F = 0.0$, $p = 0.98$). Despite the biased loss, remaining participants did not differ significantly from the original sample on mean age, baseline cotinine concentration or baseline worm burden. See Table S2.

4.4.3. Manipulation check

According to a summary of the existing literature, the mean cure rate and egg reduction rates for a single 400 mg dose of albendazole are, respectively, 77.7% and 87.8% for *A. duodenale/N. americanus* (hookworm), 94.6% and 98.6% for *A. lumbricoides*, and 47.7% and 75.4 % for *T. trichiura* (whipworm) (Horton, 2000). Consistent with the existing literature, the albendazole treatment group experienced a dramatic reduction in worm burden, $M_{pre} = 21.2$ vs. $M_{post} = 6.7$, Cohen's $d = 0.98$ ($V = 1929.5$, $p < .001$), whereas the placebo control group experienced no significant reduction in worm burden $M_{pre} = 25.0$ vs. $M_{post} = 27.4$, Cohen's $d = -0.1$ ($V = 778.5$, $p = 0.23$). See Fig. S1 (available on the journal's website at www.ehbonline.org).

Whereas there was 1 individual in the treatment group who exhibited no evidence of worm infection at baseline, there were 16 such individuals (24.6%) at follow-up. Albendazole treatment failed to reduce worm burden in 10 individuals (15.4%), which is consistent with previous studies of albendazole treatment of hookworm and whipworm (Horton, 2000).

4.4.4. Effect of albendazole treatment on cotinine concentration

Follow-up cotinine values were highly correlated with baseline cotinine values ($r = 0.8$, $p < .001$). We therefore fit an ANCOVA with follow-up cotinine as the outcome, baseline cotinine and baseline worm burden as covariates, and intervention (treatment/control) as a factor variable, plus its interaction with worm burden (Table 2 and Fig. 2).

Table 2
ANCOVA model of follow-up cotinine concentration. Baseline worm burden is centered at the mean. RSE(123) = 3.45, Adj. $R^2 = 0.66$, $F(4,123) = 62.1$, $p < .001$.

	Estimate	Std. Error	t value	Pr(t)
(Intercept)	2.300	0.697	3.298	0.001
Baseline cotinine	0.779	0.051	15.134	0.000
Baseline worm	0.035	0.023	1.528	0.129
Intervention (treatment)	−0.091	0.619	−0.147	0.884
Baseline worm:intervention (treatment)	−0.069	0.034	−2.014	0.046

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Results supported the main prediction: compared to controls, albendazole treatment caused cotinine concentrations to decrease, moderated by baseline worm burden scores (Fig. 2 bottom). This supports the idea that nicotine exposure is regulated in response to helminth infection, although other causal mechanisms cannot be ruled out.

4.5. Cotinine vs. reinfection

As predicted, there were significant negative Spearman rank correlations between reinfection scores and both year 1 cotinine concentration ($r = -0.46$, $p = 0.014$) and year 2 cotinine concentration ($r = -0.47$, $p = 0.014$). Due to the small sample size, multivariate analysis with control variables was not feasible. These results suggest that nicotine protects against reinfection. See Fig. 3.

4.6. CYP2A6 alleles vs. cotinine concentrations

Individuals with slow-metabolizing CYP2A6 alleles should have higher cotinine concentrations than individuals with normal or fast metabolizing alleles. CYP2A6 allele frequencies from both this study and (to increase sample size) a previously unpublished study of the same population are reported in Table S3. The analysis of baseline cotinine concentration of recent smokers vs. predicted metabolic phenotype included 2 females and 13 males from the previous study, and 39 males from the current study. Although a trend of higher cotinine concentrations in slower phenotypes is apparent in Fig. S2 (available on the journal's website at www.ehbonline.org), an ANOVA of square-root transformed cotinine concentration with a first-order polynomial contrast found it was not significant ($F(1, 52) = 1.9$, $p = 0.18$), perhaps due to the small sample size.

4.7. CYP2A6 polymorphisms vs. worm burden

Regular smokers with slow-metabolizing CYP2A6 alleles should have higher nicotine exposure and thus lower worm burden than those with extensive-metabolizing alleles. The predicted metabolic phenotype (extensive/slow) of participants in this study was added to the GAM of baseline worm burden as a function of age and baseline cotinine concentration analyzed above (Fig. 1). There was no significant main effect of phenotype, but there was an unexpected yet significant curvilinear interaction with age, such that slow metabolizers, relative to extensive metabolizers, had a lower worm burden at older ages, and perhaps also at younger ages (Fig. 4; see Table S4 for model parameters). (The relationship between baseline cotinine concentration and worm burden in this model was very similar to that in Fig. 1).

5. Discussion and limitations

5.1. Helminth infection patterns

Aka suffer very high rates of helminth infection, with 99% of the men in our sample infected with at least one species. Rates of helminth infection were similar to those found in another Aka population working at a national park about 230 km to the southwest of the study field site, where 93.1% were infected with hookworms or nodular worms, 75.9% with whipworms, and 10.3% with *Ascaris*. Interestingly, those Aka had higher worm burden than local farmers and sympatric gorillas and chimpanzees (Lilly et al., 2002, cf. Froment, 2014).

