The relationship between malnutrition and tuberculosis: evidence from studies in humans and experimental animals

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SUMMARY

The oral traditions of medicine and public health have it that malnutrition is an important risk factor for the development of tuberculosis (TB). Malnutrition profoundly affects cell-mediated immunity (CMI), and CMI is the principle host defense against TB. It makes biological sense. Although most health professionals readily accept this principle, much of this belief is based on uncontrolled observations such as disaster situations or on backwards logic from the cachexia common among TB patients. In fact, the evidence in humans is surprisingly thin from the perspective of scientific rigor. And few data, if any, quantify the extent of the relative or attributable risk of TB due to malnutrition. Moreover, until recently, data from experimental animals were based on animal models that were largely not relevant to human TB infection and disease. This article reviews the scientific data supporting the contention that malnutrition is an important risk factor for TB concentrating on observations in humans and on experimental animal studies based on a highly relevant animal model. If it is true, malnutrition may account for a greater population attributable risk of TB than HIV infection, and certainly a much more correctable one.

KEY WORDS: tuberculosis; nutrition; human; guinea pig

MALNUTRITION has a profound effect on cellular immune function. Pneumocystis carinii pneumonia, for example, was first described in malnourished children following the Second World War.¹ Many of the unusual infections seen in patients with human immunodeficiency virus/acquired immune-deficiency syndrome (HIV/AIDS), certain leukemias and lymphomas, and other disorders affecting cellular immunity, also characterize malnourished individuals.²⁻⁵

At the same time, malnutrition is an important risk factor for tuberculosis (TB) because cell-mediated immunity (CMI) is the key host defense against TB.⁶⁻⁷ In malnourished individuals, the likelihood is increased of primary or latent infection progressing to active disease.⁷ In populations with substantial latent TB infection, the occurrence of malnutrition may be an important determinant of the incidence of TB. The potential public health impact of malnutrition on the global incidence of TB was summarized in the US Surgeon General’s Report on Nutrition and Health which emphasized that malnutrition was the leading cause of acquired, correctable immune system dysfunction throughout the world.⁸ The United Nations Food and Agriculture Organization (FAO) estimated that 841 million people in developing countries or 20% of the 1990–1992 population were undernourished.⁹ Modest decreases in resistance affecting such large numbers of people may result in substantial changes in TB incidence at a population level. Population groups at highest risk for poor nutrition are also at high risk for TB, poverty being the common denominator.

Despite the belief that malnutrition increases the risk of TB, the depth and quality of the evidence, especially in humans, is surprisingly thin. Moreover, it is difficult to say how much the risk of TB increases relative to specific degrees or types of malnutrition. In general, there are three streams of evidence relating the risk of TB to malnutrition: observations in humans, experimental work in animal models, and inferences from related work in microbiology and immunology. In humans, direct evidence for the risk of TB due to malnutrition is sparse, and the data have not been reviewed critically in over three decades, especially in terms of methodological rigor. Until recently, experimental animal studies have not been clearly relevant to human TB because of immunological differences between species and because the route and dose of...
infection have not mimicked human infection. In vitro studies have generated a substantial body of evidence documenting the negative effects of malnutrition on cell-mediated immune function, and on the immunology of TB (reviewed by Ellner). Although one can reason from the in vitro evidence, it cannot replace in vivo data. The purpose of this paper is to summarize the evidence 1) from observations in human populations with special attention to their methodological validity, and 2) from experimental animal models with special attention to their relevance to human tuberculosis.

TB risk is of two kinds: the risk of becoming infected with Mycobacterium tuberculosis and the risk of that infection progressing to active disease. This paper focuses on the risk of infection progressing to disease, because that is where CMI comes into play and the link with malnutrition is most direct. There is no evidence for a direct relationship between malnutrition and the risk of initial infection. Although both TB and malnutrition are linked to poverty, the data reviewed below suggest no independent association between malnutrition and primary or latent TB infection.

HUMAN DATA

Early research on the interaction of nutrition and tuberculosis was reviewed in the classic text by Rich up to 1950, and in the exhaustive treatise by Scrimshaw, Taylor, and Gordon up to 1968. Except for a single, controlled intervention trial in the 1940s in New York City, studies in humans have been largely either ecological or uncontrolled observations.

Chandra and Newberne point out that most of the published evidence on the interaction of nutrition and infection in humans consists of only a few types of data:

- higher point prevalence of infection in undernourished patients or community dwellers;
- worse and more frequent complications of infection in children with marasmus and kwashiorkor;
- higher mortality in undernourished populations;
- higher rates of infectious diseases in war, famine, ghetto, refugee, or natural disaster situations.

These human studies have numerous flaws in sampling, sample size, measurement (or definition) of nutritional deficiency, assessment of infection, unmeasured confounding variables, and analytic methods. Most of them, however, are simply ecological or cross-sectional studies that cannot confirm causality.

A more formal approach to grading the quality of evidence from human studies was developed by the US Preventive Services Task Force to judge evidence for or against specific clinical and public health interventions. This grading scale can be readily adapted to epidemiological risk factor studies. Grade I evidence comes from randomized, controlled trials. Grade II evidence comes from controlled but not randomized studies and is subdivided into three levels: Grade II-1 evidence comes from well-designed controlled trials without randomization; Grade II-2 evidence comes from well-designed cohort or case-control analytic studies, preferably from more than one center or research group; Grade II-3 evidence comes from multiple time series with and without the study factor of interest, but dramatic results of uncontrolled experiments, such as post-exposure vaccination against rabies, may also be considered in this group. Grade III evidence consists of expert opinion, clinical experience, descriptive studies, and case reports.

