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Research Article

Association between Biomass Fuel for Cooking and Serum Nitric Oxide among Women in Karachi, Pakistan

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Abstract

Objective: The study determines the association between biomass fuel use and serum nitric oxide (NO) levels among women in villages of Gadap town, Karachi, Pakistan.

Materials and methods: A cross sectional study was conducted in Gadap town, Karachi, Pakistan. A total of 83 women between 15-45 years of age were recruited with 43 biomass users and 40 using natural gas as primary cooking fuel. Particulate matter ($PM_{2.5}$) and Carbon monoxide (CO) was measured in kitchens using an aerosol monitor *Sidepak* and *Monoxor II*, respectively. Serum Nitric Oxide (NO) was measured through Griess reaction. Multivariate regression was conducted to determine the association between serum NO levels and biomass.

Results: CO and $PM_{2.5}$ levels in kitchens among biomass [CO: 20.22 (±12.2), $PM_{2.5}$:4.46 (±3.6)] users were significantly higher compared to natural gas users [CO: 1.22 (± 1.22), $PM_{2.5}$:0.05 (± 0.02)]. Blood serum NO levels among women were significantly ($p<0.001$) higher in biomass users (280.8±25 μmol/L) compared to natural gas users (230.4±10 μmol/L). Multivariate regression analysis found unit change in biomass use associated with 0.25 unit ($p<0.02$) change in NO levels, after adjusting for age and socioeconomic status.

Conclusion: Women who are using biomass as fuel are exposed to high levels of CO and $PM_{2.5}$ pollutants. High NO levels in biomass users may indicate enhanced *in vivo* inflammatory responses. Further studies are needed to identify the role of serum NO levels in causing respiratory and cardiovascular diseases among biomass users.

Introduction

Increase in population and limited access to clean energy resources has compelled almost half of developing nations to rely on solid fuels [1]. Solid fuels are economical and conveniently available; however, their constant use is found to be associated with various adverse health effects [1]. Indoor air pollution (IAP) mainly due to solid fuels, like wood, coal, cow dung etc, contribute approximately 1.6 million deaths annually[1].Smoke released from the burning of biomass fuels include carbon monoxide (CO), nitric oxides (NOx), sulphur oxides (SOx), particulate matter (PM), formaldehyde and polycyclic hydrocarbons. Among these, CO and $PM_{2.5}$, are the most commonly studied pollutants showing association with chronic obstructive lung diseases (COPD), acute lower respiratory infections, lung cancers, tuberculosis, blindness, cardiac diseases and interstitial lung diseases and adverse pregnancy outcomes [2-3].

Systematic review by Naeher et al [2] found chronic exposure to biomass leads to worsening of localized and systemic inflammatory effects. Experimental animal studies demonstrated various inflammatory changes secondary to biomass exposure: like damage to respiratory epithelial lining, altered pulmonary morphology, increased number of alveolar macrophages and oxidative stress, increased cytokine influx and airway hyper reactivity, altered blood indices level, metaplasia and DNA damage [4,5].

Studies have found significant increase in proinflammatory and inflammatory markers in biomass users [6-8]. Ghio et al. exposed volunteer human subjects to prolonged wood smoke (>20 hrs) in a controlled environment. Thebronchoalveolar lavage of volunteers showed neutrophilic influx suggesting pulmonary inflammatory response [9]. Among different inflammatory markers, cytokines and nitric oxide have received special attention by environmental scientists [6-8,10]. It may be because serum NO plays an important role in many biological processes in the body and its levels are affected in various diseases like respiratory, cardiovascular and inflammatory [10,11]. Banerjee et al found serum nitric oxide levels almost twice as higher in rural Indian women using biomass as compared to gas users [8].

Several studies have determined detrimental effects of biomass exposure though experiments involving animals or *in vitro/in-vivo methods* [2,4];however few studies are available on human subjects with simultaneous measurements, both in the environment and the body [6-8]. An estimated 87% of rural and 62% of overall households in Pakistan, rely on biomass fuel for their daily household energy requirement hence exposing women to increased health risk [1]. The natural experiment of getting exposed to high levels of biomass in a real life situation, especially during winters is commonly available in the sub-urban and rural areas of a developing country like Pakistan. This study explored the association between biomass and serum NO levels in women residing in villages of sub-urban Gadap town, Karachi.

