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Original Research Article

Toward Quantifying the Usage Costs of Human Immunity: Altered Metabolic Rates and Hormone Levels During Acute Immune Activation in Men

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ABSTRACT There is a paucity of data on the energetic demands of human immune functions, despite the fact that both clinical medicine and evolutionary biology would benefit from further clarification of these costs. To better understand the energetic requirements of mounting a mild immune response, as well as some of the major hormonal changes underlying these metabolic changes, we examined changes in resting metabolic rate (RMR) and hormones during and after respiratory tract infection in young adult men. An epidemiologic passive detection design was used to recruit 25 nonfebrile subjects naturally infected with respiratory tract pathogens. Symptomology, percent body fat, RMR, salivary testosterone and cortisol, and other information were collected at a minimum of three time points during and after convalescence. Comparisons of the differences in RMR, testosterone, and cortisol between sampling days within individual cases were made using paired *t*-tests. Participants experienced 8% higher RMR during illness, and a subset of these men experienced a mean increase greater than 14%. The participants also experienced 10% lower testosterone levels during illness, and a subset of these participants experienced a mean decrease of 30%, although cortisol levels did not change significantly. These results document elevated RMR following natural pathogen exposure in adult humans, demonstrating that even mild immune reactions can elicit significant increases in energy expenditure. Understanding the costs of immunity and the immunomodulatory actions of hormones are central to understanding the role of immunity in human life history evolution. Am. J. Hum. Biol. 22:546–556, 2010.

IMMUNE FUNCTIONS ARE ENERGETICALLY COSTLY

Immunological research has traditionally focused on clinical and molecular studies to characterize the structure and function of various immune responses used for allostasis. More recently, the study of ecological immunology has focused on explicating the physiological and ecological determinants of variation in immune functions and ultimately the fitness consequences of this variation. One broad perspective is that because immunocompetence is an integral part of organismal life histories, it is involved in physiological trade-offs with other functions (Barnard and Behnke, 2001; Gustafsson et al., 1995; King, 1973; Lockmiller and Deerenberg, 2000; Norris and Evans, 2000; Sheldon and Verhulst, 1996). Organisms require a relatively steady supply of energy to sustain biological functions, but because resources are finite, they must be allocated between a number of competing functions, most notably growth, reproduction, work, storage, temperature regulation and all other forms of maintenance, including immune responses. Under conditions of resource restriction, diversion of metabolic energy to support one function will reduce the availability of energetic resources for other needs. Organisms will therefore be under selection to develop and maintain physiological systems that allow for the efficient regulation of resources between these functions. Such a system operates under the assumption that the ability to mount an effective immune response is energetically costly.

A large body of research in nonhuman animals now concludes that development, maintenance, and activation of immune responses generate a substantial energetic burden [see Sheldon and Verhulst (1996), Lockmiller and Deerenberg (2000), Schmid-Hempel (2003), Zuk and Stoehr (2002), Demas (2004), and Muehlenbein and Bribiescas (2005) for reviews]. As stated by Derting and Compton (2003), "understanding the cost of immune function is essential for more accurate characterization of energy budgets of animals and better understanding of the role of immunity in the evolution of life-history strategies" (p 744). Surprisingly, there is a paucity of data on the energetic demands of human immune functions relative to other species like rodents and birds. A selection of works on nonhuman animals is compiled in Table 1. In these cases, prolonged energy restriction and strenuous participation in energetically demanding tasks can impair immune functions, and activation of immune responses can alter metabolic rates and reproductive functions in most species examined to date.

In humans, prolonged energy and nutrient restriction as well as intense physical exercise can lead to immunosuppression (Chandra, 1992; Chandra and Newberne, 1977; Gershwin et al., 1984; Kumae et al., 1994), and supplementation with calories, micro- and macronutrients can offset age-related declines in immunity (Wouters-Wesseling et al., 2005). In adult humans, physically and psychologically stressful military training is associated with reduced natural killer cell counts and increased incidence of upper respiratory tract infection (Gomez-Merino et al.,

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TABLE 1. Numerous studies describe the energetic costs of immune activation in nonhuman organisms