Ecological studies

Studies using the ecological approach are generally considered to generate hypotheses rather than providing evidence upon which to base conclusions. Thus, the grading scale described above may not apply. In the present instance, nutritional deficiency on a broad scale is inevitably linked to many other adverse circumstances that may be strong, independent risk factors for tuberculosis. Taking this into account, a wealth of ecological associations link tuberculosis with malnutrition in populations affected by famine, war, natural disasters, poverty, mass migration, and confinement in prisons or ghettos. The value of these observations lies not so much in conclusive contributions to medical knowledge; rather, their value lies in suggesting causal links that can be investigated fruitfully. At the same time, their value lies precisely in the difficulty of conducting controlled studies of TB and malnutrition in human populations. These studies take advantage of historical circumstances in an attempt to learn about the interaction of nutrition and tuberculosis.

In general, ecological studies do not attempt to distinguish deficiencies of various macro- or micronutrients. Instead, they can be thought of as addressing multiple simultaneous nutritional deficiencies, the most common type of malnutrition in humans. Furthermore, in these complex circumstances, the effects of malnutrition cannot be disentangled from the effects of poor housing, overcrowding, lack of medical care, poor hygiene, massive social disruption, etc. For example, sharp increases in tuberculosis morbidity and mortality in Paris or in Germany during the two World Wars cannot be ascribed to malnutrition (which was pervasive) apart from the social upheaval, collapse of public health programs, mass trauma, crowding, and psychosocial stresses that were part of these wars. Similarly, the heroic studies carried out by Jewish physicians in the Warsaw ghetto during the Second World War do not control for the extreme crowding, psychological stress, and catastrophic social circumstances. We do not wish to devalue these works, nor the impact of starvation on tuberculosis in
these situations, but the role of malnutrition independent of other circumstances cannot be isolated in these studies. Although from the perspective of methodological rigor much of this evidence is weak, it constitutes a large body of observation that supports the contention that inadequate nutrition adversely influences either the incidence or the severity of tuberculosis.

In contrast to most of the ecological studies, three of these studies present fairly convincing evidence that nutrition, isolated to some extent from other historical circumstances, played a direct role in tuberculosis morbidity and mortality. Thus, the quality of the evidence from these three studies could be considered to be Grade II-3 evidence. The first is Faber’s report of tuberculosis epidemiology in Denmark during the First World War. During most of the war, neutral Denmark exported the bulk of its meat, fish, poultry and dairy products to the extent that the local diet lacked these protein, vitamin, and mineral rich foods. Tuberculosis rates climbed, as they did in the warring countries. The German blockade of Denmark in 1918, however, created a surfeit of these foods, and tuberculosis rates plummeted. In contrast, tuberculosis rates in the neighboring warring countries continued to climb unabated.

The second study involves the Trondheim, Norway, Naval Training School where the high rate of tuberculosis among recruits in the early 20th century was ascribed to crowded, poor housing and unhygienic conditions. However, tuberculosis rates did not decrease after improved housing and hygiene were implemented. Diet was subsequently fortified with margarine, cod liver oil, whole wheat bread, fresh fruits and vegetables, and milk, and tuberculosis morbidity promptly dropped to the prevailing level for young adults of that area.

The third important contribution to the early literature was Leyton’s study of tuberculosis morbidity among British and Russian prisoners of war held in German (POW) camps during the Second World War. The prisoners shared the same prison diet, but the British received Red Cross food supplements amounting to 30 g protein and 1000 Kcal per day. In a subsequent radiographic survey, the tuberculosis rate among the British was only 1.2% and their plasma proteins were higher than in the Russians, who had a tuberculosis rate of 15–19%. Both groups shared the same living and working conditions and chance for infection. In the malnourished prisoners, tuberculosis was more severe, the onset was more rapid, and patients died rapidly with large pulmonary cavities and massive tissue breakdown. Granuloma formation was poor in the malnourished prisoners, supporting the idea that there was a deficit of CMI in this group.

Lastly, McKeown’s seminal work elaborated the concept that the decline in TB mortality in England and Wales from 1770 to 1900 was most likely due to improving standards of living in general and to the nutritional status of the population in particular.

Through extensive critical reasoning, McKeown excluded the alternative explanations: 1) advances in medicine and medical effort, or 2) natural selection. What remained, according to McKeown, was that the environment, and thereby the resistance of the host, improved. The death rate from TB at the beginning of the 19th century was approximately 40/1000 person-years. It declined to 14/1000 by the end of the century, and accounted for nearly half of the overall decline in mortality in the 19th century. McKeown concludes, ‘Having confidently excluded therapy and genetic selection, with reservations, we are left with changes in the environment as the most acceptable reason for the trend of mortality from tuberculosis.’ Of the features of the environment to be considered, ‘the evidence in respect of diet seems . . . highly suggestive.’

Case series
Nutrition, immune function, and infection interact in complex and dynamic patterns. Protein-energy malnutrition compromises CMI and increases susceptibility to or severity of infections. Conversely, in-fection can rapidly lead to nutritional stress and weight loss, thereby worsening nutritional status and immunologic function. Therefore, understanding the temporal relationship between the onset of malnutrition and the development of the infectious disease is crucial to correctly assess any possible cause-effect relationship.

