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# Cross-Cultural Association Between Dietary Animal Protein and Hip Fracture: An Hypothesis

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# Cross-Cultural Association Between Dietary Animal Protein and Hip Fracture: An Hypothesis

A Thesis Submitted to the Yale University School of Medicine in Partial Fulfillment of the Requirements for the Degree of Doctor of Medicine.

by Benjamin Jac Abelow

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**ABSTRACT**

CROSS-CULTURAL ASSOCIATION BETWEEN DIETARY ANIMAL PROTEIN AND HIP FRACTURE: AN HYPOTHESIS. Benjamin J. Abelow, Theodore R. Holford, and Karl L. Insogna. Department of Epidemiologgy and Public Health, and Department of Medicine, Section of Endocrinology. Yale University School of Medicine, New Haven, Connecticut.

Age-adjusted female hip fracture incidence has been noted to be higher in industrialized countries than non-industrialized countries. A possible explanation which has received little attention is that elevated metabolic acid production associated with a high animal protein diet might lead to chronic bone buffering and bone dissolution. In an attempt to examine this hypothesis, cross-cultural variations in animal protein consumption and hip fracture incidence were studied. When female fracture rates derived from 34 published studies in 16 countries were regressed against estimates of dietary animal protein, a strong, positive association was found. This association could not plausibly be explained by variations in either dietary calcium or total caloric intake. Recent studies suggest that the animal protein-hip fracture association could have a biologically tenable basis. We conclude that further study of the metabolic acid-osteoporosis hypothesis is warranted.

## ACKNOWLEDGMENTS

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# INTRODUCTION

Hip fracture, the most devastating of the osteoporosis-associated fractures, has been noted to be more common in industrialized countries than non-industrialized countries [1]. A variety of explanations have been offered to account for this distribution. Most have focused on the possibility that variations in genetic or environmental factors result in decreased bone mass and therefore predispose to fracture [2]. This thesis focuses on one causal hypothesis that has received little attention in the published literature: the bone buffering hypothesis. This hypothesis holds that fracture incidence may reflect osteopenia resulting from chronic bone buffering of excess metabolically produced acid.

Protein catabolism is the source of most metabolically produced non-volatile acid [3]. Much of this acid appears to be generated during the net metabolism of amino-acid sulfur to sulfuric acid [3]. The majority of metabolically produced acid is buffered in the body fluids by the bicarbonate buffer system, a process that consumes bicarbonate. Homeostasis is maintained primarily by the kidney, which generates new bicarbonate and secretes it into the blood, a process quantitated by measuring Net Acid Excretion. Net Acid Excretion is defined as the sum of urinary ammonium plus urinary titratable acid minus urinary bicarbonate [3].

Consistent with this understanding of metabolic acid production and the homeostatic renal response, it has been found that raising dietary protein intake increases urinary net acid excretion [4-6]. This finding suggests that increasing dietary protein intake augments metabolic acid production, titrates bicarbonate in body fluids, and secondarily stimulates the homeostatic production of new bicarbonate. It appears that acid production and excretion are especially elevated when protein from animal sources comprises a large part of the total protein intake [7].

In addition to fluid-borne bicarbonate, it is known that buffers in bone can, at times, play a role in maintaining acid-base homeostasis. Specifically, it has been observed that a substantial elevation of endogenous acid production, as, for example, occurs during clinically apparent metabolic acidosis, results in buffering by alkali in bone, which normally exist as metallic salts [8]. The buffering process mobilizes calcium and other metallic cations in bone and results in bone loss [8].

Wachman and Bernstein considered the possibility that a degree of bone buffering and bone dissolution might occur in healthy individuals consuming diets that produce relatively high levels of metabolic acid [9]. Specifically, they hypothesized [9] that diets rich in meat and protein might

chronically increase endogenous acid production and cause ongoing, low-level osteoporotic bone loss by the same mechanism known to exist in clinically apparent metabolic acidosis [9].

A variety of epidemiologic, metabolic, and animal studies have produced results consistent with this hypothesis [10]. Of epidemiologic studies, most [11], but not all [12], of the data produced suggest that ageing-associated bone loss is accentuated in women eating diets high in meat and protein. For example, among post-menopausal women in the seventh through ninth decades of life, it has been found that ovo-lacto-vegetarians experience less radial bone loss than omnivores [11]. The finding of diminished bone density in ovo-lacto-vegetarians versus omnivores is not observed in either premenopausal women or in men of any age, and suggests the possibility of a combined effect of sex, age, and diet [11].

