Yeast-derived beta 1,3/1,6 glucan, upper respiratory tract infection and innate immunity in older adults

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Key words: beta 1,3/1,6 glucan; upper respiratory tract infection; elderly; immune function; innate immunity

Abbrerviations used: BMI, body mass index; GCSF, granulocyte colony stimulating factor; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; MCP, monocyte chemoattractant protein; MIG, monocyte induced by interferon-gamma; MIP, macrophage inflammatory protein, SIgA, secretory immunoglobulin A; TNF, tumor necrosis factor; URTI, upper respiratory tract infection; WURSS, Wisconsin Upper Respiratory Tract Severity Score

ABSTRACT

Objective: This study aimed to test whether yeast-derived beta 1,3/1,6 glucan can prevent the occurrence or reduce the severity of upper respiratory tract infection (URTI) and modulate innate immune responses during winter months in community dwelling older adults.

Methods: This was a double-blind placebo-controlled trial of community-dwelling adults aged 50 to 70 years randomised to once daily beta 1,3/1,6 glucan (Wellmune 250 mg daily; n=50) or identical placebo capsule (n=50) over 90 days during winter. URTI episodes were medically-confirmed. Symptom severity was recorded via self-reported daily Wisconsin Upper Respiratory Tract Infection Score 21. Blood and saliva samples were collected at days 0, 45 and 90 for measurements of innate immune parameters.

Results: Forty-nine participants completed the trial in each group. Supplementation was well-tolerated. A total of 45 URTIs were confirmed, 28 in the placebo group and 17 in the Wellmune group (odds ratio 0.55 (95% CI 0.24, 1.26); p=0.149). There was a strong trend for Wellmune to decrease the number of symptom days (p=0.067). Symptom severity was not significantly different between groups. Compared to the placebo group, lipopolysaccharide-stimulated blood from participants in the Wellmune group showed an increase in interferon-gamma concentration from baseline at day 45 (p=0.016) and smaller decreases in monokine induced by interferon-gamma concentration from baseline at days 45 and 90 (p=0.032 and 0.046). No difference was seen in serum or non-stimulated blood cytokines and chemokines or in salivary IgA.

Conclusion: Daily oral beta 1,3/1,6 glucan may protect against URTIs and reduce the duration of URTI symptoms once infected in older people. This may be linked to effects on innate immune function. Larger studies are needed to confirm the benefits of beta 1,3/1,6 glucan on URTIs in older people.

Introduction

Upper respiratory tract infection (URTI) is the most frequent infectious illness in humans, with an estimated prevalence of two to four episodes per person per year in adults [1]. While usually self-limiting, URTIs have marked social and economic consequences due to sickness-related absence, care-giving and primary care attendance [2,3]. Effective antiviral treatment strategies remain elusive and the wide variety of causal pathogens and associated antigenic shift provide significant challenges to the development of vaccines [4]. Within primary care in the UK the proportion of antibiotic prescriptions for cough and cold presentations increased from 39% to 51% between 1999 and 2011 [5], despite considerable attention to appropriate prescribing, with respiratory tract infection reported as the leading indication for antibiotic prescription [6]. Novel strategies to prevent URTI are urgently required in order to reduce the burden of infection, associated antibiotic use and the subsequent development of antimicrobial resistance [7].

Innate immune cells including neutrophils and macrophages form the front line of host defence to pathogenic viral and bacterial challenges [8]. Modification at the initial, non-specific, level of innate immunity may confer benefit in the prevention or amelioration of URTI. Wellmune is a beta 1,3/1,6 glucan derived from the cell wall of *Saccharomyces cerevisiae* ("baker's yeast"). Wellmune has European Food Safety Authority approval for use as a novel food ingredient [9], and is available to the general public as a supplement. Wellmune up-regulates phagocytosis and chemotaxis of innate immune cells via priming of lectin-sites on complement-receptor 3 and results in enhanced resistance to infection in animal models [10-15]. A previous trial in one hundred healthy young adults (mean age ~ 21 years) conducted during the winter months demonstrated reduction in the concentration of the chemokine monocyte chemoattractant protein (MCP)-1 during symptomatic URTI, a reduction in the total number of days with cold and flu symptoms, and a tendency towards reduced symptom severity in those treated daily with 250 mg Wellmune compared with placebo [16]. Furthermore, a significant reduction in reported URTI symptoms was seen post-event in marathon runners taking Wellmune [17] and in individuals with moderate lifestyle stress [18]. Effects in older people have not been investigated. Therefore, this study investigated the effect of daily Wellmune supplementation on URTIs and selected innate immune markers in older community dwelling people.