Since 1968, several case series of post-surgical patients undergoing intestinal bypass surgery for morbid obesity have provided observational data in which nutritional status and incident cases of tuberculosis were observed in the same individuals in the correct temporal sequence. These patients experience rapid weight loss and malabsorption due to their short-circuited bowel. In several series, the incidence rate of tuberculosis was 1% to 4% among post-operative patients during various durations of follow-up. This range was much higher than expected based on historical or population comparisons. Similarly, partial gastrectomy for ulcer disease was shown to predispose men to tuberculosis, but the association was 14 times more likely for men whose weight was <85% of ideal than for men whose weight was normal for their height. Although the patients in these series do not represent persons at risk for TB in general, and there are no contemporaneous controls, the observations are worth noting due to the sharply increased incidence of TB following nutritional insult. According to the definitions noted above, these studies might be considered to provide Grade III evidence.

Cross-sectional and case-control studies
Cross-sectional and case-control studies generally suffer from the same inherent fatal flaw. Patients with
and without active tuberculosis are compared in terms of their concurrent nutritional status. However, tuberculosis itself causes wasting, depression of the immune system, and other changes resembling malnutrition. Therefore, the intrinsic uncertainty over the sequence of cause and effect in case-control and cross-sectional study designs becomes intractable. One knows that having the outcome of interest (being a tuberculosis case) alters the primary exposure variables (nutritional status). Such studies do not assess the role of malnutrition in the development of tuberculosis because there have been no accurate measurements of antecedent nutritional status in comparable cases and controls. Therefore, these studies cannot be graded according to the scale noted previously because of the inadequate study design. There have been many recent variations on this theme, but they all founder on the same underlying problem.

In an attempt to circumvent this problem and determine risk of tuberculosis in relation to antecedent cobalamin deficiency, Chanarin and Stephenson compared the incidence of tuberculosis among Asiatic Indians who were life-long vegetarians to that of Indians who were omnivores, and found the risk to be increased nearly three fold. These investigators assumed that cobalamin intake or levels would be lower in the vegetarians but did not measure them. Nor did they consider other nutrients that might differ between vegetarian and meat-eating groups.

One study from China, available in English only in abstract form, measured zinc, copper, and iron in the hair of patients with active tuberculosis compared to controls in an attempt to determine past nutritional status with respect to these minerals. The authors report significantly lower zinc content and zinc/copper ratios in the hair of tuberculosis patients compared to controls, but no difference in iron content. Even so, numerous intervening variables prevent us from assessing the timing of tuberculosis infection versus growth of the hair segments that were assayed, and the effects on the mineral content of hair due to water, soaps, shampoos, lotions, dyes, etc., applied to the hair.

Although these studies demonstrate substantial macro- and micronutrient deficits in tuberculosis patients, one cannot infer a causal role for nutritional deficiency in the development of disease from these data because the chronological sequence is unclear and tuberculosis itself plays a role in the development of the nutritional deficits.

Two studies on vitamin D metabolism in relation to TB used a cross-sectional design, but the focus was on the molecular and cellular mechanisms of the interaction, rather than on the direction of causality. These two studies examined the dynamics of 1,25(OH)2 vitamin D3 in lymphocytes and macrophages from patients with tuberculosis compared to patients without tuberculosis. Investigators in France determined that lymphocytes obtained by bronchoalveolar lavage from patients with tuberculosis expressed specific receptors for 1,25(OH)2 vitamin D3, but not 25(OH)D3. These were primarily CD4+ T-lymphocytes. Peripher al blood lymphocytes did not express these receptors. Furthermore, uncultured cells recovered by bronchoalveolar lavage and blood mononuclear cells from nornalcemic patients with tuberculosis both produced 1,25(OH)2D3. The amount correlated with the number of CD8+ T-lymphocytes present but not other cell types. Purified T-lymphocytes from all patients with tuberculosis produced 1,25(OH)2D3 which correlated closely with that produced by unseparated lavage cells. Since 1,25(OH)2D3 can improve the capacity of macrophages to kill mycobacteria, these results support the conclusion that cellular interactions mediated in part by 1,25(OH)2D3 may be important in the anti-tuberculosis immune response. These data are consistent with work in experimental animals noted below, and may provide the only Grade II-2 evidence from case-control and cross-sectional studies.

Another study focusing on vitamin D and vitamin D receptor genetic polymorphism was conducted among vegetarians of Gujarati, Indian heritage in the UK. They found lower serum levels of 25(OH)-cholecalciferol in TB patients than in tuberculin-positive controls. In the context of low serum 25(OH)-cholecalciferol, two distinct genotypes of the VDR gene, TT/Tt and ff, were also associated with active TB. These authors conclude that vitamin D deficiency may contribute to the relatively high levels of TB in that population. This conclusion exemplifies the classic blunder alluded to previously, because the development of clinically active TB may lead to decreased blood vitamin D levels. Vitamin D metabolism is linked to granulomatous inflammation both in terms of the paracrine effects of vitamin D metabolites on macrophage function and in terms of calcitonin metabolism, osteoclast activity, and the calcification of granulomas.

Another variation on case-control methodology that has been applied in this context is based on tracing the contacts of tuberculosis cases, i.e., persons who have been exposed to an index case with active tuberculosis. The general approach has been to compare the incidence of tuberculosis in this high risk group with the incidence of tuberculosis in non-exposed controls in terms of their nutritional status (or other risk factors). The problem with contact tracing studies is that they apply only to cases of tuberculosis that can be linked with a known exposure to an identified active case. In the past, this accounted for only 10% of tuberculosis cases; up to 90% arise sporadically from reactivation of latent infection or progressive primary infection in individuals without identifiable tuberculous contacts.