Species	Manipulation	Result	References
Collared flycatcher (*Ficedula albicollis*)	Increased brood size	Reduced antibody response against Newcastle virus	Nordling et al., 1998
Zebra finch (*Taeniopygia guttata*)	Increased brood size	Reduced antibody response against sheep red blood cells	Deerenberg et al., 1997
Tree swallow (*Tachycineta bicolor*)	Increased brood size	Reduced antibody response against sheep red blood cells	Ardia et al., 2003
Great tit (*Parus major*)	Increased brood size	Increased prevalence of plasmodium	Richner et al., 1995
Bumblebee (*Bombus terrestris*)	Injected with lipopolysaccharide and latex beads	Reduced survival compared with controls	Moret and Schmid-Hempel, 2000
Mosquito (*Anopheles gambiae*)	Injected with lipopolysaccharide	Reduced egg production	Ahmed et al., 2002
House sparrow (*Passer domesticus*)	Injected with lipopolysaccharide	Reduced reproductive success	Bonneaud et al., 2003
Chicken (*Gallus domesticus*)	Injected with sheep red blood cells	Lowered fat deposition despite increased food consumption	Henken and Brandsma, 1982
Tree lizard (*Urosaurus ornatus*)	Injected with follicle stimulating hormone to increase reproductive investment	Suppressed wound healing following punch biopsy	French et al., 2007
Rat (*Rattus norvegicus*)	Infected with nematode *Nippostrongylus brasiliencsis*	Decreased body weight compared with controls	Ovington, 1985
Chicken (*Gallus domesticus*)	Infected with *Eimeria sp*.	Less weight gain compared with controls	Takhar and Farrekk, 1979
Chicken (*Gallus domesticus*)	Selected for resistance to Marek's disease	Lowered adult body weight compared with controls	Warner et al., 1987
Chicken (*Gallus domesticus*)	Selected for high antibody response against sheep red blood cells	Smaller comb size	Verhulst et al., 1999
Sheep (*Ovis aries*)	Selected for reduced intestinal helminth load	Lowered lamb growth rate compared with controls	Bisset et al., 2001
Pig (*Sus domesticus*)	Vaccinated against porcine respiratory and reproductive syndrome	21% decrease in body weight	Spurlock et al., 1997
West African dwarf goat (*Capra hircus*)	Infected with *Trypanosoma vivax*	28% increase in heat production	Zwart et al., 1991
House mouse (*Mus musculus*)	Injected with keyhole limpet hemocyanin	20–30% increase in oxygen consumption	Demas et al., 1997
Guinea pig (*Cavia porcellus*)	Infected with *Legionella pneumophila*	33% increase in oxygen consumption	Cooper et al., 1989
White cabbage butterfly pupa (*Pieris brassicae*)	Nylon implant	8% increase in metabolic rate	Freitak et al., 2003
Blue tit (*Parus caeruleus*)	Immunized with diptheria-tetanus vaccine	8–13% increase in metabolic rate	Svensson et al., 1998
Great tit (*Parus major*)	Injected with sheep red blood cells	9% increase in metabolic rate	Ots et al., 2001
Collared dove (*Streptopelia decaocto*)	Injected with sheep red blood cells	8.5% increase in metabolic rate	Eraud et al., 2005
Common sparrow (*Passer domesticus*)	Injected with phytohemagglutinin	29% increase in metabolic rate	Martin et al., 2003
White-footed mouse (*Peromyscus leucopus*)	Injected with sheep red blood cells	17% increase in metabolic rate	Derting and Virk, 2005
Black rat (*Rattus rattus*)	Infected with *Fasciola hepatica*	56% increase in metabolic rate	Magnanou et al., 2006

2005). The stress of physical exertion during elite athletic competitions is also associated with increased incidence of upper respiratory tract infections (Nieman et al., 1990; Peters and Bateman, 1983).

Resting metabolic rate (RMR) is the amount of energy expended on basic cellular functions in the absence of physical activity, digestion, thermogenesis, and activation of the sympathetic nervous response. It is the largest component of total daily energy expenditure and is determined by many factors, including body mass, body composition, temperature, age, etc. (Schofield, 1985). Severe perturbations like sepsis, burns, trauma, and surgery are associated with a 25–55% increase in RMR compared with healthy subjects, as well as a reduction in body weight and total body protein (Arturson, 1978; Biolo et al., 1997; Carlson et al., 1997; Frankenfield et al., 1994; Kreymann et al., 1993; Long, 1977), and increase in nitrogen excretion (Carlson et al., 1997; Hasselgren and Fischer, 1998). Similarly, sickle cell disease and cystic fibrosis are both associated with elevated RMR in adults (Borel et al., 1998; Buchdahl et al., 1988). Vaccination for typhoid and yellow fever both result in increased RMR (Barr et al., 1922; Cooper et al., 1992; Gandra and Scrimshaw, 1961). In children, mild immune activation can also produce significant increases in metabolic rate (Duggan et al., 1986; Fleming et al., 1994).

To fuel the body with protein, glucose and amino acids during immune activation, tissue catabolism, proteolysis, lipolysis, glycolysis, and gluconeogensis are increased (Beisel, 1977; Crouser and Dorinsky, 1996; Duke et al., 1970; Klasing, 1988; Michie, 1996). Both skeletal muscle and adipose tissue serve as significant sources of energy for immunity (Demas and Sakaria, 2005; Newsholme, 2001; Rooyackers and Nair, 1997). These resources are necessary not only for activation of immune responses but also for maintenance of the immune system. In humans, the rapid, constant turnover of T and B cells is very likely energetically demanding. Peripheral naïve B cells have an average rate of division of 0.46% per day, and memory B cells proliferate at 2.66% per day (Macallan et al., 2005). CD4+ effector-memory T cells exhibit an average proliferation rate of 4.7% per day, with an average doubling time

of 15 days for adults (Macallan et al., 2004). The exact energy consumption of this maintenance is equivocal. In general, though, developing, maintaining, and activating adequate immune responses certainly require significant energetic resources.