5.2. Smoking patterns

By any measure, the 94% prevalence of male smoking among the Aka is extraordinarily high. The prevalence of male smoking in the Congo basin is 10–14%, and in sub-Saharan Africa ranges from 10% to 27% (Pampel, 2008). The highest national male smoking prevalences are in the range of 60–70% in e.g., Russia, Indonesia, and some Pacific islands. Moreover, Aka men report earning the equivalent of USD 0.50 a day working for the farmers (a day's work being about 4–5 hours of physical labor), and spending half of that, about USD 0.25/day, on tobacco (unpublished data, Roulette et al.). Smoking is obviously very important to Aka men.

Cotinine values confirm a low prevalence of tobacco use in Aka women (5% were recent smokers), consistent with rates seen in women in other developing countries (Guindon & Boisclair, 2003). Hence, the sex difference in Aka smoking is also dramatic, with an odds ratio of 94.5 based on self-report and 311.7 based on salivary cotinine concentration.

The pharmacophagy hypothesis received support from all studies, with some limitations. None of the four studies included a behavioral measure of smoking. Thus, differences in cotinine concentration in these studies, including the treatment effect in the RCT, could be a consequence of differences in nicotine intake (e.g., differences in smoking) and/or differences in clearance of nicotine and cotinine.

5.3. Observational studies of smoking vs. worm burden

The three observational studies all found that higher nicotine exposure was correlated with lower worm burden. The cross-sectional relationship between nicotine and worm burden (Fig. 1B) appears similar to a dose–response curve, with worm burden beginning to drop for cotinine concentrations exceeding about 200 ng/ml (roughly the level found in 1 pack/day smokers), controlling for age, region, wealth, and acculturation, which supports the chemotherapy hypothesis.

Individuals with slow metabolizing CYP2A6 alleles had marginally significantly higher cotinine values (see Fig. S2, available on the journal's website at www.ehbonline.org) and significantly lower worm burden scores than those with normal or fast metabolizing alleles, the latter result holding only at younger and older ages (Fig. 4). The marginal significance of the metabolic phenotype effect on cotinine concentration is not surprising, given the small sample size and limited number of saliva samples. Over years of smoking, the cumulative effect of increased exposure to nicotine could be substantial. Regarding the unexpected curvilinear interaction with age: in populations with endemic worm infections, worm burden usually peaks during middle childhood or adolescence (Anderson & May, 1985), and again in old age (see, e.g., Fig. 1A). One interpretation, therefore, is that male tobacco use is relatively constant across metabolic phenotypes, leading to higher lifetime levels of nicotine exposure in slow metabolizers. This higher lifetime exposure protects against the increase in worm burden that typically occurs in younger and older men.

Finally, in a sample treated with albendazole in year 1, individuals with higher cotinine concentrations in year 1 and/or year 2 had lower reinfection by year 2, supporting the chemoprophylaxis hypothesis.

5.3.1. Limitations of observational studies

It is possible that differences in worm burden caused differences in cotinine concentration, a direction of causation contrary to the hypotheses we tested here, or that cotinine concentration and/or worm burden were influenced by confounding factors that we did not control for, including the consumption of dietary or medicinal plants that contain nicotine. It is unlikely that plant foods would contribute much to the overall cotinine concentrations, however, because the nicotine concentrations of dietary plants are very low (Domino, Hornbach, & Demana, 1993).

The effects of grapefruit juice, other citrus juices, and a variety of plant compounds on drug metabolism are well documented. It is possible that some Aka plant foods or traditional medicines alter nicotine metabolism, leading to higher or lower cotinine concentrations. It is also possible some dietary foods prevent and/or treat infection

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(cf. Etkin, 1986; Etkin & Ross, 1982; Jackson, 1990, 1996; Moerman, 1996), altering worm burden values.

The negative relationship between cotinine concentration and worm burden (Fig. 1) was primarily due to the minority of individuals with high cotinine concentrations. Both the reinfection and CYP2A6 allele studies had small sample sizes, which limited our ability to control for confounds with multivariate statistical models. In addition, CYP2A6 metabolizes other xenobiotic substrates, such as coumarin. It is possible that some other CYP2A6 xenobiotic substrate in the Aka diet is protective against helminths. Further, CYP2A6 plays some role in estrogen metabolism, is induced by estrogen via the estrogen receptor, and is inhibited by other steroids and neurotransmitters (Higashi, Nakajima, Katoh, Tokudome, & Yokoi, 2007). As there is bidirectional interaction between gonadal steroids and the immune system (Grossman, 1985), CYP2A6 polymorphisms could, in principle, directly influence immunity to helminths via their effect on estrogen metabolism (although this effect appears small), or another physiological pathway. Nevertheless, because slow metabolizing alleles of other P450 enzymes occur at relatively high frequencies in some populations, it is worth considering that these alleles provided protection against parasites by slowing the clearance of dietary toxins.