Technically, ‘malnutrition’ means ‘bad nutrition’,
which could mean a harmful excess of certain nutrients as well as nutritional deficiencies. In this context, work in rural Zimbabwe suggested that increased dietary iron increases the risk of developing tuberculosis, even taking into account that 69% of the TB patients in the study population had HIV infection. However, their measure of iron status was consumption of the traditional beer, which was fermented in iron pots. Although the authors assessed iron status from several blood tests, measurement of iron status in the blood after TB is diagnosed of course bears little relationship to iron status leading up to the time when the tuberculous infection began developing into clinically active TB. Moreover, the analysis does not control for alcohol consumption, which can be strongly immunosuppressive and substantially affect nutrition as well.

Cohort studies

Very few follow-up studies have been executed with the explicit purpose of understanding the relationship between nutrition and the incidence of tuberculosis. The unique strength of cohort studies is that nutritional status is measured prior to the onset of tuberculosis. In general, well-designed and well-executed cohort studies provide Grade II-2 evidence, although in the Finnish and US Navy studies described in this section, the published interpretations of the results are debatable.

Only two cohort studies examined the relationship between micronutrients and TB incidence. Both of these included vitamin C. In the 1940s, Getz et al. followed 1100 men who were free of TB at baseline, by clinical and radiographic criteria, for up to 5 years with serial clinical, radiographic, and laboratory examinations. Among 16 men who developed active TB, blood levels of vitamins A and C were consistently lower than in those who remained free of TB. Plasma vitamin A levels were low in 13 of 16 men who developed active TB compared to 30% (318/1058) of those who did not. Similarly, plasma vitamin C levels were low in all of the subjects who developed active TB compared to only 11% (117/1013) of those who did not. Exposure to TB did not differ between the men who developed TB and those who did not.

Investigators in Finland randomized 26 975 healthy male smokers aged 50–69 years to supplementation with tocopherol, beta-carotene, both or neither. The subjects were followed for a mean 6.7 years for diagnoses of cancer identified through a registry of all hospital discharges and the associated diagnoses in the region. Hemilä et al. analyzed the dietary data in this cohort for vitamin C and vitamin C-rich foods and the discharge registry for diagnoses of TB. In over 173 000 person-years of follow-up, 167 cases of TB were detected. Higher intake of fruits and vegetables was associated with lower risks of TB. Among those with increased intakes of vitamin C and fruits and vegetables, the adjusted relative risk of TB decreased to 0.4 (95% confidence interval 0.24–0.69). This study is noteworthy for its size and quality of data. However, detecting TB through hospital discharges selects TB patients who were sick enough to require hospital admission. Lower intakes of fruits and vegetables and vitamin C may be associated with higher rates of hospitalization rather than higher rates of TB.

No other studies on individual vitamins or minerals in humans were identified by Scrimshaw et al. nor by exhaustive review of the literature since. Numerous experimental studies from that era carried out in various animal species support the conclusions noted previously, i.e., that multifaceted malnutrition, protein deficiency, and deficiencies of vitamins A and C increase susceptibility to tuberculosis.

Turning to macro indicators of nutritional status, as part of the long-term follow-up of participants in the large scale BCG vaccine trials in Georgia and Alabama, Comstock and Palmer reported that the incidence of tuberculosis was 2.2 times higher in children with 0–4 mm subcutaneous fat than in those with 10 mm subcutaneous fat. Cegielski et al. examined the relationship between undernutrition and the incidence of TB based on data from the first National Health and Nutrition Examination (NHANES-1) and the NHANES-1 Epidemiological Follow-up Study (NHEFS). NHANES-1 was a cross-sectional survey of a representative sample of the US population from 1971 to 1975. In the NHEFS, the adult subjects of NHANES-1, aged 25–74 years at baseline, were followed longitudinally with serial waves of questionnaires and examinations. Follow-up exceeded 95%. Through 1987, 64 cases of TB were detected. In proportional hazards analysis, having body mass index (BMI), average skin-fold thickness, or upper arm muscle area in the lowest decile of the population increased the adjusted hazard of TB from six to ten fold, controlling for other known risk factors for TB.

Palmer et al. studied the relationship of TB incidence to naturally acquired delayed-type tuberculin sensitivity among US Navy recruits. Nearly all navy recruits from 1949 to 1951 were skin tested and followed longitudinally. Of 68 754 subjects with follow-up data, tuberculin sensitivity was recorded as >0 mm for 8704 (12.7%). During 4 years of follow-up, 109 developed tuberculosis: 28/100 000 among those with 0 mm skin test reactions, 29/100 000 among those with 1–9 mm reactions, and 157/100 000 among those with 10 mm or greater reactions. Later, these investigators related the risk of tuberculosis to ‘body build’ by obtaining height and weight data from the entrance medical examination on a stratified random sample of 1138 subjects. A weight-height index was constructed based on deviation of weight from the median weight for height of the study sample. There were no significant differences in tuberculin sensitivity by weight, height, or weight-height index. In contrast,
the weight-height index was strongly associated with tuberculosis incidence: TB incidence was 75/100 000 for those 15% or more below the median weight for their height and decreased to 19/100 000 for those at least 5% overweight for their height ($P < 0.01$ for both purified protein derivative [PPD] groups). The trends were the same regardless of the degree of tuberculin sensitivity, although incidence rates were higher among those with $\geq 10$ mm PPD reactions. Edwards et al. extended Palmer et al.'s study to over 823 000 navy recruits, and found that tuberculosis developed three times more often in young men 10% or more below their ideal body weight than those 10% or more above it.69,70