HORMONES ARE IMPORTANT MODERATORS OF IMMUNE AND METABOLIC FUNCTIONS

Increased metabolic demands during infection are met largely through the actions of various hormones and immune factors. In fact, many molecules exhibit pleiotropic actions on metabolic, immune and reproductive functions, including thyroid hormones, cytokines, glucocorticoids, and androgens (Frayn, 2003). Thyroid hormones and several cytokines, particularly tumor necrosis factor-α and interleukin-6, play important roles in altering RMR (de Lange et al., 2001; Freake and Oppenheimer, 1995; Johnstone et al., 2005; Matarese and La Cava, 2004; Stouthard et al., 1995; Tsigos et al., 1997). Nutritional restriction (as during illness) usually results in elevated cortisol levels (Bergendahl et al., 2000) that stimulate gluconeogenesis, tissue catabolism, insulin resistance, amino acid metabolism, and RMR (Brillon et al., 1995; Khani and Tayek, 2001; Tataranni et al., 1996). Cortisol also inhibits inflammation (Elenkov and Chrousos, 1999; Elenkov et al., 1996), affects cytokine production (DeRijk et al., 1997; Turnbull and Rivier, 1999), and increases monocyte apoptosis (Norbiato et al., 1997), which may translate into increased susceptibility to infections. Cortisol, like testosterone (Braude et al., 1999), may also affect the differential trafficking of leukocytes to tissues or areas when they are needed, as during infection.

Cortisol can directly suppress Leydig cell function (Gao et al., 2002; Hardy et al., 2005), downregulate testicular luteinizing hormone receptors (Aakvaag et al., 1978; Bambino and Hsueh, 1981), and suppress the production and secretion of gonadotropins from the hypothalamus and pituitary (Attardi et al., 1997; Breen and Karsch, 2006; Doerr and Pirke, 1976; Kalantaridou et al., 2004; Mitchell et al., 2005). In contrast, testosterone's actions on male reproductive physiology and most other somatic functions are quite the opposite. Testosterone facilitates muscle anabolism by increasing protein synthesis and glucose uptake in muscle cells and increasing metabolic rates of muscle cells (Bhasin et al., 1996; Tsai and Sapolsky, 1996). In this manner, testosterone would augment male reproductive effort, primarily through its actions on musculoskeletal function (e.g., skeletal muscle mass, red blood cells, cortical bone density, etc.), which would facilitate inter- and intrasexual competition (Bribiescas, 2001). At the same time, testosterone stimulates fat catabolism and adipose tissue redistribution (Marin et al., 1992; Welle et al., 1992), and altered somatic composition combined with increased energetic costs could compromise survivorship (Bribiescas, 2001; Ketterson et al., 1992; Marler and Moore, 1988; Marler et al., 1995). Other costs of elevated testosterone levels include increased risk of prostate cancer (Soronen et al., 2004), elevated production of oxygen radicals (Zirkin and Chen, 2000) and reduced resistance against oxidative damage (Alonso-Alvarez et al., 2007), and increased risk of injury due to hormonally augmented behaviors such as aggression, violence, and risk taking (Dabbs, 1996; Wilson and Daly, 1985). Furthermore, testosterone's immunomodulatory actions appear to be primarily suppressive, increasing suppressor T-cell populations, reducing T-helper cell function, inhibiting cytokine and antibody production, and impairing natural killer cell and macrophage activity (Burger and Dayer, 2002; Chao et al., 1994; Daynes and Araneo, 1991; Giltay et al., 2000; Grossman et al., 1991; Grossman, 1995; Lin et al., 1996; Olsen and Kovacs, 1996; Smithson et al., 1998; Straub and Cutolo, 2001; Weinstein and Bercovich, 1981; Wunderlich et al., 2002). See Muehlenbein and Bribiescas (2005) for a review.

In addition to directly causing immunosuppression as well as increasing energetic costs via elevated metabolic rates, increased testosterone levels could also compromise survivorship by decreasing the amount of energy and nutrients available for somatic repair and the maintenance and activation of immune responses (Muehlenbein, 2008; Muehlenbein and Bribiescas, 2005; Sheldon and Verhulst, 1996; Wedekind and Folstad, 1994). To avoid such costs, testosterone levels typically decrease during injury and infection (Boonekamp et al., 2008; Spratt, 2001; Spratt et al., 1993). In brief, various hormones (including testosterone and cortisol) and immune factors likely play important roles in regulating energy investment into different physiological systems including reproduction and immunity.