Materials and Methods

The cross sectional study was conducted during February, 2012 in semi-urban community of Gadap town of Karachi. Gadaptown is located in the north-west of Karachi and according to 2002census has population of 289,732 [https://www.google.com/fusiontables/DataSource]. The town is divided into eight union councils, each having several villages. Almost half of Gadap town lacks access to basic facilities like drinking water and gas. The area comprises small villages with mud houses and brick made streets.

Two separate groups of women were invited to participate, the first group; Biomass users (n=42, who were using wood, cow dung, crop residues or coal (locally purchased or collected) as cooking fuel for atleast the last five years) and the second group Current gas users (n=41, who were using gas as cooking fuel for at least the last 5 years). The biomass users were selected consecutively from six villages of Gadap town and natural gas users were selected from the nearest adjacent village with availability of gas as cooking fuel. A team of two local residents was formed to identify the households in the village according to the inclusion criteria. Only those households that had a properly constructed (with four walls and roof) external kitchen (not linked internally with rooms), were selected for this study. One woman, doing the major cooking (the primary cook) for the family, was selected from each house. Females who were suffering from high grade fever, physician diagnosed infectious disease (hepatitis, typhoid, tuberculosis) or chronic disease (diabetes and hypertension), or were pregnant, were current smokers, were not included in the study. The study was approved by Aga Khan University Ethics Review Committee (Human Subject Assurance Number: FWA00001177). Due to cultural reasons, signed informed consent was taken from the woman and her husband (elder of the house in case husband was away) before starting the interviews/measurements (thumb impression was taken in case of illiterate woman). The data collectors first explained to the women about the procedure of the study which included structured interviews, anthropometric measurements, and blood sample collection and kitchen air measurements. Participation was on voluntarily basis and there were no refusals. All participants were assigned a unique identifier number (questionnaire and the blood samples) in order to maintain confidentiality.

A team of two health persons, one male environmental scientist and one female phlebotomist approached each selected house. Male data collector was responsible for recording $PM_{2.5}$ and CO measurements, and female phlebotomist conducted the interview, took anthropometric measurements and blood samples. All interviews were conducted in Sindhi language, which is locally understood and spoken. Detailed interviews comprised of socio-demographic variables (age, education, occupation, number of family members, income, source of water supply); kitchen information(duration of kitchen use, type of kitchen, type of stove, presence of chimney, number of windows and doors and time spent in cooking); smoking history (current smoker, passive smoker, other burning sources such as burning of garbage or incense inside the house); medical history related to allergic symptoms (coughing, sneezing, wheezing, redness of eyes, watery eyes or sticky eyes). Anthropometric measurements included weight and height. Weight was measured by using the electronic weighing scale whereas height was measured using measuring tape according to the standard procedure.

Trained phlebotomist collected and stored 3ml blood sample from each woman using aseptic venipuncture technique. Whole blood samples were transported to laboratory in cold boxes within 3 hours of collection. Blood was centrifuged at 800g for 10 minutes to separate serum, which was subsequently collected, aliquoted and stored at -70^{0}C until used for NO level analysis.

Exposure to indoor air pollution was determined by measuring fine particulate matter ($PM_{2.5}$) and carbon monoxide (CO). Women were asked to do cooking in a manner similar to their daily routine. All measurements were taken exactly five minutes after the fire was lit to its maximum (mentioned according to participant) and by standing at a distance of 1 meter from the stove. Real-time measurement of particulate matter of <2.5micron aerodynamic diameter ($PM_{2.5}$) was made using a handheld laser operated monitor (Sidepak-Model AM510, TSI Inc., MN, USA), a laser guided photometer with an inbuilt 2.5 micro impactor. Prior to each measurement, the device was zero-calibrated with a HEPA filter according to manufacturer's specifications and air flow rate was set to 1.7 L/min. CO level was measured using portable CO Analyzer (Bacharach, Monoxor-II USA). CO monitor had the ability to measure between 0 and 2,000 parts per million (ppm) with an accuracy of ±2 ppm in low CO concentrations. Spot monitoring for $PM_{2.5}$ and CO measurement were done.