These studies merit special interest because of the large sample size and consistency of the findings. Yet several methodological flaws stand out. First, post-hoc analyses of data collected for other purposes are subject to bias and confounding which cannot be controlled because the necessary information was not measured appropriately. Second, height and weight had to be gleaned from entrance medical examinations because this aspect of the study was not planned in advance and the data were not recorded on the study forms. Thus, the accuracy and precision of the major predictor variables may be questioned. Third, of the nearly 100 000 recruits tested, 25 000 were excluded because their service number was not recorded on the study forms and could not be linked to their entrance medical examination. Their characteristics in comparison to the study sample were unknown, and the representativeness of the study sample should be questioned. In addition, recruits who were not white (3.8%) and outside the age range 17–21 years (2.7%) were excluded because the initial sample was nearly homogeneous with respect to age and race aside from these small percentages. At best the results apply to young white men, although it is unclear whether even this narrow slice of the population is represented fairly in the study population. Fourth, weight as a per cent of the median weight for height has drawbacks as a weight-height index. It is not normalized relative to a standard population and, as a consequence, the interpretation of a given value of the index differs in different age and height groups.71 It is impossible to say whether recruits below a certain percentage of the median weight-for-height were thin or whether they were just thinner than other recruits.

Curiously, these authors avoid any reference to inadequate nutrition in the recruits. Instead they conclude that the results demonstrate an association between ‘body build’ and tuberculosis disease, that some unknown factor associated with body build is an important determinant of host susceptibility to active disease, but not to primary infection. A small but consistent body of literature has accumulated on the relationship of body build to tuberculosis, reviewed by Snider in 1987.72 One study stands out. The country of Norway attempted to screen all persons over the age of 14 years for TB with compulsory mass miniature radiography from 1963–1975. This screening program covered 42% to 85% of the population, the percentage varying by age group. Height and weight were measured accurately for nearly 80% of those screened. From these data, Tverdal reported results from over 1.7 million Norwegians with follow-up through the national notification system through 1982 (i.e., 8–19 years follow-up, mean 12.1 years).73 A total of 2531 incident cases of tuberculosis were identified. The incidence of pulmonary tuberculosis, both sputum smear-positive and smear-negative, declined logarithmically with increasing BMI for both sexes, all age groups, and at all durations of follow-up: the age-adjusted incidence of new pulmonary tuberculosis was five times higher in the lowest BMI category than in the highest. Interestingly, this relationship was not observed for extra-pulmonary TB. Tverdal argued that the association could not be explained by pre-existing nutritional status or tuberculosis. As with the US navy studies, this author argues that this relationship is a function of body build and, aside from this single mention, does not discuss nutrition. Comstock suggested that body build may influence susceptibility to tuberculosis because of differences in pulmonary mechanics,74 but no studies have attempted to address this hypothesis.

On the other hand, interpreting the findings of these large studies only in terms of body build rather than nutritional status disregards the well established concept that body weight is a function of the balance over time between caloric intake and energy expenditure. Clearly, increased or decreased intake can transform a thin individual into an overweight one or an obese person into an underweight one. With physical training and appropriate intake, a person with either body type could become muscular and fit. Therefore, the concept of body build as a fixed phenotype that, by itself, predisposes to or protects against TB may be inadequate. Re-interpreting the findings of these studies in terms of nutritional status may be valid. A more inclusive view might be that body habitus as a function of genetic and early environmental influences, and nutritional status as a function of ongoing nutrient intake and physical activity, each affect the incidence of TB. Sorting out the mechanisms and relative contribution of each remains a challenge for future research.

**Intervention trial**

Micronutrient deficiencies in relation to tuberculosis are difficult to study in isolation in human beings. In this respect, a unique study of the effect of micronutrient supplementation on tuberculosis incidence was reported by Downes in 1949.18 In a controlled trial among the families of black tuberculosis patients in Harlem, New York City, 194 of 218 families under
public health supervision in 1941 were examined and divided into two groups matched for family size. The families were allocated alternately to receive vitamin and mineral supplements versus no supplements along with the health department's standard health education program. Since the allocation was not randomized, the quality of the evidence from this trial could be considered to be Grade II-1. The education program included intensive nutrition education. The two groups were similar in prior attack rates and mortality from tuberculosis, prevalence of primary and re-infection tuberculosis at the start of the study, sputum smear positivity among the index cases, and relation of the index case to the rest of the family. In addition, the groups were similar in terms of their income, the proportion receiving welfare, the degree of crowding within the home, and their food habits. After 5 years follow-up, using an intention to treat analysis, the risk of tuberculosis in the control group was 2.8 times the risk of tuberculosis in the vitamin group. However, there was substantial non-compliance with the supplements. The relative risk of tuberculosis among the controls (1096 person-years of follow-up) compared to those who actually took the vitamin supplements for the entire follow-up period (644 person-years of follow-up) was 5.9. The relative risk compared to those who did not take the supplements despite being allocated to that group (27% of the supplement group) or who only took them for some of the follow-up period (33% of the supplement group) (total 598 person-years of follow-up) was only 1.82. Therefore, vitamin supplementation substantially reduced the risk of tuberculosis among family contacts of active tuberculosis cases.

