## **Perspective**

# How Much Calcium Does an Indian Postmenopausal Woman Need?

Meeta Meeta

Department of Gynaecology, Tanvir Hospital, Hyderabad, Telangana, India

ABSTRACT

Calcium is needed for many biological processes. The serum concentration is of calcium is regulated very finely. The absorption of calcium and excretion maintains the calcium balance. Net calcium excretion must be replaced, but the amount of calcium needed has been debated for decades. The debate is what is the adequate calcium in Indian postmenopausal women to maintain bone health and as an intervention to prevent and treat osteoporosis. In this article experts give their opinion on the increase in the Recommended Dietary Allowance (RDA) of the ICMR 2020 committee. Based on the present evidence, the Indian menopause Society guidelines 2020, retains the ICMR 2010 recommendation of 800 mg of calcium as RDA until new Indian data emerges. The positive impact of increasing calcium and vitamin D intake is most evident in calcium-deficient individuals.

**KEYWORDS:** Calcium, dietary reference intakes, postmenopause

## Accepted: 28-Mar-2022 Published: 02-May-2022

Submitted: 23-Mar-2022

Revised: 25-Mar-2022

## Introduction

The role of calcium (Ca) in bone health and metabolism of almost every cell in the body is established. Globally, the requirement of adequate calcium in postmenopausal women to maintain bone health and as an intervention to prevent and treat osteoporosis is inconclusive.

The calcium needed for skeletal growth and remodeling varies by life stage. The Dietary Reference Intake (DRI) of calcium for adequacy of health is influenced by life stages, gender, ethnicity, genetic, geographical factors, a variety of hormones, ions, nutrients, diseases, and drugs that affect absorption and excretion. Absorption of dietary calcium is 20%-50% which is facilitated by Vitamin D. The endogenous calcium excretion does not change appreciably with age, is estimated to be 2.1 mg/kg from the fecal loss and minor loss in sweat.[1] The urinary loss of calcium is influenced by various factors and is primarily involved in calcium retention. The kidney maintains a balance between the calcium load filtered by the glomeruli and the efficiency of reabsorption (approximately 98%) from the renal tubules and is about roughly 100-400 mg/day with an average intake of calcium.[2] There is uncertainty as to the actual retention rates as there is a wide variability seen in various countries such as China, where zero retention rate has been reported to be 400 mg, Japanese have reported 600 mg, and the World Health Organization (WHO) at 540 mg. Based on Hunt and Johnson's study in 2007, where the calcium zero retention was 741 mg, the Institute of Medicine (IOM) has set the Estimated Average Requirement (EAR) as 800 mg for calcium.

## Estimated average requirement

Reflects the estimated median requirement and is particularly appropriate for applications related to planning and assessing intakes for groups of persons. Recommended Dietary Allowance (RDA): derived from the EAR and meets or exceeds the requirement for 97.5% of the population. DRI recommendations for calcium per day are not uniform globally, varying from 800 mg to 1300 mg/day for women over the age of 50 years, but all health authorities recognize the increased need for calcium in postmenopausal women and older adults [Table 1].

In 2003, the WHO's report on diet, nutrition, and the prevention of chronic disease concluded that in

Address for correspondence: Dr. Meeta Meeta, Department of Gynaecology, Tanvir Hospital, Hyderabad, Telangana, India. E-mail: drmeeta919@gmail.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow\_reprints@wolterskluwer.com

How to cite this article: Meeta M. How much calcium does an Indian postmenopausal woman need? J Mid-life Health 2022;13:9-14.



DOI: 10.4103/jmh.jmh\_70\_22

Table 1: Recommended daily allowance for women over the age of 50 years estimated average requirement is not defined

Organization	RDA (mg)	Country
MHLW 2015	650	Japan <sup>[3]</sup>
BNHS	700	$UK^{[3]}$
NORDIC	800-900	Nordic <sup>[4]</sup>
INFH-CAPM (1991)	800	China <sup>[5]</sup>
KBMS	800-1000	Korea <sup>[6]</sup>
NHMRC	1300	Australia <sup>[3]</sup>
USPSTF 2018	1000	USA <sup>[7]</sup>
NIH, IOM 2011, NOF	1200	$USA^{[3]}$
ICMR NIN 2020	1200	India <sup>[8]</sup>
ICMR NIN 2010, IMS 2020	800	India <sup>[9,10]</sup>