Of human metabolic studies, most, but not all [13], have found that increasing protein from animal and vegetable sources can adversely affect calcium status. For example, diets containing high levels of animal protein [7,14,15] or enriched with purified protein [4-6,14] can elevate urinary calcium excretion and produce a negative calcium balance.

Animal studies [10], too, have produced results consistent with the hypothesis. For example, one study found that chronic, low-level feeding of ammonium chloride, a substance metabolized to equimolar amounts of hydrochloric acid, produced marked changes in bone reabsorption. Taken together, these epidemiologic, metabolic, and animal studies suggest that the bone-buffering hypothesis warrants continued study.

The purpose of this study was to characterize the epidemiologic association between dietary animal protein and hip fracture in women over 50. Women over age 50 were considered because, world wide, the vast majority of hip fractures occur in this population [1]. Our goal was to assess whether the metabolic acid-osteoporosis hypothesis helps to statistically explain the well-recognized cross-cultural variation in hip fracture incidence. In doing so, our goal was simply to assess the explanatory power of the hypothesis. We did not attempt to rule out competing epidemiologic or statistical hypotheses. Nonetheless, we did assess the the potentially confounding role of two factors: dietary calcium and total caloric intake.

## METHODS

Surveys of the incidence of hip fracture were identified by MEDLINE and several manual literature searches. A total of 37 published reports were located. These 37 were then assessed in terms of the following inclusion criteria: (1) the report must be published in a peer-reviewed journal, (2) the report must provide fracture incidence data for a geographically defined area, and (3) the report must contain age-specific data, i.e., the fracture rates must be reported (or derivable) for different age groups within the population studied. Of the 37 surveys identified by our literature searches, 34 surveys in 29 publications [16-44] from 16 countries met the inclusion criteria and were analyzed in this thesis.

For each survey analyzed, hip fracture rates for women over age 50 were expressed as fractures/100,000 person-years and age-adjusted by the direct method [45,2]. This age-adjustment process allows populations of different age distributions to be compared, without differences in age distribution affecting the comparison. This process requires that a reference population be selected, and that data for each population studied be statistically normalized to the

reference population. The end result is that populations of effectively identical age distributions are compared. This approach is commonly used, and has been described in detail elsewhere [45,2].

The distribution of women in the United States for 1987, as estimated by the U.S. Census Bureau [46], was taken as the reference population. The selection of a reference population can, in certain settings, introduce bias into the final comparisons. For this reason, we performed an additional set of analyses using cumulative incidence rates [45]. This approach has the statistical effect of using a different reference population, one with equal numbers of members in each 5-year age group. By using both approaches, and comparing the results generated with each, we were able to assess if the analysis was biased by the selection of a particular reference population. In addition, in the cumulative incidence analysis, we excluded women over age 85, so that data only for women between ages 50 and 85 were analyzed. This was done because, for some countries, incomplete data prevented us from precisely determining the age of some of the oldest women. By truncating the data at age 85, we were able to compensate for this.

Dietary estimates came from two sets of publications produced by the Food and Agriculture Organization (FAO) of the United Nations: Food Balance Sheets [47,48] and Per Caput Food

Supplies [47]. These two FAO publications are the only sources available that contain dietary data for all 16 countries studied here. These FAO estimates have some shortcomings. For example, the calculations do not account for food that is wasted in the production and distribution process, and there are country-to-country differences in the quality of data analyzed to arrive at the estimates [47]. Nonetheless, the estimates are generated with a standard algorithm and appear to provide good relative measures of dietary intake in different countries [47].

To assess the association between dietary animal protein and hip fracture incidence, we used the statistical technique of regression analysis. Whenever possible, we used dietary data and fracture rates that were derived for exactly coincident time periods. For example, if a fracture survey covered the years 1968-1975, we tried to use dietary estimates that also were calculated for the years 1968-1975. In some settings, precisely co-temporal data were not available. In these cases, we used dietary data for the closest available years. In most cases, there was considerable overlap between the time frames for the dietary and hip fracture data.