This study probably underestimates the efficacy of micronutrient supplements for two reasons related to an underlying secular trend: the economic status and food habits of both groups improved substantially during the period of the study. First, as a consequence of World War II, opportunities for employment increased. The per cent of families whose income was from welfare alone decreased from 51% to 18%, and the proportion who depended only on earnings increased from 37% to 75% over the 5 years of the study (nearly equal for both groups). Likewise, the mean family income per person increased from approximately $524 per year to $967 per year, with a slightly greater increase in the control group. Therefore, the overall economic status of both groups improved. Second, the proportion of families with marginal or unsatisfactory food habits decreased from 40.5% in the vitamin group in 1942 to 12.8% in 1947, and even more in the control group, from 50% to 6.8%. Both these secular trends would diminish the apparent effect of vitamin and mineral supplements. A third consideration, unrelated to secular trends, was that non-compliance with the supplements by over half of the individuals in the supplemented group would further bias the measure of effect toward the null. With these three factors working against the experimental intervention, it is likely that the effect may be greater than the findings demonstrated.

**Nutrition and the immune response to BCG vaccine in humans**

One study design permits prospective evaluation of the effects of malnutrition on the immune response to mycobacterial proteins closely related to *M. tuberculosis*, namely, delayed-type hypersensitivity (DTH) responses following bacille Calmette-Guérin (BCG) vaccination. Satyanarayana et al. showed that milder grades of malnutrition did not affect the skin test response to PPD 6 months after immunization with BCG, but that children with kwashiorkor were skin test negative.73 Chandra and Newberne demonstrated that the DTH skin test response to tuberculin and to numerous other antigens is reduced in protein-energy malnutrition in children and adults.19 Among tuberculosis patients, PPD skin test reactivity was directly proportional to serum transferrin level, a sensitive indicator of protein malnutrition.19 Similarly, malnourished individuals do not develop skin test responses to tuberculin as often or as large after BCG vaccination as well nourished children. Importantly, this effect has been demonstrated even in modest protein energy malnutrition.36,76

**Nutrition—immunity—tuberculosis: limitations of human data**

While the Surgeon General’s report summarizes data on the interaction of nutrition and infection, it cautions that few reliable data have been obtained in human subjects on the influence of individual essential nutrients or of protein-energy nutrition on specific immune system functions and their interactions.9 Nutrition, immune function, and infection interact in complex and dynamic patterns. Many intervening and unknown variables affect the relationship. Protein-energy malnutrition (PEM) impairs CMI and worsens infections.4,6,7,10–12,33–37 Conversely, infection can lead rapidly to weight loss, malnutrition, and immunologic dysfunction.2,6,38 Indeed, PEM is only partly due to food deprivation. Common infectious disease such as diarrhea diseases, respiratory and parasitic infections are major contributing and precipitating factors in PEM.77 However, in patients with TB it is nearly impossible to determine their nutritional status prior to the onset of TB accurately and, therefore, determine whether malnutrition led to TB or whether TB led to malnutrition. This problem naturally leads to the use of experimental animal models to elucidate the causal links between nutritional deficiencies, immune system function, and tuberculosis.
Experimental animal data

Experimental animal models in general
In their classical review of the interaction between nutrient deficiencies and infection, Scrimshaw, Taylor, and Gordon summarized the results of 40 experimental studies published prior to 1968 which specifically examined the relationship between diet and tuberculosis.81 These experiments were conducted in several experimental animal species (mouse, rat, guinea pig, hamster, chicken), and with at least two species of mycobacteria (M. avium and M. tuberculosis). The nutrients examined varied, but included protein, and vitamins A, C and D. While the details of the experimental protocols varied widely, and the read-outs were crude by modern immunological standards, the overwhelming majority of these studies (31/40, or 78%) demonstrated a synergistic relationship, meaning that the nutrient deficiency was accompanied by an exacerbation of tuberculosis disease following experimental infection. Thus, the weight of the evidence supported a detrimental effect of malnutrition on the pathogenesis of tuberculosis. However, the nature of these experimental infection models detracts from the relevance of these observations. In most cases, very large numbers (millions) of tubercle bacilli were injected by a parenteral route (e.g., intravenously), a protocol which in no way approximates the nature of these experimental infection models detracts from the relevance of these observations. In most cases, very large numbers (millions) of tubercle bacilli were injected by a parenteral route (e.g., intravenously), a protocol which in no way approximates the inhalation of a very small number of bacilli, which characterizes the initiation of tuberculous infection in humans. In addition, the field of immunology was in its infancy when most of these experiments were performed, and thus few inferences can be drawn regarding the nutrient-associated immune defects that led to the observed loss of disease resistance.

Guinea pig model of pulmonary tuberculosis
In the past 30 years, a modest body of literature has accumulated regarding the link between diet, antimycobacterial immunity and disease resistance in tuberculosis. The vast majority of this work has been conducted in a highly relevant guinea pig model of low-dose pulmonary tuberculosis.78–80 The pathogenesis of tuberculosis in this model mimics essentially all of the important aspects of tuberculosis in humans. Following the deposition of a few viable, virulent M. tuberculosis into the alveolar spaces by means of a specially-designed aerosol chamber,81 guinea pigs develop typical pulmonary granulomas, experience extra-pulmonary dissemination with subsequent reseeding of the lung by the blood stream,82 convert their tuberculin (PPD) skin tests and lymphocyte proliferation tests to positive, exhibit fever and weight loss, and eventually succumb to disease.81,83 Vaccination with BCG protects the guinea pigs, as measured by reductions in bacillary loads in the lung and spleen within 3–5 weeks post-challenge, and significant improvement in long-term outcome.81,84 Early studies established that moderate, chronic deficiencies of protein and other nutrients (e.g., zinc) could be induced in guinea pigs, and that the resulting nutritional states had many of the metabolic hallmarks of human dietary deficiencies.85,86 In general, the design of these experiments called for vaccinating guinea pigs in different diet treatments with BCG vaccine and measuring a number of antigen-specific immune responses in vitro and in vivo several weeks later. Groups of vaccinated and non-vaccinated animals from each diet group were then challenged with an aerosol containing a low dose of virulent M. tuberculosis, and the ability of the guinea pigs to control the infection was assessed quantitatively by culture of viable mycobacteria from the lungs and spleens.87