Materials and Methods

Study design, subjects and sample collection

This study was a randomized, double-blind, placebo-controlled trial of 250 mg Wellmune (Biothera, Eagan, MN, USA) (n=50) once daily for 90 days versus an identical in appearance rice-flour placebo capsule (n=50). The study was approved by the South Central Hampshire B Research Ethics Committee (approval number 11/SC/0520), received Clinical Trial Authorisation from the Medicines and Healthcare Products Regulatory Agency and is listed on the European Clinical Trials Database (EudraCT number 2011-004910-41).

Recruitment and study commencement were completed over a three week period during January 2012 at a single National Health Service Primary Medical Care site in Hampshire, UK. Written invitations and participant information sheets were posted to approximately 600 registered patients who met the principal inclusion criterion (age 50 to 70 years). Invitations to an assessment appointment and to undertake completion of written informed consent were sent in time order of initial patient response.

Inclusion criteria were age 50-70 years, general good health, body mass index (BMI) 18-40 kg/m^2, agreement to attend all study visits and undergo all procedures, community-dwelling, and at least one self-reported URTI in the last 12 months. Exclusion criteria were current symptomatic respiratory illness; current use of oral steroids, antibiotics or immunosuppressant medication; known immune or auto-immune disorders (including HIV infection, ankylosing spondylitis, Crohn's Disease, ulcerative colitis); having low BMI or an eating disorder; severe renal or liver disease; symptomatic heart failure. Medical records and patient history were used to assess recruitment criteria. Symptomatic respiratory and cardiac conditions were excluded to prevent difficulty assessing URTI symptoms. The lower age limit of 50 years was selected as a generally accepted threshold for the development of age-related immune decline ("immunosenescence") [19] and the upper limit of 70 years to prevent wide heterogeneity in general health and frailty. Smoking status and influenza vaccine uptake over the previous 12 months were recorded to control for potential confounding.

Randomization to Wellmune or placebo was blinded and used a random block allocation sequence generated by the University of Southampton Research Design Service. The packaging and capsules used for Wellmune and placebo were identical in appearance and were labelled with a study identifier code 001-100. Participants were allocated to the

lowest available study identifier on completion of the consent process. Participants were asked to self-administer the intervention once daily before food.

Saliva and blood samples were collected at study entry, at day 45 and at day 90. Heparin was used as an anticoagulant. Whole blood was used for culture (see below) and for preparation of plasma which was stored at minus 80^{o}C prior to analysis

Health diary and Wisconsin Upper Respiratory Tract Severity Score 21

The presence or absence of URTI symptoms was recorded in a daily health diary using the following numerical categories: "1" no health problems today; "2" cold symptoms (listed as runny nose, blocked nose, sore throat, coughing, sneezing, coloured discharge); "3" flu-like symptoms (fever, headache, general aches and pains, fatigue and weakness, chest discomfort). The presence of any two or more URTI symptoms for two consecutive days triggered telephone or face-to-face review to medically confirm URTI. Participants were instructed to complete the validated Wisconsin Upper Respiratory Tract Severity Score 21 (WURSS-21) for each symptomatic day. WURSS-21 contains a Likert scale 0-7 for specific URTI symptoms and the impact of symptoms on activities of daily living.

Adherence, side effects (self-classified by symptom and severity as mild/moderate/severe) and concomitant medication use were self-recorded in the health diary which was reviewed along with an unused capsules count during face-to-face interviews at days 45 and 90. Non-adherence was classified as more than 9 or more missed capsules in either 45 day period.