MHLW 2015: Ministry of Health, Labour and Welfare, BNHS: Britain's National Health Service, INFH-CAPM: Institute of Nutrition and Food Hygiene, Chinese Academy of Preventive Medicine, KBMS: Korean Bone and Mineral Society, NHMRC: National Health and Medical Research Council, USPST: United States Preventive Services Task Force, NIH: National Institutes of Health, IOM: Institute of Medicine, NOF: National Osteoporosis Foundation, ICMR NIN: Indian Council of Medical Research-National Institute of Nutrition, IMS: Indian Menopause Society, RDA: Recommended Daily Allowance

countries with a high fracture incidence, a minimum of 400–500 mg of calcium intake is required to prevent osteoporosis.<sup>[11]</sup>

### Global intake of calcium

There is a vast gap between recommendation and consumption, especially in the developing Asia-Pacific region with a deficient calcium intake of 350 mg versus 1000 mg/day by developed countries.<sup>[12]</sup>

#### Indian scenario

Based on NNMB, Report of Urban Surveys National Nutrition Monitoring Bureau, National Institute of Nutrition (NIN) Indian Council of Medical Research (ICMR), Hyderabad 2016,the median milk consumption in Urban and Rural India is 122 ml/ consumption unit (CU) and 85 ml/CU, respectively. Calcium intakes from the same data show intakes of 402 mg/CU (Urban) and 331 mg/CU (Rural).

# THE INDIAN COUNCIL OF MEDICAL RESEARCH COMMITTEE RECOMMENDATIONS ON CALCIUM REQUIREMENT FOR AN INDIAN POSTMENOPAUSAL WOMAN

The ICMR Committee in 2010, 2020 has based their recommendations on the same studies described below, and there has been no new data on balance, factorial, or bone health since the recommendation of 2010.

# The Indian Council of Medical Research Committee in 2010

Given the balance studies<sup>[12,13]</sup> and ICMR multicentric study data,<sup>[14]</sup> the upper value of calcium requirements (RDA) for postmenopausal women is 800 mg/day. There is no recommendation on EAR.

# The Indian Council of Medical Research Committee in 2020

The current committee, based on the evidence from balance studies[13,14] and bone density studies,[15] has considered an RDA of 1200 mg and an EAR of 1000 mg for postmenopausal women. Adding 25% coefficient of variance (CV), RDA has been set at 1000 mg for adult males and females. It is only logical to recommend sufficient additional calcium (+200 mg/ day) proportionate to what is recommended by the Food and Agriculture Organization/WHO after menopause to cover at least the obligatory extra loss of 30 mg/day of calcium in the urine.[11] An additional allowance of 200 mg is added to the EAR, and the RDA has been set at 1200 mg for postmenopausal women. The ICMR Task Force study found that parallel to a decrease in calcium intake; bone densities were also lower with a decreasing income. Those above 50 years suffered from much worse bone densities than those <50 years in the same group.<sup>[15]</sup> Studies on the three socioeconomic groups at NIN show that even after the age of 50 years, the extent of osteoporosis in the spine is only 16% in the high-income group (with higher calcium intakes of around 1 g) compared to the low-income group with 65% osteoporosis (calcium intakes around 400 mg).[14]

Bone mineral density (BMD) was measured in women aged 20–90 years, with median intakes of calcium of 800 (with lifetime milk consumption) or 480 mg/day (who consumed no milk or less). The group with a higher intake of calcium entered osteoporotic and fracture zones of bone density 10 years later than those with lower intake. In addition, women engaged in hard work and those with lifetime vegetarian habits were at reduced risk of osteoporosis.<sup>[16]</sup> The fracture rate at the neck of the femur was shown to occur 12–15 years earlier in women from the low-income group as compared to that in high-income group.<sup>[17]</sup>

We invited experts to comment on the increased RDA and EAR by ICMR 2020 for calcium intake in postmenopausal women in India to understand their perspectives.

Dr. Raman Marwah: Endocrinology, Scientific Advisor and Senior Consultant Endocrinology International Life Sciences Institute (India), New Delhi. Dr. Sanjay Kalra: Department of Endocrinology, Bharti Hospital Karnal, India.