Data on hip fracture incidence and dietary animal protein intake were analyzed in four major ways. In all four, the regression line and $R^2$ values that were produced reflect the

statistical association between dietary animal protein and fracture incidence. In the first regression method, age-adjusted summary rates from all surveys meeting the inclusion criteria were regressed, unweighted, against dietary animal protein. In other words, a single fracture incidence rate was calculated for each of the 34 surveys meeting the inclusion criteria, and these rates were regressed against the appropriate dietary data for the closest overlapping years.

In the second regression method, we analyzed only those surveys containing data that were sufficiently detailed to permit the calculation of an overall variance. The calculation of a variance makes it possible to carry out a weighted regression. Of the 34 surveys analyzed, 26 contained data detailed enough to produce a weighted regression. The initial 34 surveys covered populations in 16 countries; the 26 surveys analyzed in this phase of the study covered populations in 14 countries. This weighted regression was done using fracture rates derived from both the directly weighted populations and the cumulative fracture rates method.

In the third regression method, we attempted to emphasize the cross-cultural association between diet and fracture incidence. Note that in the previous two methods, a data point was generated for each survey. However, in this third

analysis, a single estimate of fracture incidence was generated for each country. This estimate was regressed against an estimate of dietary intake for the country as a whole. The details of this third regression method are as follows:

The estimate of fracture incidence for each country was calculated as the weighted mean of age-adjusted fracture rates from all surveys within that country. The reciprocal of the variance (1/variance) was used as the weighing factor. Surveys not sufficiently detailed to calculate an overall variance were excluded. (This meant that no estimates could be calculated for Holland and Ireland.) When only a single survey was available for a given country, the age-adjusted rate from that survey was used. The estimates for each country were weighted by 1/(variance of the mean) and regressed against similarly weighted means of dietary animal protein for that country. This analysis was repeated using cumulative rates for ages 50-85 instead of age-adjusted rates

In the fourth regression method, dietary calcium and total caloric intake were considered as regression variables. These variables were considered both independently and in various combinations, which are described more fully in the results section.

## RESULTS

Table 1 presents the data from each of the 34 fracture surveys that met the inclusion criteria. The table includes data on age-adjusted hip fracture incidence (derived from data contained in the 34 included surveys), the years during which fracture data were collected, the closest overlapping years for which FAO data on dietary animal protein are available, and estimated per capita dietary animal protein for those years. FAO estimates of dietary calcium are also presented for each of the 16 countries where fracture surveys were carried out.

As expected, values for animal protein intake in the United States, and in western and northern Europe, are higher than those for Asia and Africa. Countries of southern Europe tend to have intermediate levels of animal protein consumption. Some of the more industrialized countries have been the subject of several fracture surveys, while none of the lesser-industrialized countries has been surveyed more than once in sufficient detail to generate age-adjusted values.

Of the countries studied more than once, it can be seen on Table 1 that in some (e.g., United Kingdom) there is considerable inter-survey variation in the fracture rates, while for others (e.g., Denmark) the variation is relatively

minor. In some cases, the variations may result partially from the fact that not all surveys carried out in a given country studied the same geographic region. In addition, the years of the surveys in a given country did not always coincide, or even overlap.

As indicated in Table 1, black women in the United States have a lower incidence of fractures than white women in the U.S. and Europe. However, black women in the U.S. still experience a much higher rate than black women in South Africa. An exhaustive analysis of all the possible genetic or environmental causes for this pattern was beyond the scope of this study and was not undertaken.

In general, the data show that women over 50 years of age tend to experience higher rates of hip fracture in industrialized countries than in lesser-industrialized countries.

When age-adjusted summary rates for all 34 surveys meeting the inclusion criteria were regressed, unweighted, against dietary animal protein (the first of the four regression methods), a positive association was found. The best fitting regression equation is $y = -18.0 + 2.29x$, where y is hip fracture rate per 100,000 person-years, and x is animal protein in grams/day. The fractional variation of the hip fracture rate that is explained by dietary animal protein,

$R^2$, is 0.42 ($p<0.001$). This indicates that 42 percent of the observed variation in fracture incidence can, on a statistical basis, be explained by variations in dietary animal protein.

Given the limitations inherent in an unweighted regression analysis, weighing of the data sets was undertaken. When a weighted regression line was fitted to the age-adjusted summary rates from all 29 surveys for which an overall variance could be calculated (the second of the regression methods), a strong, positive association between fracture and animal protein remained. The best fitting regression equation is $y = -38.7 + 2.51x$ ($R^2 = 0.66$; $p<0.001$). As the $R^2$ value indicates, 66 percent of the variation in fracture incidence can, on a statistical basis, be explained by variations in dietary animal protein. This association is shown graphically in Figure 1. Using the cumulative incidence of fractures for women between ages 50 and 85, the strength of the association was essentially the same: $y = -4350 + 314x$ ($R^2 = 0.71$; $p<0.001$). This suggests that the selection of the original reference population did not introduce substantial bias into the analysis.

