

# Evaluation of a food frequency questionnaire for the assessment of dietary nitrate intake

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## Introduction

The discovery of nitric oxide (NO) and its catabolism to nitrate has shed some light on the complex metabolism of nitrate in humans. Nitrate is present in food in different concentrations and examples of food with high nitrate content are beetroot, lettuce, rocket or cabbage (1). However, nitrate content in these foods can be variable and influenced by factors such as seasonality, fertilizers, contamination, water supply (2). This is one of the main reasons for lack of valid tools to assess nitrate intake in humans. Two studies have attempted to validate a food frequency questionnaire (FFQ) in 1989 (3) and 2009 (4). The studies showed a modest correlation between the FFQ and urinary nitrate ( $r=0.19$ ) (3).

## Objectives

This study attempted to overcome this gap in research by developing a new simple FFQ to estimate nitrate intake in humans. We used data collected in three different studies and correlated nitrate intake with urinary nitrate concentrations.

## Methods

The results from three different human studies were combined to evaluate the association between nitrate intake and urinary nitrate concentrations. All studies were approved by local ethics committees.

A semi-structured FFQ was used to estimate nitrate intake. The FFQ contained 16 questions focused on the frequency and amount of consumption of food with high and moderate nitrate concentrations. A fixed amount of 14mg/day was attributed to water intake due to the large variability in water consumption and nitrate content.

Nitrate concentrations in urine were measured by Griess colorimetry assays (Cayman Chemicals, UK) (5).

Data were analysed using SPSS 21 for Windows. Mean and SD were used to describe the data. Pearson's correlation analysis was used to evaluate the association between nitrate food content and urinary nitrate concentrations. In addition, we evaluated the association with factors such as age, BMI and blood pressure (BP).

## Results

Table 1: Characteristics of study population

	N	Mean	SD	Min	Max
Age (years)	137	62.1	5.5	49.0	71.0
BMI (kg/m <sup>2</sup> )	137	28.6	4.5	19.8	42.9
Systolic BP (mmHg)	137	131.6	17.6	96.7	212.7
Diastolic BP (mmHg)	137	75.6	9.5	50.0	101.0
Dietary Nitrate Intake (mg/day)	137	259.9	146.1	21.2	749.9
Urinary Nitrate (mmol/L)	137	0.7	0.6	-0.003	3.1