Dr. B. Sesikeran MD: Former Director, NIN (ICMR).

Dr. R. Hemalatha: Director, ICMR-ICMR-NIN Department of Health Research Ministry of Health and Family Welfare.

Prof. Anura Kurpad: MD, PhD, FRCP (Lond), FAMS, FASc, FIUNS, St John's Medical College, Bengaluru, India, IAEA Collaborating Centre in Nutrition.

Dr. Jagmeet Madan: National President, Indian Dietetics Association, Principal, Director Research, Consultancy and Collaboration Center, Professor, Department of Food Nutrition and Dietetics, Sir Vithaldas Thackersey College of Home Science (Autonomous), SNDT Women's University, Mumbai.

Dr. Vibha Hasija: Head of the Department, Associate Professor, Nirmal Niketan College of Home Science, Mumbai.

 Comment on the recommended increase in RDA from 800 mg to 1200 mg amounting to an additional 400 mg/day in women above the age of 50 years and an EAR of 1000 mg?

**Dr. Raman Marwah:** (a) No calcium balance studies have been done in this age group, (b) no clinical trials investigating the response of change in calcium balance or BMC/BMD or fracture rates have been undertaken to evaluate the requirement in postmenopausal women.

**Dr. Sanjay Kalra:** Calcium is an essential micronutrient that contributes to musculoskeletal and metabolic health. However, its need is most pronounced in postmenopausal women, who are at greater risk of osteoporosis.

*Dr. B. Sesikeran:* Menopause is accompanied by a sustained rise in obligatory urinary calcium of about 30 mg daily and decreased calcium absorption leading to a negative calcium balance. An additional allowance of 200 mg has been added to the EAR, and the RDA has been set at 1200 mg for postmenopausal women.<sup>[11]</sup>

*Dr. R. Hemalatha:* The calcium EAR for women of reproductive age (nonpregnant and nonlactating) is 800 mg/day. For the reasons explained below, an additional allowance of 200 mg/day is the recommended EAR for postmenopausal women, and accordingly, the RDA is adjusted to 1200 mg/day.<sup>[11]</sup>

**Prof. Anura Kurpad**: There is an interchanging of RDA and EAR here. It is critical to understand that the present EAR (the correct requirement value) is 800 mg/day, increasing to 1000 mg/day for postmenopausal women. This is a 200 mg increase — not 400 as suggested above. The RDA is not used much now-it is an intake

that should be monitored by a dietician regularly, as it is higher. Higher does not mean better. The average absorption of Ca is about 40%, which might be lower in postmenopausal women. There are some indications that this happens in later ages (75 years) - rather than immediately in 40–50-year-old postmenopausal women, but there is a relation between calcium absorption and Vitamin D status with aging. For safety, I would consider taking a much lower absorption-say half of the usual 40%; then 30 mg (loss) divided by 0.2 (absorption) gives 150 mg/day as required to balance this loss. For additional safety, as age increases the EAR for postmenopausal women is 1000mg/day.

**Dr. Jagmeet Madan:** My response to the question is from a nutritionist perspective and thus would emphasize a food-based approach. To get 1000 mg of calcium, which is the EAR for postmenopausal women, how many calcium-rich sources do the women need to consume daily and how practical are they? [Table 2].

Total as per table 2 = 300 + 50 + 170 + 250 + 180 = 950 mg of calcium per day.

The above is wishful thinking for a minimalistic idealistic intake scenario for all postmenopausal women. This is not going to be true for all women. In addition, only 40–50% of this amount (500 mg) may be available to the body keeping in mind the factors that inhibit calcium absorption, including phytates in cereals, nuts and oilseeds, and oxalates in vegetables. Thus, adding supplements of 300–500 mg/day of calcium seems mandatory with the present recommendations of the EAR. To my mind, the increased EAR may be justified keeping in mind the ideal physiological needs of calcium for postmenopausal women.

**Dr. Vibha Hasija:** Indian population sees a very high rate of Vitamin D deficiency leading to a compromise in bone health. Calcium adequacy must be handled to offset the bone loss and replenish the bone.