When a weighted regression line was fitted to the 14 age-adjusted country estimates (the third regression method), a strong cross-cultural association was found between fracture incidence and animal protein intake. The best fitting

regression equation for this cross-cultural association is y =

$-38.4 + 2.50x$ ($R^2 = 0.67$; $p<0.001$). This association is shown graphically in Figure 2. Using estimates derived from cumulative rates, the results were again substantially the same: $y = -4307 + 313x$ ($R^2 = 0.72$; $p<0.001$).

In summary, when age-adjusted and cumulative fracture rates were regressed against estimates of dietary animal protein on either a study-by-study or purely cross-cultural basis, a strong, positive association was found.

In addition to these regression analyses, which involved hip fracture and dietary animal protein as the only variables, a number of other analyses were carried out using dietary calcium and total caloric intake as variables. When FAO estimates of dietary calcium were regressed on country estimates of hip fracture incidence, a significant positive association was noted: $y = -41.0 + 0.14z$, where z equals milligrams/day calcium ($R^2 = 0.62$; $p<0.001$). This finding is consistent with a previously reported association between hip fracture and dietary calcium [49].

It is interesting that this calcium-fracture association is positive; based on the likely protective role of dietary calcium on bone mass and fracture, a negative association

might have been expected. To further clarify this relationship, dietary calcium and animal protein were simultaneously regressed against fracture incidence. The resulting equation is: $y = -42.5 + 1.83x + 0.041z$ ($R^2$ = 0.68). Comparing the regression coefficients with their standard errors, the effect of calcium in the presence of animal protein is not significant ($p = 0.59$). Likewise, the effect of animal protein in the presence of calcium is not significant ($p = 0.19$), although this value falls closer to significance. Dietary calcium and animal protein are highly correlated ($r = 0.91$; $p<0.001$); thus, the problem of multicolinearity makes it difficult to distinguish between the effects of these variables.

When estimated total caloric intake was regressed against country estimates of hip fracture, a moderate association was found ($R^2 = 0.50$; $p = 0.005$). Caloric intake was then regressed with all combinations of dietary animal protein and calcium. When caloric intake and animal protein were simultaneously regressed, the association between animal protein and hip fracture remained significant ($p = 0.024$) whereas the calorie-fracture association did not ($p = 0.87$). When caloric intake and dietary calcium were simultaneously regressed, the association between fracture and calcium remained marginally significant ($p = 0.049$), whereas the calorie-fracture association did not ($p = 0.58$). As expected, when all three dietary variables were

simultaneously regressed, none of the associations was significant.

This work represents a collaboration by Mr. Benjamin Abelow, Dr. Theodore R. Holford, and Dr. Karl Insogna. The hypothesis and idea for this work were conceived entirely by Benjamin Abelow. Mr. Abelow undertook the primary research, data collection, and initial statistical analyses. These data were then reviewed in their entirety by Dr. Insogna. Under his guidance, a more rigorous and expanded statistical analysis was undertaken. The biological limitations of the data were also discussed and clarified. Dr. Theodore Holford's assistance was then sought to further strengthen the statistical analysis, and the statistical methodology was modified to its present form. The final statistical analyses of the data, using weighted regression analyses and cumulative incidence rates, were performed by Dr. Holford.

## DISCUSSION

### Epidemiologic Considerations:

A variety of factors have previously been hypothesized to account for cross-cultural differences in fracture incidence. For instance, variables such as physical exercise and sunlight exposure have been shown to be plausibly correlated with female hip fracture incidence rates [2]. A cross-cultural association between total dietary protein and hip fracture has been shown by Hegsted, a finding the author suggests might be due to protein-induced damage of renal calcitriol regulation [49]. Low rates among Blacks in South Africa [30] has raised the possibility that genetic factors may be involved.