5.4. Randomized controlled trial

Treating Aka men for helminthiasis with albendazole reduced cotinine concentrations 2 weeks later, compared to placebo controls (controlling for baseline cotinine concentration and worm burden scores). This causal effect was moderated by baseline worm burden score, with a larger reduction in cotinine concentration seen in men who, at baseline, had higher worm burden scores. At 20 points above the mean worm burden score, for example, men in the treatment group had cotinine levels consistent with about 8.7 cigarettes/day vs. 11.4 cigarettes/day in the control group (Fig. 2 and Table 2). Other aspects of the model in Table 2 and Fig. 2 largely support the pharmacophagy hypothesis, with some caveats. In the sample as a whole, cotinine concentration decreased from baseline to follow-up (coef = 0.78). Although unexpected, the decrease is not too surprising as there are numerous factors that affect access to tobacco in this population. For instance, 40 men reported spending time hunting in the forest during the two-week period between collection of baseline and follow-up samples, which would decrease access to tobacco.

Despite this overall decrease in cotinine concentration, in the control group there was a positive relationship between baseline worm burden and follow-up cotinine (coef = 0.03; Fig. 2B), whereas this coefficient was predicted to be 0 (i.e., in the control group, follow-up cotinine should not depend on baseline worm burden; Fig. 2A). However, this coefficient, although positive, was not significantly greater than 0 ($p = 0.13$).

Another prediction was that because treatment would have caused little change for individuals with low baseline worm burden, the follow-up cotinine concentration of such individuals in the treatment group should have been similar to comparable individuals in the control group. Instead, individuals in the treatment group with low worm burden had slightly higher follow-up cotinine than individuals in the control group (left hand side of the panels in Fig. 2B). Again, however, this difference was not significant.

5.4.1. Limitations of the RCT

The RCT design does not elucidate the underlying causal mechanism. The treatment effect could be due to mechanisms unrelated to self-medication, or to population specific mechanisms, contrary to the hypothesis tested here. For instance, treatment improved health, which could have encouraged some men to spend more time in the forest, reducing access to tobacco (an hypothesis we were able to rule out: there was no significant difference between the treatment and control groups in days spent in the forest from intervention to follow-up, $M = 1.2$ vs. $M = 1.3$, $t = 0.22$, $p = .83$). Alternatively, albendazole slightly induces CYP2A6 in the rat (Souhaili-El Amri, Fargetton, Benoit, Totis, & Batt, 1988), so there is a possibility that albendazole itself influences nicotine or cotinine metabolism. It is unlikely that such P450 induction explains our treatment effect, however, because participants received a single 400 mg dose of albendazole; albendazole and its active metabolite have a short half-life of about 12 hours whereas follow-up cotinine was measured about two weeks after receiving albendazole; and the treatment effect was moderated by baseline worm burden, which is not clearly consistent with an induction-mediated pathway. Finally, cannabis is known to contain anthelmentic compounds that are potentially toxic to nematodes. In the RCT study, treatment could have affected cannabis smoking or THC metabolism. However we did not quantify cannabis use and THC, which is a limitation of our study.

5.5. Candidate self-medication mechanisms

An evolved mechanism to self-medicate with psychoactive substances should up- and down-regulate consumption and elimination of such substances in response to infection and/or infection risk, either by increasing consumption of a plant substance and/or by physiologically down-regulating drug metabolism. Intriguingly, infection and inflammation are associated with a broad down-regulation of xenobiotic-metabolizing enzymes and transporters in humans and laboratory animals (albeit with complications for CYP2A6), which often results in a pronounced increase in plasma concentrations of various drugs. This well-documented but poorly understood phenomenon (Morgan et al., 2008) could be evidence for a chemotherapy mechanism.

The "proinflammatory hypothesis of drug abuse" has emerged from growing evidence of immune involvement in drug reinforcement. Bidirectional neural-immune interactions are also well established (Wrona, 2006). More specifically, opioids, perhaps acting as xenobiotic-associated molecular patterns, activate toll-like receptor 4 (TLR4) signaling, which surprisingly reinforces opium consumption via the mesolimbic dopamine reward pathway (Hutchinson et al., 2012). Especially intriguing is direct evidence that the immune system modulates intake of the psychoactive drug ethanol (Blednov et al., 2011, 2012). One genome-wide association study found that smoking behavior might be regulated by IL-15 (Liu et al., 2009). Such results indicate an intimate relationship between psychoactive drug use and immunity, and importantly that central immune signals can mediate drug consumption. (The prevailing view, however, is that drug abuse increases infection risk by impairing immunity [Friedman, Newton, & Klein, 2003].)

Keeping in mind study limitations and uncertainty about mechanisms, if tobacco use in this population is motivated, in part, by therapeutic benefits, it implies that signaling pathways exist between the immune and nervous systems that might be exploited to reduce smoking. The project also provides a novel model system to test evolutionary theories of self-medication by humans and other primates. Lastly, these results, to our knowledge, are the first to suggest an important relationship between smoking and helminthiasis, two of the world's most pressing health problems.

Supplementary Materials

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.evolhumbehav.2014.05.005.

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