Nitric Oxide Assay Kit Thermo (R&D Systems, UK) was used and total nitrate (Nitrite and Nitrate) was estimated using Griess Reagent I and Griess Reagent II followed by measurement at dual wavelength (540 nm/ 690 nm). For the Nitrate Reduction assay, NADH and Nitrate Reductase were added to all wells prior to addition of Griess reagents and subsequent reading at dual wavelength was done. Range of detection for Nitrite was 3.125µM -200µM and for Nitrate 3.125µM -100µM (Sensitivity of assay: 0.222µM).

Data were analyzed using SPSS computer statistical software package (IBMSPSS statistics version 21).The continuous variables were calculated as mean (±SD), whereas, categorical were measured as percentages. Serum NO levels, and air pollutants $PM_{2.5}$ and CO were analyzed as continuous variable. Comparison between two groups was done using student's t-test for independent samples with the significance level at<0.05. Correlation coefficient was estimated for $PM_{2.5}$ and CO levels in the kitchen during cooking and serum NO levels. Multivariate linear regression was performed to determine the association between serum NO and type of fuel used, after adjusting for potential confounders. Model fit was used assessed using the R square value.

Results

The mean age of participants was 25.4 (±7.5) years, and no significant difference in age was observed between the two groups.

Similarly, no statistically significant difference was found for education, marital status, number of children and income between the two groups. Question on the type of biomass used found that almost all used dry wood as primary cooking fuel except for 2 participants who were using cow dung as primary and wood as secondary fuel. Around 40% of biomass users had no window in the kitchen as compared to 33% gas users (p=0.29). Among those who had windows, majority of them (93% gas users and 75% biomass users) kept their windows and doors open while cooking (p=0.09). Around 50% of biomass users and 32% of gas users mentioned burning incense inside their homes. More women (28%) in biomass group were passive smokers as compared to natural gas users (12%). Allergic symptoms like watery eyes were more frequent among natural gas users whereas upper respiratory symptoms although not statistically significant were more frequent among biomass users (Table I).

Table 1: Comparison of socio-demographic characteristics of women using biomass or gas as cooking fuel in Gadap town, Karachi.

Ari	Biomass users (n=42) (%)	Gas users (n=41) (%)	p value
Age (years)			
(Mean+/- SD)	25.4 (±8.5)	23.7 (±6.9)	0.31
Educational status			
Intermediate*	1 (2.4)	1 (2.4)	0.98
Secondary	12 (28.6)	12 (29.3)	
Primary	14 (33.3)	12 (29.3)	
Illiterate	15 (35.7)	16 (39.0)	
Marital status			
Single**	20 (47.6)	20 (48.8)	0.92
Married	22 (52.4)	21 (51.2)	
BMI			
(mean +/- SD)	21.1 (±3.5)	20.4 (±3.7)	0.44
Passive smoker			
No	30 (71.4)	36 (87.8)	
Yes	12 (28.6)	5 (12.2)	0.05
Drinking water supply			
Tap water	30 (71.4)	13 (31.7)	
Well water	12 (28.6)	28 (68.3)	<0.01
Animals in the household			
No	5 (11.9)	7 (17.1)	
Yes	37 (88.1)	34 (82.9)	0.54
Monthly income (PKR)			
>10 000	17 (40.5)	18 (43.9)	0.98
6000-10 000	16 (38.1)	14 (34.1)	
<6000	9 (21.4)	9 (22.0)	
Eye & and respiratory symptoms			
Itching of eyes during cooking			
No			
Yes	27 (63.4)	27 (65.8)	0.88
	15 (36.6)	14 (34.1)	
Redness of eyes during cooking			
No			
Yes	21(51.2)	23(54.8)	0.72
	20(48.8)	19(45.2)	
Tears while cooking			
No	16(36.8)	23 (54.7)	0.71
Yes	26(63.2)	19 (45.2)	
Sneezing			
No	29 (69)	31 (75.6)	0.5
Yes	13 (31)	10 (24.4)	
Sore throat			
No	24 (57.1)	27 (65.9)	0.36
Yes	18 (42.9)	14 (34.1)	
Cough			
No	32(78)	32 (78)	0.16
Yes	9 (22)	9 (22)	
Hoarseness			
No	30 (71.4)	32 (78.0)	0.25
Yes	12 (28.6)	9 (22.0)	

Table II: Univariate linear regression analysis between NO levels (μgm/dl) and sociodemographic, allergic and type of fuel used by women in Gadap town, Karachi, Sindh (n=83).