Zinc
Chronic dietary zinc deficiency was found to exert a profound suppressive effect on T-lymphocyte functions in BCG-vaccinated guinea pigs. Thus, there was significant anergy in response to PPD skin tests in zinc-deficient animals, and dramatic loss of PPD-induced lymphoproliferation in vitro.86 The activity of a cytokine, macrophage migration inhibitory factor (MIF) was also impaired by zinc deficiency.88 Taken together, these data implied that zinc deficiency had interfered with the ability of BCG vaccine to induce protection against virulent pulmonary challenge. However, no differences were observed between the bacillary loads of zinc-deficient and normally nourished guinea pigs 4 weeks following aerosol infection, and BCG exerted the same protective effect regardless of zinc status in this model.89

Vitamin D
As mentioned above, 1,25(OH)2 vitamin D3 (calcitriol) is a potent co-activator of macrophages. Several in vitro studies have demonstrated that the addition of calcitriol to cultured human macrophages enhanced the ability of the cells to control the intra-cellular replication of virulent M. tuberculosis over several days in culture.90,91 The role of dietary vitamin D deficiency was examined in the guinea pig model of pulmonary tuberculosis. Feeding a diet completely devoid of vitamin D for several weeks resulted in marked depletion of serum levels of the calcitriol precursor, 25(OH) vitamin D3, and resulted in significant loss of some T-cell functions in BCG-vaccinated guinea pigs. However, vitamin D deficiency did not alter the course of tuberculous disease in non-vaccinated guinea pigs, nor did it impair the protective efficacy of BCG vaccination in this model.92

Protein
Most of the work with this model has been carried out with moderate, chronic protein deficiency. Feeding a 10% ovalbumin-based diet over several weeks resulted in a dramatic loss of T-cell functions in BCG-
vaccinated guinea pigs. Thus, protein-deprived animals had much smaller PPD skin tests and their lymphocytes proliferated poorly to mitogenic and antigenic stimuli in vitro.\(^3\,8,88\) PPD-stimulated T-cells from low-protein animals produced significantly less interleukin (IL)-2,\(^9,93\) and interferons (IFN),\(^9,94\) and macrophage-lymphocyte co-cultures from malnourished animals produced less tumor necrosis factor-alpha (TNF-\(\alpha\)) in response to infection of the macrophages with virulent M. \textit{tuberculosis}.\(^9,94,95\) Following virulent, pulmonary challenge, protein-deficient guinea pigs were unable to form mature, well-circumscribed granulomas in the lung,\(^9,96\) and expressed significantly less BCG-induced resistance in the lung and spleen.\(^9,97\) Not only was BCG-induced protection diminished by protein deficiency, but the response to exogenous reinfection was impaired as well.\(^9,98\) Furthermore, while immune cells from normally nourished guinea pigs adoptively protected syngeneic, protein-deficient guinea pigs against aerosol infection, the reverse was not true, i.e., immune lymphocytes from low-protein animals did not protect naive, normally nourished recipients.\(^9,96\)

Protein malnutrition altered the absolute and relative numbers of total T-lymphocytes and various subpopulations, including CD2\(+,\)\(^9,99\) CD4\(+,\)\(^9\) CD8\(+,\)\(^10\) and Fc receptor-bearing\(^10,1\) T-cell subsets in the circulation and lymphoid organs (e.g., spleen and bronchotracheal lymph nodes draining the infected lung). Taken together, these results imply that protein deficiency is accompanied by alterations in the ability of guinea pigs to regulate the normal recirculation and trafficking of T-lymphocytes which would be required for the formation of protective granulomas.\(^1,10,2\) These phenomena could be explained by diet-induced changes in the production or function of chemokines, which have been observed to be altered in tuberculosis,\(^10,3\) or by perturbations in the expression of adhesion molecules on T-cells or endothelial cells.

Finally, macrophages from tuberculosis patients are known to produce suppressive factors for T-cells, including transforming growth factor-beta (TGF-\(\beta\)),\(^1,10,4\) Alveolar macrophages are particularly effective at down-regulating T-cell proliferation in many species, including humans.\(^1,0,3\) While protein deficiency was not associated with loss of some macrophage functions in the guinea pig model,\(^1,0,6,10,7\) we did observe that alveolar macrophages suppressed the mitogen-induced proliferation of autologous splenic lymphocytes at macrophage-to-lymphocyte ratios of 1:4 or greater.\(^9,0,8\) More importantly, the intrinsic suppression by alveolar macrophages was enhanced ten fold in this system when the cells were derived from protein-deficient guinea pigs.\(^1,0,9\) In a separate series of experiments, we demonstrated that TGF-\(\beta\) was produced in higher amounts by cells from protein-deprived guinea pigs,\(^9,4\) and that recombinant human TGF-\(\beta\) injected daily into guinea pigs infected with virulent \textit{M. tuberculosis} suppressed T-cell functions and impaired bacillary control in the lungs and spleens of treated animals.\(^1,10\) Thus, macrophages from protein-deprived guinea pigs appear to be more suppressive for T-lymphocyte functions, and this suppression may be mediated, in part, by overproduction of TGF-\(\beta\).