2. What is the rationale for increasing RDA at postmenopause and its effect on health outcomes?

*Dr. Raman Marwah:* The increase has been done because postmenopausal women have an obligatory extra loss of 30 mg/day of calcium and their absorption

Table 2: Calcium-rich foods	
Food	Calcium (mg)
250 ml of milk and curd	300 mg
50 g of pulses	50 g
15 g of til seeds	170 mg
15 g of green leafy vegetables	250 mg
50 g of millets especially ragi	180 mg

of calcium is also low, which leads to negative calcium balance. Therefore, based on the reference, it was logical to recommend an additional 200 mg proportionate to what is recommended by the WHO after menopause (1000 mg) to cover for at least an extra obligatory loss of 30 mg/day in the urine.<sup>[11]</sup> Studies on three socioeconomic groups at NIN show that even after 50 years, the extent of osteoporosis in the spine is only 16% in the high-income group with calcium intakes of 1000 mg compared to the low-income group with 65% osteoporosis with Ca intake of 400 mg/day. It was, therefore, logical to recommend an additional 200 mg proportionate to what is recommended by the WHO after menopause (1000 mg) to cover for at least an extra obligatory loss of 30 mg/day in the urine.<sup>[15]</sup>

*Dr. Sanjay Kalra:* Pan-India studies from the ICMR have demonstrated poor bone health in Indians with low calcium intake. This has led to an enhanced emphasis on ensuring adequate calcium intake of 800–1000 mg/day, through dietary sources such as milk, in all individuals. Postmenopausal women experience higher urinary calcium losses due to impaired calcium absorption. Therefore, it makes sense to increase the recommended daily allowance of calcium to 1200 mg, as advocated by the NIN.

Dr. B. Sesikeran: It may not be possible to reverse or stop the bone resorption but taking additional calcium through low-fat milk and Vitamin D supplements if the serum Vitamin D levels are below 30 ng/dl will delay the risk of BMD entering the fracture risk zone based on Dual-energy X-ray absorptiometry (DXA) measurement. In advancing age, better muscle mass and muscle strength through regular physical activity and making the environment conducive to lower fall risk and physical activity to improve coordination and balance are additional measures to lower fracture risk.

Dr. R. Hemalatha: Postmenopausal women have higher bone resorption than bone formation and an increased obligatory loss, resulting in bone loss with an increased bone turnover leading to osteoporosis and its associated complications. In addition, fractional calcium absorption decreases considerably after menopause, further decreasing with advancing age. In general, absorption of most nutrients, including calcium, is reduced with advancing age in the elderly, which necessitates the recommendation of higher RDAs. Higher calcium intake and Vitamin D and magnesium have improved BMD and have an overall beneficial effect on health outcomes.

**Prof. Anura Kurpad:** Certainly, the significant rationale is the prevention of bone loss, osteoporosis, and

fractures, which are very problematic at older ages. Indeed, a factor that also affects this is the bone density present before menopause sets in which depends on peak bone density accrued as a young adult. The key is to reduce the osteoclastic effects through additional calcium, which is better if there is adequate Vitamin D to assist in the absorption.

**Dr. Jagmeet Madan:** The rationale is documented to compensate for the physiological age-related bone loss, which is not supported by osteoblastic activity.

**Dr.** Vibha Hasija: Preserving bone health by maintaining nutrition in the postmenopausal woman, with declining estrogen levels is a good proactive policy. However, if we are looking at overall bone health for postmenopausal women, a single-point focus of only an increase in calcium may not be adequate.

3. What impact is the changed RDA has in clinical practice?

**Dr. Raman Marwah:** No one knows the impact of the changed RDA in clinical practice unless studies are undertaken.

**Dr. Sanjay Kalra:** The changed RDA will have a beneficial impact on clinical practice. It calls attention to menopause health and encourages efforts to promote the nutritional management of women living with menopause. Health-care professionals will provide adequate calcium intake as part of the comprehensive lifestyle and nutritional optimization management.

Dr. B. Sesikeran: The concern that higher calcium intake could increase the risk of stone formation in the kidneys is not borne out from the various studies. There may be a few stone formers to whom this may contribute but not in a large majority. Taking calcium supplements and foods with higher oxalates could lead to oxalate stones in the urinary tract, especially in stone formers. Dietary sources can achieve as much as 1000 mg. Supplements for postmenopausal may be unavoidable. In terms of prevention, ensuring that adolescent girls receive the required amount of calcium so that they would still have adequate calcium despite physiological losses when they are in the postmenopausal stage. Difficult to achieve a recommended intake does not justify lowering the recommended levels. We have to find ways of enhancing their milk and other sources of calcium intake.