Variables	B	Beta	95% CI
Biomass use	43.2	0.32	(16.9, 69.3)
Literacy level	36.5	0.42	(20.0, 53.0)
Body Mass Index (BMI)	21.9	-0.2	(-0.10, 43.3)
Passive smoking	16.4	-0.08	(57.2, -24.4)
Age of respondent (in years)	3.4	0.29	(1.1, 5.8)
Household income level	-0.002	-0.35	(-0.004,-0.001)

Table III: Multivariate linear regression analysis between NO levels and type of fuel used by women in Gadap town, Karachi, Sindh (n=83).

Variables	B	Adjusted Beta	95% CI
Biomass use	35.03	0.25	(3.4, 66.7)
Total household income	-0.001	-0.13	(-.003, 0.001)
Age of respondent (years)	0.35	0.03	(-1.9, 2.6)

The mean CO levels inside the biomass and natural gas users kitchen was 24.9 (±15.0)mg/m^3 and5.50(±4.4) mg/m^3, respectively. The mean $PM_{2.5}$ levels inside the biomass and natural gas userskitchen was [1.22 ppm (±0.8) and 0.05 ppm (±0.02)] respectively. There was significant correlation between CO and $PM_{2.5}$ levels (correlation coefficient r=0.73, p<0.01). Mean serum NO levels were higher among biomass users (280.8 ±10 μmol/L) as compared to natural gas users (230±10μmol/L).

Univariate analysis found biomass use (0.32, 95%CI 16.9, 69.3), literacy level (0.42, 95%CI 20, 53), age in years (0.29, 95%CI 1.1, 5.8) and income level (-0.35, 95% CI -0.004, -0.00) were significantly associated with serum NO levels (Table II). Multivariate linear regression analysis found significant association between serum NO levels and biomass use after adjusting for income level and age; biomass use led to 0.25 times increase in serum NO levels compared to natural gas users(0.25, 95%CI 3.4, 66.7 μmol/L) (Table III). The adjusted R^2 for the model was 0.28, explaining 28% variance in the serum NO levels due to biomass use.

Discussion

Similar to previous studies, high mean levels of CO and $PM_{2.5}$ levels were found in kitchens of biomass users during peak cooking hours. These values were consistent with studies conducted by Siddiqui et al (8 hr mean CO: 29.4 ppm, 8 hr mean $PM_{2.5}$: 2.74 mg/m^3) [12-14] (24 hr mean $PM_{2.5}$ 1169 μg/m^3). Immediate exposure to these pollutants may cause watery and red eyes and upper respiratory symptoms; however, no significant difference was noted in the number of women belonging to both groups.

Our study demonstrated high levels of Nitric oxide among the biomass users. Nitric oxide was first discovered as a biologically active molecule in the late 1980s. It is produced from L-arginine in the body at numerous sites such as the vascular endothelial cells, and plays an important role in neurotransmission and in relaxation of vascular smooth cells [15,16]. However, high levels of NO, referred as inducible or iNOS is expressed only under the influence of certain substances such as cytokines, and/or endotoxins that are released during inflammatory and infectious processes resulting in a sustained production (hours to days) of NO [8]. This inducible NO acts on the non specific host defense mechanism and its raised levels have been observed in a number of conditions including sepsis, asthma, rheumatoid arthritis, tuberculosis, inflammatory bowel disease, Alzheimer disease, multiple sclerosis, etc. [1-2,17,18]. The inorganic free radical (NO) act as an important mediator in a number of physiological as well as pathological processes. In our study, initiation of inflammation secondary to biomass use is one of the major proposed mechanisms leading to adverse health outcomes [6-8,17].