We considered the possibility that differences in animal protein intake, a relatively specific marker for metabolic acid production, might help explain the cross-cultural variability in hip fracture incidence. Our data demonstrate a strong cross-cultural association between estimated per capita dietary animal protein and fracture incidence in women over 50. While these results do not necessarily imply a causal association, they suggest that the metabolic acid-osteoporosis hypothesis warrants serious study as a possible

explanation of cross-cultural variations in hip fracture incidence.

Bone loss usually occurs from about age 40 until death, with accelerated losses in most women occurring for about ten years after menopause [50]. Environmental modifiers of the rate of bone loss might thus be expected to act over many years. For this reason, if one wished to assess the role of diet, it would be most desirable to evaluate chronic dietary patterns for the years before fracture occurred. Thus, ideally, for this study, it would have been desirable to analyze dietary data for several decades prior to the periods studied in the hip fracture surveys. However, this was not possible because reliable dietary data were not consistently available for most countries those years. As a result, we used data for the dietary patterns prevailing during the periods when the fractures occurred.

Although less than ideal, a number of factor suggest that this approach is nonetheless satisfactory. All industrialized countries studied here have had relatively high and stable levels of dietary animal protein over the past three decades [47,48]. Thus, the diets consumed during the periods when fractures occurred can be considered reasonable approximations of the diets consumed chronically before the fractures occurred. Of the lesser-industrialized countries included in our assessment, some have shown a

gradual increase in dietary animal protein over time [47,48], and there is little reason to suspect that animal protein intake in any of these countries was higher than it is currently [47,48]. Thus, for the lesser-industrialized countries, animal protein intake during the periods when fracture occurred was either approximately the same, or was somewhat higher, than that of the chronic pre-fracture diet. The effect of this would be to make the overall slope of the diet-hip fracture plot less steep than it otherwise would have been. In other words, the overall effect of using contemporaneous dietary and fracture data may well have been to weaken the statistical association we observed, thereby making our analysis more conservative.

The aim of this study was to evaluate the explanatory power of dietary animal protein. It was not our intention to rigorously rule out other potential causal factors. Nonetheless, we did analyze data on two dietary factors: calcium and total caloric intake. We chose to study dietary calcium because, of all the factors assumed to have an effect on osteoporotic disease, calcium is the best studied, has a validated protective effect, and is commonly assumed to be of substantial importance. Thus, if a population consuming high levels of animal protein also consumed low levels of calcium at the time when fracture incidence was high, it would be reasonable to assume that a substantial confounding effect by calcium might have occurred.

Perhaps surprisingly, our data showed a positive cross-cultural association between dietary calcium and hip fracture incidence. In other words, those countries with high fracture rates also tended to have high calcium intake. This association is biologically implausible, and appears to contradict a substantial body of data that suggests a protective role for dietary calcium. This implausibility suggests two things. First, it suggests that, cross culturally, calcium intake is likely acting as an epidemiologic marker for one or more factors that are causally related to fracture incidence via a biologically plausible mechanism. Second, it suggests that on a cross-cultural basis, variations in dietary calcium probably do not play a major role in explaining the variability in fracture incidence. It must be stressed, however, that this does not preclude a possible protective effect on an individual (as opposed to population) basis. These two interpretations appear to be supported by a recent prospective study showing an inverse relationship between dietary calcium and hip fracture incidence, a finding that suggests a protective effect of increasing dietary calcium intake on fracture rate [51].

If dietary calcium is acting as an epidemiologic marker, what is it marking? Our data can say little in this regard. Given the high cross-cultural correlation between dietary animal

protein and calcium, it seems plausible that calcium may be a marker for an animal protein-rich diet. However, because of the problem of multicolinearity, simultaneous analysis of calcium and animal protein could not assess this possibility. Other genetic or environmental factors [2] are also possible. The epidemiologic evaluation of these factors is beyond the scope of this study.

The second factor whose potential confounding effect we assessed was total caloric intake, a non-specific marker of nutrient intake. Although we know of no hypothesized link between total caloric intake and fracture, we studied caloric intake because it seemed likely to be highly correlated with many other markers for industrialization. If the association between animal protein and fracture could be fully explained by total caloric intake, this would have suggested that animal protein may itself have only been a marker for other factors; that is, it would have tended to devalue the importance of our epidemiologic findings. When total caloric intake was simultaneously regressed with animal protein, the fracture-animal protein association remained significant, whereas the fracture-calorie association did not. This indicates that the cross cultural-association between animal protein and fracture incidence can not be explained by differences in caloric intake between the countries studied.