**Dr. R. Hemalatha:** In clinical practice for elderly and postmenopausal women, higher calcium intake and Vitamin D and magnesium would benefit optimal bone health outcomes. It is preferable to derive the EAR of any nutrient to the extent possible from dietary sources, failing which supplementation may be necessary.

**Prof. Anura Kurpad:** Not much. A diverse diet with dairy, greens, ragi, legumes, and fruits is excellent if one can measure the Ca intake and get it to 1000 mg. If it is not possible (say someone does not like dairy), supplements should be considered, along with Vitamin D.

**Dr. Jagmeet Madan:** The increase in EAR puts more responsibility on the clinician and nutritionist to increase awareness of regular consumption of calcium-rich sources, sun exposure, and regular physical activity in postmenopausal women to decrease the regular dosage of calcium through supplements. Not necessarily adding supplements will translate into improved bone health as it is dependent on many factors.

**Dr. Vibha Hasija:** There will surely be a positive impact on clinical practice. There will start a greater supplement prescription because it is difficult to achieve very high levels of calcium in the diet.

#### DISCUSSION

Osteomalacia is a nutritional disorder with defective mineralization due to low calcium intake, low Vitamin D concentrations, and secondary hyperparathyroidism. Osteoporosis is a multifactorial disorder and maintains mineralization, unlike osteomalacia. Hence, increased calcium intake to overcome the effects of bone loss related to menopause or normal aging may not be adequate.

Indicators used to assess calcium requirements include balance studies, factorial estimates of requirements, or assessment of changes in BMD and bone mineral content. It is shown that developing countries subsist on a much lower calcium intake of about 500 mg without any ill effects. Long-term balance studies in such populations indicate that they are in positive calcium balance even on much lower intakes. This is because the body can adapt to different levels of calcium intake and maintain a positive calcium balance. As explained by Nordin, the paradox of low fracture rates associated with low calcium intakes in the developing world against the higher fracture rates in the developed world with higher calcium intake is due to the positive calcium balance maintained with low intakes. This is due to enhanced absorption with low calcium intakes and decreased absorption with higher dietary levels of both animal protein and sodium. It was estimated that a reduction in animal protein from 60 to 20 g or sodium from 150 to 50 mmol/day could decrease calcium requirement by about 200 mg/day; a combination of both could be additive, accounting for differences up to 400 mg.[18]

The RDA for calcium in Japan is lower than in most other industrialized countries, and Japanese women consume less calcium than those in the United States (400 mg vs. 650 mg) yet have a lower rate of hip fractures. [19] Two reviews on dietary calcium intake on BMD and fracture did not translate into clinically meaningful reductions in fractures. [20,21] A 2016 meta-analysis from the National Osteoporosis Foundation showed a significant reduction in total fractures with calcium plus Vitamin D supplementation and concluded that the use of calcium plus Vitamin D supplements to reduce fracture risk was supported in both community-dwelling and institutionalized older adults. [22]

## **CONCLUSIONS**

Based on the evidence, it is prudent to follow the ICMR 2010 recommendation of RDA of 800 mg until new Indian data emerges. The positive impact of increasing calcium and Vitamin D intake is most evident in calcium-deficient individuals. Indian data on bone health and calcium intake studies show the need to be vigilant for deficiency or inadequate intake. The increase in RDA to 1200 mg and the reality of low dietary calcium intake in India may lead to enhanced use of supplementary calcium to reach the EAR in healthy women, as explained by the experts.

## **Acknowledgment**

Thanks to Dr. Sunila Khandelwal and Dr. Sonia Malik for reviewing the article.

## Financial support and sponsorship

Nil

## **Conflicts of interest**

There are no conflicts of interest.