Salivary immunoglobulin A and plasma cytokine concentrations

Secretory immunoglobulin A (SIgA) concentration in saliva was measured using a commercially available ELISA kit (Immundiagnostik AG, Bensheim, Germany). Saliva was centrifuged at 3000 rpm for 10 min prior to assay. The manufacturer's instructions were followed. The detection limit of the assay is 13.4 ng/ml.

Plasma was collected from fresh blood collected into heparin and then stored at minus 80^{o}C until analysis. Cytokines (interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, IL-17a, IL-22, interferon (IFN)-γ, tumor necrosis factor (TNF)-α) and chemokines (IL-8, macrophage inflammatory protein (MIP)-1α, MIP-1β, MCP-1, granulocyte

colony stimulating factor (GCSF), monocyte induced by interferon-γ (MIG)) were measured in plasma using flow-cytomix 6-plex and 13-plex kits (eBiosciences, Hatfield, UK). The manufacturer's instructions were followed. The detection limits of the assays are 0.9 to 20.8 pg/ml; any value lower than the limit of detection was assigned half of the value of the lowest detectable standard.

Whole blood cultures and supernatant cytokine concentrations

Whole blood was diluted one in ten with RPMI culture medium containing 2 mmol/l glutamine and antibiotics. Cultures were stimulated with the toll-like receptor 4 ligand lipopolysaccharide (LPS (ultrapure *Escherichia coli* K12 lipopolysaccaride; InvivoGen, San Diego, CA, USA) final concentration 1 ng/ml). Medium was collected after 24 hr and then stored at minus 80^{o}C until analysis. Cytokines and chemokines (as listed above for plasma) were measured in plasma using flow-cytomix 6-plex and 13-plex kits (eBiosciences, Hatfield, UK). The manufacturer's instructions were followed.

Sample size and statistical analysis

Given the lack of data regarding Wellmune use in older adults, the pragmatic sample size of 100 participants was selected for this trial, with the aim of further informing effect size, mechanisms of action and potential benefit of a larger trial; therefore this trial should be regarded as a pilot study. Data were collated in Excel (Microsoft, Redmond, WA, USA) and then transferred to Stata (Stata, College Station, TX, USA) for analysis on an intention-to-treat basis. The Chi-Squared test was used to determine the odds ratio for between-group difference in the number of URTI episodes and an Anderson-Gill model was used to analyse difference in the number of symptom days due to heterogeneity in the distribution of data between groups. The Mann Whitney U test was used to analyse between-group difference in mean daily WURSS-21 symptom severity score and between-group difference in the change from baseline at day 45 and day 90 in plasma cytokines, LPS-stimulated whole blood cytokines and salivary IgA. In all cases statistical significance was set at $P<0.05$.

Results

Participant characteristics

Table 1 lists the characteristics of the 100 participants who entered the study. The groups did not differ according to age or age distribution, sex distribution, weight or BMI, cigarette smoking or recent seasonal influenza vaccination. Figure 1 shows the flow of participants through the study.

Forty-nine participants completed the study in each group and supplementation was well-tolerated. One participant in the placebo group left the trial due to constipation as a reported side effect within days 0-45. One participant in the Wellmune group failed to take the supplement on more than 9 days during days 0-45 and so was excluded from the study; this participant did not report any adverse events from supplementation. Table 2 provides a summary of reported side effects. Side effects were more common in the placebo group.

Upper respiratory tract infections

A total of 45 URTI episodes were confirmed. Smokers were more likely to experience an URTI than non-smokers (odds ratio 2.81 (95% CI 0.67, 11.67)), but this difference was not statistically significant, likely due to small sample size (n = 10 smokers). Twenty-eight URTI episodes were in the placebo group while 17 were in the Wellmune group (odds ratio 0.55 (95% CI 0.24, 1.26); this difference was not statistically significant (p=0.149) (Table 3). After controlling for both seasonal influenza vaccine uptake within the previous 12 months and smoking status, the odds ratio for URTI in the Wellmune group was 0.66 (95% CI 0.28, 1.57; p=0.346). Six participants in the placebo group (12%) reported two URTI episodes over the 90 days of the study compared to two participants (4%) in the Wellmune group (Table 3). There was a trend towards fewer symptom days in the Wellmune group (median 3.0 (IQR 2, 9)) than in the control group (median 3.5 (IQR 1, 9)) (p=0.067). No difference was seen in symptom severity between groups in either global or mean daily WURSS-21 scores (data not shown).