It should be noted that the profound loss of T-cell-mediated resistance that accompanies chronic dietary protein deprivation in this model is substantially and rapidly reversible. BCG-vaccinated guinea pigs maintained on a low protein diet during the entire 6-week period post-vaccination, but given a normal diet beginning on the day of virulent pulmonary challenge, displayed PPD skin test reactivity and vaccine-induced control of bacillary loads in the lungs and spleens 2–4 weeks later that were indistinguishable from BCG-vaccinated animals which had never been protein-deficient.\(^1,11\) One possible interpretation of these observations is that protein deficiency interferes with the expression, but not with the development, of T-cell-mediated protective mechanisms in tuberculosis.

These basic observations were recently confirmed and extended by studies performed in protein malnourished mice. Using a high-dose intravenous challenge model, Chan et al. observed many of the same T-cell defects that have been reported in low-protein guinea pigs, including loss of control of the virulent infection and impaired granuloma formation, and recovery of resistance following refeeding with an adequate diet.\(^1,12\) They concluded that loss of resistance to tuberculosis in their model was a result of diminished nitric oxide (NO) production by activated macrophages which occurred secondary to an IFN-gamma (IFN-\(\gamma\)) defect in malnourished animals.\(^1,13\) These are important studies because they confirm the fundamental nature of the effects of protein deprivation in tuberculosis even when such crucial variables as host species and infection dose and route are altered.

**Conclusions from experimental animal studies**

Taken together, these published studies confirm that protein deficiency, in particular, can have devastating consequences in both innate and vaccine-induced resistance against tuberculosis in animal models. The precise mechanisms by which diet exerts these effects remain to be elucidated. However, the results of the experiments summarized above point to defects in T-cell trafficking and antigen-induced proliferation, inability to form mature granulomas, diminished production of ‘protective’ cytokines (e.g., IL-2, IFN-\(\gamma\), TNF-\(\alpha\)) and antimycobacterial effector molecules (e.g., NO in mice), and increased suppression by adherent cells, perhaps secondary to increased TGF-\(\beta\) production.

**CONCLUSION**

This review critiques known studies in human populations and in relevant animal models to cover the in
vivo evidence concerning the risk of tuberculosis due to malnutrition. Although TB is clearly related to malnutrition, the risk relative to specific levels and types of both protein-energy and micronutrient malnutrition remain to be defined. Only the NHANES-I Epidemiological Follow-up Study provided plausible relative risk data in a representative nationwide sample of adults. To the extent that the six to ten fold increase in relative risk reflects mild to moderate as well as severe undernutrition, these results suggest that nutritional support of undernourished populations at high risk of TB may reduce the incidence of TB in such groups.

In this regard, the distinction between relative risk and attributable risk must be emphasized. Although the risk of TB in severe malnutrition may be higher than in mild or moderate malnutrition, severe malnutrition occurs in a small fraction of the population even in poorer countries except in famine, war, or disaster-type situations. Mild to moderate malnutrition or micronutrient deficiencies may affect large fractions of the population at risk for TB so that prevention efforts will not be highly successful if they target only severely undernourished groups.

The questions we would like answered are not only how much TB is due to malnutrition, but how is TB due to malnutrition. As suggested by work in the aerosol-infected guinea pig model, protein undernutrition in particular impairs host defense against TB, and the impairment is rapidly reversed with nutritional rehabilitation. Changes in the movement and proliferation of T-lymphocyte subpopulations in response to specific antigens, and changes in the production of key cytokines, in the formation of organized granulomas, and in macrophage activation, have been identified as important components of the process. Further work is needed to identify the optimal points for intervention in terms of cost and effectiveness.

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The relationship between malnutrition and tuberculosis


Les traditions orales de la médecine et de la santé publique considèrent que la malnutrition est un facteur de risque important pour le développement de la tuberculose (TB). La malnutrition atteint profondément l’immunité à médiation cellulaire (IMC), et l’IMC est la principale défense de l’hôte contre la TB. C’est donc biologiquement vraisemblable. Bien que la plupart des professionnels de la santé acceptent facilement ce principe, une grande partie de cette croyance repose sur des observations non contrôlées comme des situations de désastre ou sur une logique rétrospective provenant du caractère fréquent de la cachexie parmi les patients TB. En fait, les preuves chez l’homme sont étonnamment minces dans une perspective de rigueur scientifique. Et peu, voire même aucune donnée, ne quantifie l’étendue du risque relatif ou attributable de TB dû à la malnutrition. De plus, des données provenant d’animaux d’expérience étaient basées jusqu’il y a peu sur des modèles animaux qui dans l’ensemble n’étaient pas pertinents pour l’infection et la maladie TB humaines. Cet article passe en revue les données scientifiques qui soutiennent l’affirmation que la malnutrition est un facteur de risque important pour la TB en se concentrant sur des observations chez l’homme et sur les études animales expérimentales basées sur un modèle animal hautement pertinent. Si ceci s’avère vrai, la malnutrition pourrait représenter un risque attributable de TB plus important pour la population que ne l’est l’infection VIH ; elle serait certainement un risque beaucoup plus remédiable.