SIGNIFICANCE

The metabolic responses to mild, acute infections and injury in humans have been relatively unexplored, despite the fact that much work in evolutionary anthropology relies on the assumption that immune maintenance (including immune tissue and cell turnover) and activation (responding to a challenge) impose costs. Understanding the costs of immunity is central to understanding the role of immunity in human life history evolution. Furthermore, because hormones influence and regulate immune, metabolic, and reproductive functions, measuring changes in hormone levels and determining how they interact with immune and metabolic factors may have important implications for understanding the optimization of hormonal activity under varying environmental conditions, and consequently the evolution of the life history trade-offs between endocrine and immune functions.

Better understanding the immunomodulatory actions of hormones may inform treatment patterns for hypogonadism and other endocrine dysregulation during illness. Changes in metabolism and immune-endocrine interactions during infection may serve as valid biomarkers for understanding differential disease severity and recovery. Evidence would suggest that individuals with certain hormone profiles (e.g., high-androgen levels) should be more susceptible to infection or require longer periods of convalescence, and more severe physiological perturbation should be accompanied by greater metabolic changes. Degree of reproductive suppression and altered metabolism likely represent valid, but understudied, biomarkers of stress associated with infection, injury, and immune activation. Understanding the precise energetic costs of acute immune activation in adults will also facilitate better treatment plans for metabolic dysregulation during illness, and a more complete understanding of the immunomodulatory actions of hormones will benefit clinicians who utilize hormone supplementation to treat a variety of conditions.

HYPOTHESES

To our knowledge, the metabolic costs of mild immune activation (as reflected by changes in RMR in the absence of fever or changes in body composition) have not been examined in adult humans under natural infection conditions, nor have the correlative changes in androgens or glucocorticoids been adequately examined. The energetic requirements of mounting an acute, mild immune response in adults have only been investigated following vaccinations, including typhoid and yellow fever (Barr et al., 1922; Cooper et al., 1992; Gandra and Scrimshaw, 1961). To better understand the energetic requirements of mounting a mild immune response, as well as some of the major hormonal changes underlying these metabolic changes, we examined changes in RMR and hormones during and after respiratory tract infection in young adult men. We hypothesized that (1) RMR would be higher during illness compared with samples taken following recovery, (2) testosterone levels would be lower during infection compared with samples taken following recovery, and (3) cortisol levels would be higher during infection compared with samples taken following recovery.

METHODS

Location and participants

Participant recruitment and sampling took place between August 28, 2006 and April 20, 2007 at the Norris Health Center on the University of Wisconsin-Milwaukee campus. This health center provides general health care services to the University of Wisconsin-Milwaukee student body. Inclusion criteria for this study were adult males, ages 18–40 years, of any ethnicity, not currently taking any medications for any disease or disorder other than respiratory tract infection, free of all known endocrine, metabolic and immunosuppressive disorders, free of all other known chronic diseases (e.g., chronic obstructive pulmonary disease, congestive heart failure, etc.), no recent surgery or injury, and currently diagnosed with acute respiratory tract infection of viral or bacterial origin. This protocol (#07.02.014) was approved by the Institutional Review Board, Department of University Safety and Assurances, University of Wisconsin-Milwaukee.

Measurements and sample collection

To analyze changes in RMR following natural pathogen exposure in adult humans, we utilized an epidemiologic passive detection design: potential participants who sought treatment and/or advice for respiratory tract infections and met all other inclusion criteria were referred to the study by healthcare providers at Norris Health Center. Following explanation of the project and a signed informed consent document, participants completed a confidential questionnaire that recorded basic demographic information, present diagnosis and history of symptoms, history of prescription and nonprescription medication usage, history of illnesses and injuries, and sleep, exercise, diet (including alcohol), and tobacco usage in the preceding 24 h. Confirmation of current diagnosis at initial visit was always verified via access to electronic medical records.

Weight was recorded to the nearest 100 g and height to the nearest 0.1 cm. Height and weight were used to calculate body mass index (kg/m^2). Chest (diagonal pinch halfway between the nipple and armpit), subscapular (diagonal pinch below shoulder blade), and tricep skinfolds (vertical pinch halfway between elbow and shoulder) were measured in duplicate on both sides of the body using a Lange caliper while the participant was standing erect (American College of Sports Medicine, 2008). The duplicate measures were averaged and used to calculate body density $\{1.1125025 - [0.0013125 \times \text{sum of three skinfolds}] + [0.0000055 \times (\text{sum of three skinfolds})^2] - [0.0002440 \times \text{age}]\}$ and percent body fat $[(4.95/\text{body density}) - 4.50]$ (Heyward and Wagner, 2004). All measurements were made by the same investigator.

Duplicate saliva samples were obtained via passive drool into sterile cryovials labeled with the subjects' unique identifiers. Samples were immediately frozen at $-80^{\circ}C$ until later analyses. Time of sample collection was always recorded.

RMR was assessed using a FitMate indirect calorimeter (Cosmed, Rome, Italy) according to manufacturer's instructions. Subjects were allowed to relax for ~15 min before measurements were made. A reclined, awake participant breathed normally through a facemask (over the nose and mouth) equipped with a turbine flowmeter and galvanic fuel cell oxygen sensor for 12 min. This system and procedure have been previously validated against the Douglas bag system for accurately measuring RMR (Nieman et al., 2006). The masks and turbines were disinfected with a bleach solution as well as automatically calibrated between subsequent measurements. Participants received monetary compensation for their participation.