In the countries studied here, most hip fractures in women over 50 results from low or moderate trauma [17,18,21,24,25,31,33-35,39-41,44]. This appears to be true in the lesser-industrialized countries as well [28-30], although a higher proportion of fractures may due to severe trauma [30]. In general terms, this means that in the non-industrialized countries, the incidence of those hip fractures classically considered to have an osteoporotic component was even lower than the data suggest. Thus, if bias was introduced by fractures caused by severe trauma, one would have expected it to make the analysis presented here more conservative.

The quality of data on fracture incidence analyzed in this study is determined by that of the surveys whose results we analyzed [16-44]. In these various surveys, inclusion criteria for fractures varied slightly, but the authors of almost all surveys present evidence that all or most fractures were identified [2]. In our own study, fracture rates surveyed regionally were taken to reflect national rates, an assumption that might produce some distortions. Similarly, FAO dietary estimates are per capita averages and do not reflect potential ethnic, regional, sex, age or individual differences. While these factors may have affected the quantitative accuracy of individual estimates, it is unlikely that the overall patterns observed in this study were artifactual.

Biologic Plausibility:

In this study, we used dietary animal protein as a marker for metabolic acid production. A number of studies, both *in vitro* and *in vivo*, suggest that this approach is valid. When typical diets were analyzed, it was found that relative dietary ash-acidity varies in the order omnivore > ovo-lacto-vegetarian > vegan [52]. Ash-acidity quantitates the net acid produced when foodstuffs are completely oxidized *in vitro*. Although this *in vitro* quantitation is highly accurate and reproducible, acid production so calculated generally overestimates that generated when the same foods are oxidized endogenously. The major reason for this is that, in the body, foodstuffs are not always metabolized to their highest oxidation state. For example, some carbohydrates and fats are oxidized to organic acids, and a fraction of ingested sulfur is oxidized to products other than sulfate.

It is well-known that dietary protein, irrespective of its origin, is a source of metabolic acid [4-6]. It thus seems possible that the above differences in ash-acidity might be due simply to differences in protein content. Although protein-adjusted comparisons of ash-acidity have not been

carried out, a carefully controlled study suggests that, *in vivo*, protein from animal sources is more acidogenic than protein from vegetable sources. When individuals consuming protein-matched diets containing different levels of animal protein were studied on a metabolic ward, urinary net acid excretion was found to increase with animal protein content [7]. In terms of our own study, this finding suggests that dietary animal protein is a more specific marker for metabolic acid production than total protein, of which it is also a marker.

The high acidogenicity of animal protein is due partly or wholly to the fact that animal protein typically contains more sulfur than protein from vegetable sources [53,15]. This is relevant because most amino-acid sulfur undergoes net metabolism to sulfuric acid [54].

An important, additional, finding is that on a cross-cultural basis dietary animal protein is strongly associated with total protein intake [47,48]. Thus, populations consuming diets high in animal protein also tend to consume high levels of total protein. This is important, as noted, because total protein is itself an important source metabolic acid [4-6].

In addition to the link between dietary animal protein and metabolic acid production, the hypothesis being considered in this study requires that diet-associated changes in metabolic

acid production result in bone dissolution. A number of studies support this possibility. A variety of studies indicate that dietary protein supplementation leads to calciuresis [4-7,14,15]. Some of these studies suggest further that changes in calcium excretion are mediated by alterations in acid-base status. For example, protein-induced negative calcium balance is accompanied by large increases in urinary net acid excretion [4-6] and is halted by the ingestion of sodium bicarbonate [6]. Omnivore diets can induce a more negative calcium balance than less-acidogenic vegetarian diets matched for total protein, and do so in association with relatively suppressed levels of urinary cyclic-AMP, serum PTH and 1,25-dihydroxy vitamin D [7]. These latter biochemical findings are consistent with acid-induced bone dissolution [7].

Finally, there is evidence that fixed acid loads currently considered physiologic may produce bone loss. When ammonium chloride is ingested at rates that elevate urinary acid excretion to levels just above those attained with high protein diets, fasting calcium excretion is increased [55]. Although there is no direct evidence linking this relative calciuresis to alterations in acid-base status, it has been independently shown that endogenous acid production in excess of 1 mEq/kg/day, well within the range considered physiologic, can lead to sustained mild metabolic acidosis and positive hydrogen ion balance [56]. Furthermore, it has

been shown that among older individuals, the group most prone to hip fractures, the capacity to excrete an acid load is decreased [57,58]. Thus, while it is well established that clinically apparent metabolic acidosis can result in the chronic titration of buffers in bone [8], these latter data suggest that mild, clinically inapparent changes in acid-base status might also, over time, lead to qualitatively similar results.