It is proposed that increased serum NO levels affects the activation of macrophages cytokine production by host cells via release of P-select in expressing platelets or release of reactive oxygen species (ROS) [18-20]. Review on air pollution from India and Pakistan found association between indoor air pollutants and cardio-vascular diseases [21]. Study from rural Pakistan found women using biomass for cooking purpose are 4.8 times (95% CI 1.5 to 14.8) at risk of developing acute coronary syndrome [22]. The constant and regular exposure to biomass resulted in very high serum NO levels, which were significantly higher as compared to Iranian population (mean value Iranian population 22.7 ± 0.02 μmol/L) [23]. These levels even exceeded those suffering from chronic inflammatory disease like diabetes mellitus (166.8 ± 3.2 μmol/L) [24], suggesting that biomass exposure may lead to severely poor health status, that can differ based on the duration of exposure. Trials focusing on improved stoves with less exposure to biomass smoke have shown beneficial health effects [25].

A natural experiment in which women are exposed to high biomass levels in a real life situation is the main strength of our study. Both exposure to biomass and outcome as serum NO levels were measured using validated methods. CO and $PM_{2.5}$ were measured using equipment which has been tested and used by prior studies conducted within Pakistan and in South Asian region [26,27]. Similarly, serum NO levels were measured via Griess method that gives estimates close to accurate levels.

This study had some limitations. It was a cross sectional study hence difficult to establish causality between biomass use and serum NO levels. Women who were exposed to biomass for last five years were included; hence it is difficult to comment if any other type of fuel was used prior to five years or if there was any contribution due to neighborhood burning of biomass. More than one time measurements of indoor pollutants, NO and other cytokines (Interleukins-6, Tumour necrosis factor) would have strengthened the findings. The results demonstrated high levels of NO, which may be due to the difference in the detection kit method (Banarjee, utilized the ELISA method, whereas we used Griess reaction method).

High indoor air pollutants secondary to biomass is a well-established finding, however, detection of high serum NO levels among biomass users invite large scale follow up studies to explore underlying pathophysiological mechanism responsible for causing adverse health effects in humans.

Conclusion

Women who are using biomass as fuel are exposed to high levels of CO and $PM_{2.5}$ pollutants. Immune markers, like high serum NO levels in biomass users may indicate enhanced *in vivo* inflammatory responses. Further studies are needed to identify the role of serum NO levels in causing respiratory and cardiovascular diseases among biomass users.