## REFERENCES

- Abrams SA, Sidbury JB, Muenzer J, Esteban NV, Vieira NE, Yergey AL. Stable isotopic measurement of endogenous fecal calcium excretion in children. J Pediatr Gastroenterol Nutr 1991;12:469-73.
- Weaver C, Heaney R. In: Weaver C, Heaney R, editors. Chapter 9: Food Sources, Supplements, and Bioavailability, Calcium in Human Health. Totowa, NJ: Humana Press; 2006.
- Skully R. Essential nutrient requirements of the elderly. Nutr Diet Suppl 2014;6:59-68.
- Fogelholm M, Nordic Nutrition Recommendations 2012 Integrating nutrition and physical activity, 5<sup>th</sup> edition, Nord, ISSN 0903-7004;2014:002 Copenhagen: Nordisk Ministerråd, 2014, 5. Available form: http://dx.doi.org/10.6027/Nord2014-002. [Last accessed on 2022 Feb 01].
- Stookey JD, Wang Y, Ge K, Lin H, Popkin BM. Measuring diet quality in china: The INFH-UNC-CH diet quality index. Eur J Clin Nutr 2000:54:811-21.
- Kim KM, Choi HS, Choi MJ, Chung HY. Calcium and vitamin D supplementations: 2015 position statement of the Korean Society for Bone and Mineral Research. J Bone Metab 2015;22:143-9.
- US Preventive Services Task Force (2013) Vitamin D and Calcium Supplementation to Prevent Fractures. Available

- from: http://www.uspreventiveservicestaskforce.org/uspstf/uspsvitd.htm. [Last accessed on 2022 Jan 22].
- Nutrient Requirements for Indians and Recommended Dietary Allowances and Estimated Average Requirement. A Report of the Expert Group. Indian Council of Medical Research; 2020.
- Indian Council of Medical Research: Nutrient Requirements and Recommended Dietary Allowances for Indians, A Report of the Expert Group of the. Indian Council of Medical Research; 2010.
- Meeta M, Digumarti L, Agarwal N, Vaze N, Shah R, Malik S. Clinical practice guidelines on menopause: \*An executive summary and recommendations: Indian Menopause Society 2019-2020. J Midlife Health 2020;11:55-95.
- Joint FAO/WHO. Vitamin and Mineral Requirements in Human Nutrition. A Report of the Joint FAO/WHO Expert Consultation. 2<sup>nd</sup> ed. Bangkok, Thailand: Joint FAO/WHO; 1998, 2004.
- Balk EM, Adam GP, Langberg VN, Earley A, Clark P, Ebeling PR, et al. Global dietary calcium intake among adults: A systematic review. Osteoporos Int 2017;28:3315-24.
- Ahuja MM, Mohanan P. Calcium phosphorus and nitrogen balance studies in renal calculus disease. Indian J Med Res 1970;58:444-55.
- Tandon GS, Teotia SP, Yadav SC, Garg SK. A study of balance in normal individuals and postmenopausal state. Indian J Med Assoc 1973;61:214-17.
- 15. Indian Council of Medical Research. Population Based Reference

- Standards of Peak Bone Mineral Density of Indian Males and Females-An ICMR Multicenter Task Force Study. New Delhi: Indian Council of Medical Research; 2010.
- Teotia SP, Teotia M. Nutritional bone disease: The continuing challenge to neonatal bone health. Postgrad Med 2009;23:30-8.
- Shatrugna V, Kulkarni B, Kumar PA, Rani KU, Balakrishna N. Bone status of Indian women from a low-income group and its relationship to the nutritional status. Osteoporos Int 2005:16:1827-35.
- Nordin BC. Calcium requirement is a sliding scale. Am J Clin Nutr 2000;71:1381-3.
- Fujita T, Fukase M. Comparison of osteoporosis and calcium intake between Japan and the United States. Proc Soc Exp Biol Med 1992;200:149-52.
- Bolland MJ, Leung W, Tai V, Bastin S, Gamble GD, Grey A, et al. Calcium intake and risk of fracture: Systematic review. BMJ 2015;351:h4580.
- Tai V, Leung W, Grey A, Reid IR, Bolland MJ. Calcium intake and bone mineral density: Systematic review and meta-analysis. BMJ 2015;351:h4183.
- Weaver CM, Alexander DD, Boushey CJ, Dawson-Hughes B, Lappe JM, LeBoff MS, et al. Calcium plus vitamin D supplementation and risk of fractures: An updated meta-analysis from the National Osteoporosis Foundation. Osteoporos Int 2016;27:367-76.