In conclusion, many epidemiologic, human metabolic, and animal studies have produced results consistent with the metabolic acid-osteoporosis hypothesis. While the original, epidemiologic findings presented here do not necessarily imply a causal relationship, the strength of the association we observed does lend additional weight to the hypothesis. We conclude that further study of the endogenous acid-osteoporosis hypothesis is warranted. Given the epidemic of osteoporotic fractures in the West [1] and the possibility of dietary prevention or new adjunctive therapies [6], such study may be of considerable practical value.

**Table 1.** Fracture incidence rates and dietary estimates, with source information

| Country | Fracture survey reference | Figure symbol | Years of fracture survey | Years of dietary data | Fracture rate | Animal protein (g/day) | Calcium (mg/day) |
|---|---|---|---|---|---|---|---|
| Denmark | [16] | [a] | 1971–76 | 1971–76 | 164.8 | 57.1 | 960[c] |
| | [17] | D | 1973–79 | 1973–77 | 165.3 | 58.0 | |
| Finland | [18] | F1 | 1968 | 1968 | 71.9 | 56.1 | 1332[c] |
| | [19] | F2 | 1970 | 1970 | 97.3 | 57.4 | |
| | [20] | F3 | 1980 | 1979–81 | 111.2 | 60.5 | |
| Holland | [21] | [a] | 1967–79 | 1967–77 | 87.7 | 54.3 | 1006 |
| Hong Kong | [22] | HK | 1965–67 | 1967 | 45.6 | 34.6 | 356 |
| Ireland | [23] | [a] | 1968–73 | 1968–73 | 76.0 | 61.4 | 1110 |
| Israel | [24] | IS | 1957–66 | 1957–65, 67 | 93.2 | 42.5 | 794 |
| New Zealand | [25] | NZ | 1973–75 | 1973–75 | 119.0 | 77.8 | 1217 |
| Norway | [26] | N1 | 1972–73 | 1972–73 | 148.8 | 56.4 | 1087[c] |
| | [27] | N2 | 1978–79 | 1979–81 | 220.9 | 66.6 | |
| | [26] | N3 | 1983–84 | 1979–81 | 190.4 | 66.6 | |
| Papua New Guinea | [28] | P | 1978–82 | 1977 | 3.1 | 16.4 | 448 |
| Singapore | [29] | SN | 1955–62 | 1961–65 | 21.6 | 24.7 | 389 |
| South African blacks | [30] | SA | 1957–63 | 1961–65 | 6.8 | 10.4 | 196 |
| Spain | [31] | SP | 1974–82 | 1974–77, 77–79 | 42.4 | 47.6 | 766 |
| Sweden | [32] | [a] | 1950–60 | 1951–57 | 121.6 | 57.1 | 1104[c] |
| | [33] | S1 | 1965, 70, 75, 80[b] | 1967–77, 79–81 | 191.8 | 58.2 | |
| | [34] | S2 | 1972–81 | 1972–77, 1979–81 | 187.8 | 59.4 | |
| | [35] | [a] | 1981 | 1979–81 | 214.3 | 58.9 | |
| United Kingdom | [36] | [a] | 1954–58 | 1954–57 | 77.1 | 49.0 | 977[c] |
| | [37] | UK1 | 1975, 80[b] | 1975–81 | 91.4 | 55.3 | |
| | [38] | UK2 | 1977 | 1977 | 118.2 | 56.6 | |
| | [39] | UK3 | 1978–79 | 1977 | 115.6 | 56.6 | |
| | [40] | UK4 | 1983 | 1979–81 | 131.0 | 53.9 | |
| United States | [41] | US1 | 1965–74 | 1967–74 | 144.9 | 72.0 | 973[c] |
| (Whites) | [42] | [a] | 1974–79 | 1974–79 | 132.0 | 72.5 | |
| (Non-White) | [42] | [a] | 1974–79 | 1974–79 | 33.5 | 72.5 | |
| (Whites) | [42] | USW | 1980 | 1979–81 | 118.3 | 71.5 | |
| (Blacks) | [42] | USB | 1980 | 1979–81 | 60.4 | 71.5 | |
| Yugoslavia | [43] | Y1 | 1968–73 | 1968–73 | 20.5 | 27.5 | 588[c] |
| | [43] | Y2 | 1968–73 | 1968–73 | 52.3 | 27.5 | |
| | [44] | Y3 | 1969–72 | 1969–72 | 27.6 | 27.3 | |