The sampling regimen was designed to collect specimens at a minimum of three time points: during initial visit to the Health Center as well as return visits for resampling 2 days (during recovery) and 2 weeks (complete recovery) after initial visit. Some participants were sampled a fourth time due to sustained illness at their second week follow-up appointment. Participants were instructed not to eat, drink (except water), smoke, or exercise within 4 h of their follow-up visits since such activities can alter RMR results. When possible, participants were sampled approximately at the same time of day for each visit.

During the follow-up visits, participants completed a separate health questionnaire that recorded any changes in symptoms and sleep, exercise, diet, and tobacco usage. Weight and RMR were measured and saliva samples were collected during each follow-up visit.

Laboratory analyses

Saliva samples were analyzed for cortisol and free testosterone using enzyme immunoassay kits from Salimetrics (State College, PA) according to manufacturer's instructions (expanded range, high-sensitivity salivary cortisol enzyme immunoassay kit #1-3012; expanded range salivary testosterone enzyme immunoassay kit #1-2312). The sensitivities of the assays were <0.003 μg/dl for cortisol and <1.0 pg/ml for testosterone. The correlation coefficients for each of the curves were better than 0.99. High- and low-level controls were included in each standard curve, and results for the controls in each assay were within established confidence limits. Intra-assay coefficients of variation were assessed using the mean coefficients of variation of control duplicates. Intra-assay coefficients of variation were less than 7% for testosterone and

cortisol. Interassay coefficients of variation were assessed using the mean coefficients of variation of control duplicates in two separate assays. Interassay coefficients of variation were less than 10% for testosterone and cortisol.

Statistical analyses

Baseline characteristics (demographic and clinical) were summarized as proportions of the sample with the characteristic. Continuous measures were summarized by mean, median, and standard deviation. The visits were classified into "sick" or "well" mutually exclusive categories. The initial visit was by default a "sick" visit, and the last follow-up visit (with no signs or symptoms of infection) was classified as "well." The difference in RMR, testosterone, and cortisol measured on sick and well visits (as well as the percent change in these variables between sick and well visits) was calculated for each participant.

Comparisons of the differences in RMR, testosterone, and cortisol between sampling days within individual cases were made using paired *t*-tests. Pearson correlations between RMR, testosterone, and cortisol were calculated for each measurement time point (initial visit, 48-h follow-up visit, and 2-week follow-up visit). Because of diurnal rhythms in hormone production and secretion as well as RMR measures, time of sample collection was always included as a covariate in analyses. Because of the impact of lean body mass and athletic condition on RMR, percent body fat was included as a covariate in analyses involving RMR. All analyses were conducted using SAS version 9.1 (SAS Institute, Cary, NC). *P*-values less than or equal to 0.05 were considered statistically significant.

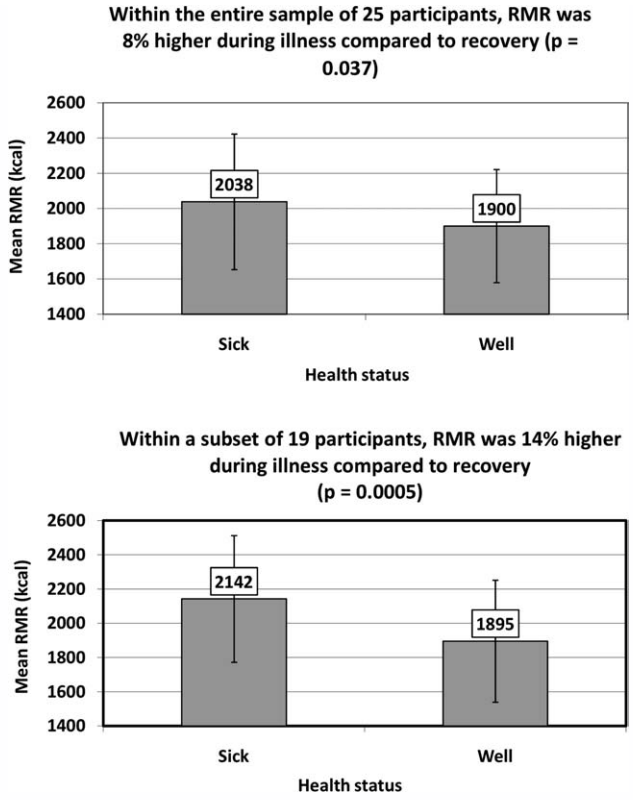

Fig. 1. Mean RMR levels during illness and recovery.