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References

1. Fatmi Z, Rahman A, Kazi A, Kadir MM, Sathiakumar N. Situational Analysis of Household Energy and Biomass Use and Associated Health Burden of Indoor Air Pollution and Mitigation Efforts in Pakistan. Int J Environ Res Public Health 2010; 7: 2940-2952.
2. Naeher LP, Brauer M, Lipsett M, Zelikoff JT, Simpson CD, Koenig JQ, et al. Wood smoke health effects: A Review. Inhal Toxicol 2007; 19: 67-106.
3. Kim KH, Jahan SA, Kabir E. A review of diseases associated with household air pollution due to the use of biomass fuels. J Hazard Mater 2011; 192: 425-431.
4. Migliaccio TC, Mauderly JL. Biomass smoke exposures: toxicology and animal study design. Inhal Toxicol 2009; 22: 104-107.
5. Tesfaigzi Y, Singh SP, Foster JE, Kubatko J, Barr EB, Fine PM, et al. Health effects of subchronic exposure to low levels of wood smoke in rats. Toxicol Sci 2002; 65: 115-125.
6. Ray MR, Mukherjee S, Roychoudhury S, Bhattacharya P, Banerjee M, Siddique S, et al. Platelet activation, upregulation of CD11b/ CD18 expression on leukocytes and increase in circulating leukocyte-platelet aggregates in Indian women chronically exposed to biomass smoke. Hum Exp Toxicol 2006; 25: 627-635.
7. Mondal NK, Roy A, Mukherjee B, Das D, Ray MR. Indoor Air Pollution from Biomass Burning Activates Akt in Airway Cells and Peripheral Blood Lymphocytes. A Study among Premenopausal Women in Rural India. Toxicol Pathol 2010; 38: 1085-1098.
8. Banerjee A, Mondal NK, Das D, Ray MR. Neutrophilic inflammatory response and oxidative stress in premenopausal women chronically exposed to indoor air pollution from biomass burning. Inflammation 2012; 35: 671-683.
9. Ghio AJ, Soukup JM, Case M, Dailey LA, Richards J, Berntsen J, et al. Exposure to wood smoke particles produces inflammation in healthy volunteers. Occup Environ Med 2011; 69: 170-175.
10. Zelikoff JT, Chen LC, Cohen MD, Schlesinger RB. The toxicology of inhaled woodsmoke. J Toxicol Environ Health B Crit Rev 2002; 5: 269-282.
11. Barregard L, Sällsten G, Gustafson P, Andersson L, Johansson L, Basu S, et al. Experimental exposure to wood-smoke particles in healthy humans: effects on markers of inflammation, coagulation, and lipid peroxidation. Inhal Toxicol 2006; 18: 845-853.
12. Population of Gadap Town. Retrieved on 30th August 2016.
13. Siddiqui AR, Lee K, Bennett D, Yang X, Brown KH, Bhutta ZA, et al. Indoor carbon monoxide and PM2.5 concentrations by cooking fuels in Pakistan. Indoor Air 2009; 19: 75-82.
14. Colbeck I, Nasir ZA, Ali Z. The state of indoor air quality in Pakistan--a review. Environ Sci Pollut Res Int 2010; 17: 1187-1196.
15. Moshage H. Nitric oxide determinations: much ado about NO-thing? Clin Chem 1997; 43: 553-556.
16. Akdeniz N, Esrefoglu M, Keles MS, Karakuzu A, Atasoy M. Serum Interleukin-2, Interleukin-6, Tumour Necrosis Factor-alpha and Nitric Oxide levelsin Patients with Behcet's Disease. Ann Acad Med Singapore 2004; 33: 596-599.
17. Rückerl R, Greven S, Ljungman P, Aalto P, Antoniades C, Bellander T, et al. Air pollution and inflammation (interleukin-6, C-reactive protein, fibrinogen) in myocardial infarction survivors. Environ Health Perspect 2007; 115: 1072-1080.
18. Dutta A, Mukherjee B, Das D, Banerjee A, Ray RM. Hypertension with elevated levels of oxidized low density lipoprotein and anticardiolipin antibody in the circulation of premenopausal Indian women chronically exposed to biomass smoke during cooking. Indoor Air 2011; 21: 165-176.
19. Codoñer-Franch P, Tavárez-Alonso S, Murria-Estal R, Megías-Vericat J, Tortajada-Girbés M, Alonso-Iglesias E. Nitric oxide production is increased in severely obese children and related to markers of oxidative stress and inflammation. Atherosclerosis 2011; 215: 475-480.
20. Dutta A, Ray MR, Banerjee A. Systemic inflammatory changes and increased oxidative stress in rural Indian women cooking with biomass fuels. Toxicol Appl Pharmacol 2012; 261: 255-262.
21. Yamamoto SS, Phalkey R, Malik AA. A systematic review of air pollution as a risk factor for cardiovascular disease in South Asia: Limited evidence from India and Pakistan. Int J Hyg Environ Health 2014; 217: 133-144.
22. Fatmi Z, Coggon D, Kazi A, Naeem I, Kadir MM, Sathiakumar N. Solid fuel use is a major risk factor for acute coronary syndromes among rural women: a matched case control study. Public Health 2014; 128: 77-82.
23. Ghasemi A, Zahedi Asl S, Mehrabi Y, Saadat N, Azizi F. Serum nitric oxide metabolite levels in a general healthy population: Relation to sex and age. Life Sci 2008; 83: 326-331.
24. Pereira FO, Frode TS, Medeiros YS. Evaluation of Tumour Necrosis Factor Alpha, Interleukin-2 Soluble Receptor, Nitric Oxide Metabolites, and Lipids as Inflammatory Markers in Type 2 Diabetes Mellitus. Mediators Inflamm. 2006; 2006: 39062.
25. McCracken JP, Wellenius GA, Bloomfield GS, Brook RD, Tolunay HE, Dockery DW, et al. Household Air Pollution from Solid Fuel Use. Evidence for links to CVD. Glob Heart 2012; 7: 223-234.
26. Nafees AA, Taj T, Kadir MM, Fatmi Z, Lee K, Sathiakumar N. Indoor air pollution (PM2.5) due to secondhand smoke in selected hospitality and entertainment venues of Karachi, Pakistan. Tob Control 2012; 21: 460-464.
27. Sabzwari SR, Fatmi Z. Comparison of exhaled carbon monoxide levels among commuters and roadside vendors in an urban and a suburban population in Pakistan. Environ Monit Assess 2011; 180: 399-408.