Incidence rates are per 100,000 person-years for women over 50, age-adjusted to 1987 U.S. female population [46]

[a] Survey data were not detailed enough to calculate variance (see text)

[b] Fracture data for discreet years were pooled to calculate a single age-adjusted rate

[c] Average for all surveys in this country

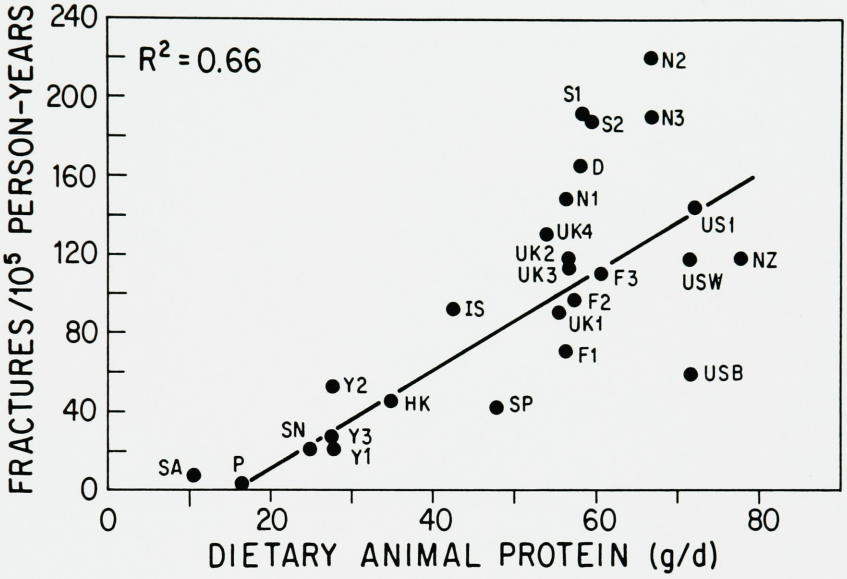


Figure 1: Plot of age-adjusted hip fracture incidence in women over 50 against estimated per capita dietary animal protein, by fracture survey. See Table 1 for symbols and sources of data.

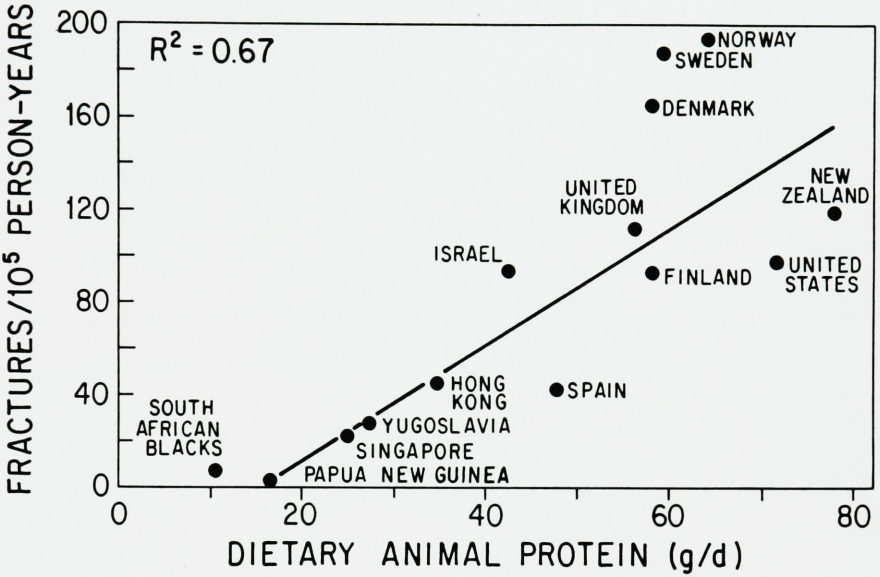


Figure 2: Plot of age-adjusted hip fracture incidence in women over 50 against estimated per capita dietary animal protein, by country. See Table 1 for symbols and sources of data.

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