RESULTS

During the sample collection period, 914 men met all of the inclusion criteria for this study. Sixty-one men were eventually enrolled in the study. Of these 61, only 31 men followed the complete instructions by not eating, drinking, smoking, or exercising in the 4 h prior to their first (sick) and last (recovered) visits. Seven participants developed fever during their infections and so were not included in the analyses presented here, resulting in a final sample of 25 men (mean age 21, range 18–30 years). Symptoms of respiratory tract infections included: nasal and chest congestion, sore throat, wheezing, sinus pressure, cough, and headache. Participants reported having symptoms for an average of 7.3 days (range 1–28) prior to visiting the health center. Ten of the 25 participants reported using over the counter medications just prior to their initial visit to the health center.

Resting metabolic rate

Figure 1 illustrates the results of the paired analysis of the RMR data. In general, participants experienced significantly higher RMR during illness (sick visit RMR: 2038 ± 385 kcal; well visit RMR: 1900 ± 321 kcal; $P = 0.037$), with a mean increase of over 8% (138 kcal). However, of the 25 participants, six experienced lower RMR during their sick visit compared with well visit. It is unknown why these individuals experienced a change in RMR in the opposite direction as predicted, but there may be underlying variation in reported confounding activities in these individuals (i.e., some may have ate before one of their visits, but refused to accurately divulge this information). Alternatively, these participants could have been less ill or in better overall physical condition than the other participants, or could have been infected with a different virus or bacteria. Differences in degree of symptoms are not reported or analyzed here because these represent subjective interpretation of individual experiences. Furthermore, actual infectious organism (viral or bacterial) was not determined by the health center.

So as to assess the average percent change in RMR in only those patients that experienced elevated RMR during illness, we further removed the aforementioned six participants from subsequent analysis. Among the remaining 19 participants, RMR was 14.2% higher during their sick visit compared with their well visit (sick visit RMR: 2142 ± 370 kcal; well visit RMR: 1895 ± 356; $P = 0.0005$).

Hormones

Figure 2 illustrates the results of the paired analysis of the testosterone data. The participants experienced significantly lower testosterone levels during illness compared with the well visit (sick visit: 129.2 ± 67.7 pg/ml; well visit: 161.6 ± 88.3 pg/ml; $P = 0.007$), with a mean decrease of nearly 10% (32.4 pg/ml). However, of the 25 participants, seven experienced higher testosterone levels during their sick visit compared with well visit. It is unknown why these individuals experienced a change in testosterone in the opposite direction as predicted, but the reasons may be the same as those listed earlier for RMR.

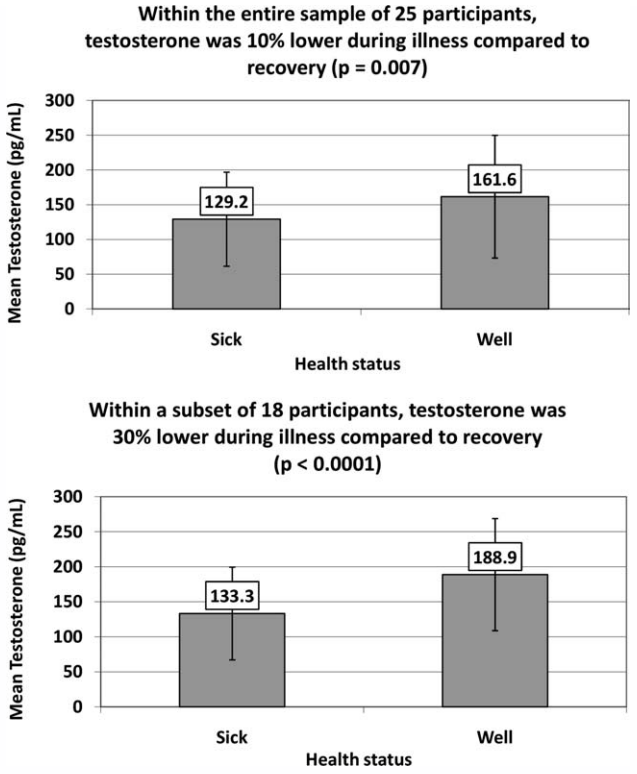

Fig. 2. Mean testosterone levels during illness and recovery.

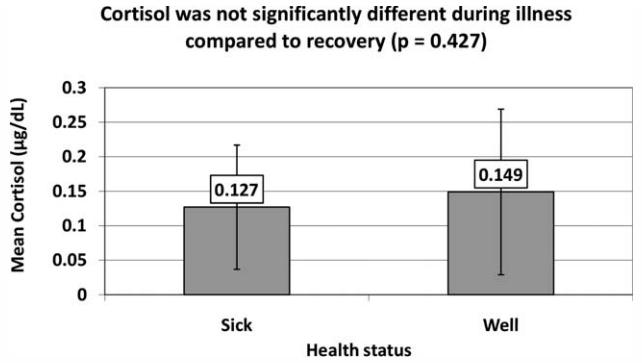

Fig. 3. Mean cortisol levels during illness and recovery.