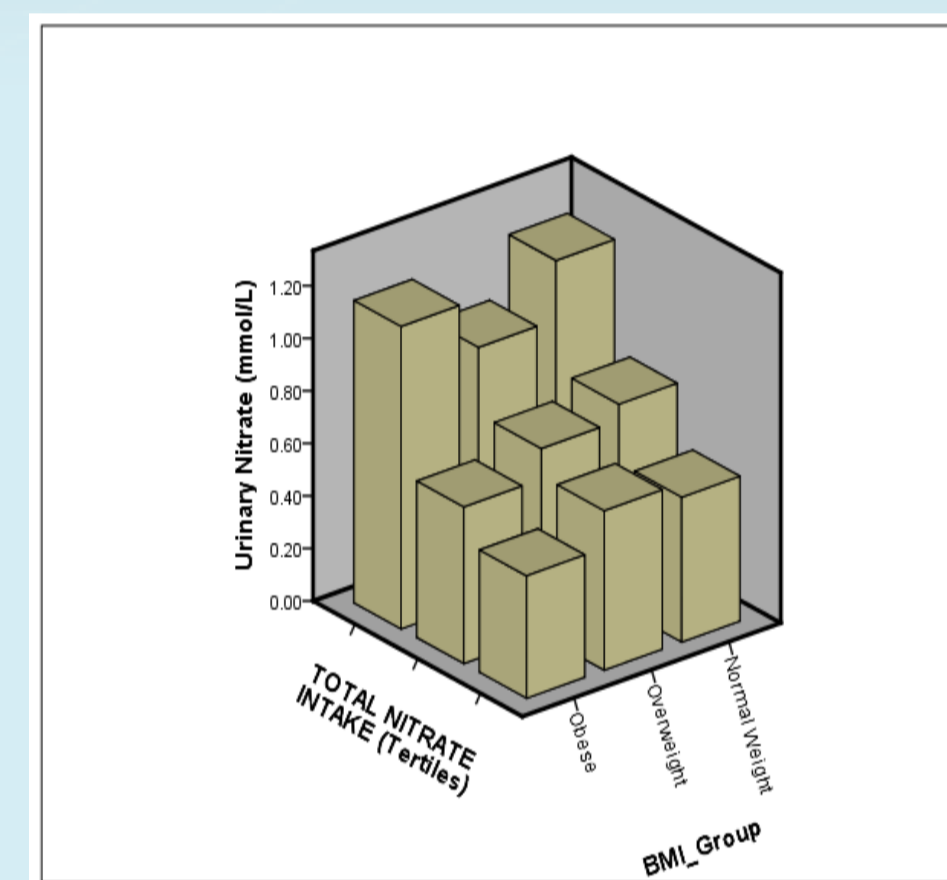
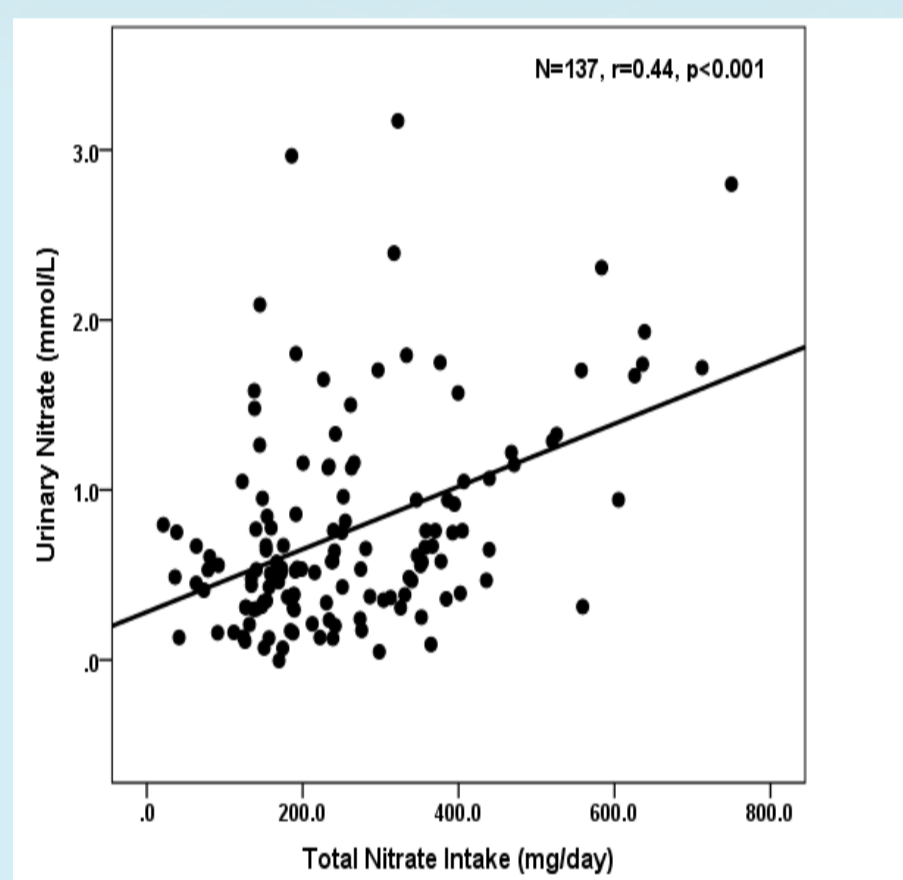


Figure 1(A) Correlation between nitrate intake and urinary nitrate (left). Figure 1(B) Evaluation of the influence of BMI on the association between nitrate intake and urinary nitrate concentrations (right)

Table 2: Correlation Analysis (\* $p<0.05$ ; \*\* $p<0.01$ )

	Age (years)	BMI (kg/m <sup>2</sup> )	Systolic BP (mmHg)	Diastolic BP (mmHg)	Nitrate Intake (mg/day)	Urinary Nitrate (mmol/L)
Age	1	-0.20*	0.26**	-0.04	-0.04	0.12
BMI	-0.20*	1	0.24**	0.29**	0.06	-0.009
Systolic BP	0.26**	0.24**	1	0.58**	-0.003	-0.06
Diastolic BP	-0.04	0.29**	0.58**	1	-0.07	-0.06
Nitrate Intake	-0.04	0.06	-0.003	-0.07	1	0.44**
Urinary Nitrate	0.12	-0.009	-0.067	-0.06	0.44**	1

The characteristics of the population are described in Table 1. 137 participants were included in the analysis. Average BMI and age of the population was 28.6 kg/m<sup>2</sup> and 62.1 years. A significant association was found between nitrate intake and urinary concentration ( $r=0.44$ ,  $p<0.001$ ). It also appears that BMI may influence the association between these two variables (Figure 1B). Further, the correlation analysis in (Table 2) shows a general lack of association between nitrate intake and urinary concentrations with age, BMI and BP.

## Discussion and Conclusion

Our results show a significant association between nitrate intake and urinary nitrate concentrations. Therefore the FFQ could be a useful and simple tool for estimating nitrate intake in humans and evaluating compliance to human interventions. It could potentially be applied in epidemiological studies to evaluate the association between nitrate intake and disease risk.

Main limitations of the study are the cross-sectional study design, the relatively small sample size and the reduced representativeness of the sample of the general population.

Future studies are therefore needed to further validate the FFQ in larger, more representative populations. If confirmed, we believe that this tool will be of great help and support to scientists and nutritionists working in the field of nitrate and nitric oxide biology.

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