To assess the average percent change in testosterone in only those patients that experienced lower testosterone levels during illness, we further removed the aforementioned seven participants from subsequent analysis. Among the remaining 18 participants, testosterone levels were on average 30% lower during their sick visit compared with their well visit (sick visit: 133.3 ± 66.3 pg/ml; well visit: 188.9 ± 80.1; $P < 0.0001$).

Figure 3 illustrates the results of the paired analysis of the cortisol data. These data suggest that the participants did not experience significantly different cortisol levels during illness compared with the well visit (sick visit: 0.127 ± 0.09 µg/dl; well visit: 0.149 ± 0.12 µg/dl; $P = 0.427$).

Testosterone and cortisol levels measured during illness and recovery were directly correlated with one another (illness: $r = 0.44$, $P = 0.026$; recovery: $r = 0.61$, $P = 0.001$). Testosterone and cortisol levels were never significantly correlated with RMR (data not shown), whether using the entire sample of 25 participants, only the 19 participants that demonstrated elevated RMR during illness, or only the 18 participants that demonstrated decreased testosterone during illness.

DISCUSSION

Change in metabolism during infection

As hypothesized (#1), RMR was elevated in young adult men during immune activation following natural pathogen exposure (in the absence of fever or changes in body mass or composition). These data suggest that mild immune reactions under natural conditions can elicit significant increases in RMRs (average of 8–14%, depending on sample subset used) indicative of increased energy expenditure. In humans, RMR is typically increased by 7–15% for every 1°C rise in body temperature during fever (Barr et al., 1922; Elia, 1992; Roe and Kinney, 1965). This study suggests that metabolic expenditure is significantly increased even in the absence of fever during infection.

From an evolutionary perspective, changes in metabolism and endocrine function during immune activation illustrate the basic nature of phenotypic plasticity in response to stochastic environments. An individual's immune system is a premier example of a reaction norm that allows for short- and long-term phenotypic plasticity in response to environmental signals such as pathogens, allergens, and injury. Immunocompetence is an integral component of organismal life histories precisely because (1) it is crucial for maximizing evolutionary fitness and (2) it is energetically expensive to produce, maintain, and activate. Optimized immune functions should trade off with other critical life history functions, like growth. Now there are several datasets that provide evidence consistent with the supposition that such a trade-off exists in humans. In children, chronic immune activation is associated with growth faltering (intestinal infections: Checkley et al., 1998; Campbell et al., 2003; Hadju et al., 1995; HIV infection: Arpadi, 2000; inflammatory bowel disease: Ballinger et al., 2003). Elevated concentrations of α-1 antichymotrypsin are associated with growth faltering in Nepalese adolescents (Panter-Brick et al., 2000). Within Tsimane children of Amazonian Bolivia, elevated C-reactive protein levels are associated with reduced gains in height across a 3-month period (McDade et al., 2008). Infants in the Philippines born small-for-gestational age exhibit slower growth rates as adolescents (McDade et al., 2001b) and are less likely to produce antibodies in response to typhoid vaccination (McDade et al., 2001a). All of these data suggest that immune activation is energetically costly. In this study, we use actual metabolic measures to demonstrate that mounting an acute response to even mild pathogens is energetically taxing for otherwise well-nourished adult men.

Change in hormone levels during infection

This study further illustrates decreased testosterone levels (hypothesis #2) during acute immune activation following natural pathogen exposure. Hypogonadism and

hypogonadotropism are common physiological responses to somatic injury, and the degree of response is often associated with the degree of disrupted homeostasis (Spratt, 2001). HIV-infected men frequently exhibit low-testosterone levels along with dyslipidemia, lipodystrophy, and sarcopenia (Poretsky et al., 1995). In women, HIV infection often results in amenorrhea (Lo and Schambelan, 2001), despite the fact that estrone and estradiol levels often increase during major illness as a result of aromatization from androgens (Spratt, 2006). Honduran men infected with *Plasmodium vivax* exhibit significantly lower testosterone levels compared with age-matched healthy controls (Muehlenbein et al., 2005). Similarly, experimental Venezuelan Equine Encephalitis virus infection in captive male macaques (*Macaca fasicularis*) is associated with significant declines in serum testosterone levels (Muehlenbein et al., 2006). Decreased testosterone levels have also been reported in response to influenza vaccination in young men (Simmons and Roney, 2009). In these situations, variation of testosterone level may be acting as a physiological mechanism regulating the differential investment in either reproductive effort (i.e., musculoskeletal performance, courtship, and copulatory behaviors, etc.) or survivorship (i.e., immunocompetence, adipose tissue, etc.) according to availability of energy (Bribiescas, 2001), availability of mates (McKean and Nunney, 2005), and disease risk in the environment (Muehlenbein, 2008; Muehlenbein and Bribiescas, 2005).

Depressed androgen synthesis and release during immune activation may be caused by negative feedback from other hormones, including glucocorticoids, endogenous opioids, and cytokines (Aakvaag et al., 1978; Attardi et al., 1997; Bambino and Hsueh, 1981; Bonavera et al., 1993; Doerr and Pirke, 1976; Gao et al., 2002; Hardy et al., 2005; Isseroff et al., 1989; Oktenli et al., 2004; Sapolsky and Krey, 1988). However, in this study, cortisol was not significantly different during illness compared with recovery (contrary to hypothesis #3). Because cortisol levels were not significantly different over time in these participants, we do not believe that there was a habituation response to the Health Center (i.e., that results are caused by changes in levels of habituation to stress of visiting the clinic). Altered cortisol levels may be identified under different conditions, such as during more severe infections like malaria (Muehlenbein et al., 2005).

Study limitations

As evidenced in Table 1, there are many ways of measuring the costs of mounting immune responses, including experimental manipulations under conditions of resource restriction. Such studies are not ethically or logically feasible in humans. The present study design represents a compromise that unfortunately does not necessarily allow for causal inference, which is in fact the case for most studies in human evolutionary biology. One major shortcoming of this study is that, because sample collection was opportunistic during initial visits to the clinic, participants most likely presented with illness at different stages of disease: early, peak, or late. It was not possible to control for differences in stage of infection between individuals. Subjects also likely differed by type of infectious pathogen (viral vs. bacterial), although all were generally classified as having "respiratory tract infection." Furthermore, subjects would have different levels of adaptive immunity against these pathogens, and so would naturally react differently during subsequent infections.

In addition to controlling for pathogen type and severity of infection, future studies would benefit by including (and controlling for) individuals of varying states of energy flux. Furthermore, although there are clear benefits to a within-subject study design, it is possible that subjects could become acclimated to the repeated sampling regime (e.g., adjusting their ventilation pattern and heart rates with repeated visits to the Health Center, which might obscure results from the indirect calorimeter). In this case, individually matched control subjects could be employed for comparative purposes.

For a more complete understanding of immune-endocrine interactions in relation to changes in metabolism and convalescence, other hormones that exhibit pleiotropic effects on androgen production, immunity, and metabolism (e.g., estrogens and thyroid hormones) should be included in analyses. Finally, because social stress has significant effects on endocrine activity and immune functions (Herbert and Cohen, 1993; van Eck et al., 1996), it would be useful to include proxies of social stress (e.g., reported life events and daily hassles) in future study designs.

Feed a cold, starve a fever?

Although we contend that transient changes in hormone levels throughout the range of physiological variation function as a basic aspect of male phenotypic plasticity and an adaptive response that facilitates the allocation of metabolic resources (Muehlenbein, 2008; Muehlenbein and Bribiescas, 2005), an alternative perspective is that changes in metabolic and endocrine functions during immune activation are simple by-products of infection/injury, or even an adaptation on the part of the pathogen to divert host caloric resources to itself (Connors and Nickol, 1991). In fact, host appetite frequently decreases when these energy demands are highest, an effect likely produced by various cytokines (e.g., TNFα) and other components of the neuroendocrine system (Wong and Pinkey, 2004). Such anorexia could reduce the risk of food-borne infections or limit energetic usage on digestion (Kyriazakis et al., 1998). Interestingly, fasting is associated with transient increases in IL-4 (a dominant Th-2, antibody-mediated immune response) (van den Brink et al., 2002), which might function to limit bacterial infections associated with fever. Fasting during a fever might even prevent the development of some autoimmune responses (Yarnell, 2001). However, caloric intake appears to result in transient increases in circulating IFNγ levels (a dominant Th-1, cell-mediated immune response) (van den Brink et al., 2002), which could function to limit viral infections associated with cold and flu.

Modulation of feeding, hydration, and sleep in response to specific pathogens are suggested to be adaptive behavioral responses in human and nonhuman animals alike (Bazar et al., 2005; Ritz and Gardner, 2006). Given the energetic requirements of mounting immune responses against respiratory tract infections, as illustrated in this study, the proverb to "feed a cold, starve a fever" may be appropriate advice. That is, fighting different pathogens may require plasticity in host behaviors. Different immune responses (i.e., cell- versus antibody-mediated responses) likely have different energetic and nutritional

needs (Long and Nanthakumar, 2004; Schmid-Hempel and Ebert, 2003; Westneat and Birkhead, 1998). Variability in behavioral, endocrine, and immune responses would allow for more efficient balancing between the costs and benefits of immune defense. Benefits obviously include fitness maximization through survivorship and reproduction, and costs include the use of protein, energy (e.g., glucose), amino acids (e.g., glutamine), and essential nutrients (e.g., carotenoids) as well as the risk of autoimmunity and immunopathology during prolonged or excessive activation. These relative costs may depend on severity, type, and duration of infection as well as sex, age, and nutritional status of the host (Lockmiller and Deerenberg, 2002). Studies on human physiological ecology and ecological immunology, although difficult compared with typical laboratory model systems which allow manipulation, will benefit from accounting for such complex interactions between the metabolic, endocrine, immune, and behavioral systems.

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