Salivary IgA concentration

No between-group difference was detected in the change from baseline at day 45 or 90 in salivary IgA concentration (data not shown).

Plasma cytokine and chemokine concentrations

No between-group differences were detected in the change from baseline at day 45 or 90 in plasma cytokine and chemokine concentrations (data not shown). However, many of these analytes were present at very low concentrations in many of the samples.

LPS-stimulated whole blood cytokine and chemokine concentrations

The concentrations of IL-2, IL-4, IL-13, IL-17a and GCSF were very low in LPS-stimulated whole blood cultures, often being below the limit of detection. In contrast, the other cytokines and chemokines were detected in most samples. The concentrations of IL-1β, IL-6, IL-8, TNF-α, MCP-1, MIP-1α and MIP-1β were easily detected in all samples. There were some changes with time that occurred in both groups, such that there were few between-group differences in the change from baseline at day 45 or 90. However, LPS-stimulated blood samples from the Wellmune group showed an increase in IFN-γ concentration from baseline at day 45 compared to a small reduction from baseline in the placebo group (p=0.016) (Table 4). However, no between-group difference was seen for IFN-γ for the change from baseline at day 90. The concentration of MIG decreased from baseline in both groups at days 45 and 90 (Table 4). However, the decreases were smaller in the Wellmune group at both time points (p = 0.032 and 0.046 at days 45 and 90, respectively) (Table 4). The concentration of MCP-1 decreased from baseline in the placebo group at days 45 and 90 and in the Wellmune group at day 90. However, the decrease tended to be smaller in the Wellmune group (Table 4).

Discussion

Finding effective prevention and management strategies for URTI remains an area of unmet healthcare need. Despite the usually self-limiting nature of illness, the high incidence of URTI causes significant health, social and economic impact which was estimated by Fredrick et al. to cost the United States economy nearly $40 billion per annum due to use of healthcare resources and lost productivity [2]. Given the scale of the problem, even interventions with a modest ability to reduce URTI incidence and/or severity may be beneficial if proven safe, effective and acceptable for widespread use. Here, we examined

the ability of Wellmune to reduce incidence, severity and duration of URTI in elderly subjects living in the community. There was a trend to fewer illness episodes with Wellmune compared to placebo (odds ratio 0.55; p = 0.149) and to fewer days of illness (p = 0.067) but there was no effect on symptom severity. In a previous study in healthy young subjects given the same dose of Wellmune (250 mg/day) for the same duration (90 d) as used here, we also found a trend towards fewer days with symptoms of URTI (p = 0.06) and no effect on severity of most symptoms as assessed by WURSS-21 [16]. It is clear that larger studies are needed in order to identify significant effects of Wellmune on URTIs, and that such studies should be performed. Using number of illness episodes as the primary outcome, in order to detect a difference between groups of about 15% with 90% power a sample size of 217 per group would be needed without any allowance for loss to follow up. Future studies may benefit from targetting populations at risk of higher incidence or severity of infection, such as the inclusion of adults aged over 70 y [20], children [21], or individuals self-reporting high frequency of URTI.

The intervention was well tolerated in the elderly subjects studied here, with no withdrawals due to adverse effects and with a better (self-reported) side effect profile compared to placebo. Informal feedback from participants during the recruitment phase of the study often cited the appeal of nutritional options as a self-care strategy to enhance immune function and prevent the burden of common infections.

Intervention with Wellmune did not modify plasma cytokine or chemokine concentrations in the current study. There was also no effect on salivary SIgA concentration. Furthermore, there was a limited effect of Wellmune on the inflammatory response of immune cells in cultured blood. In the previous study in young adults [16], there was no effect of Wellmune on plasma cytokine or chemokine concentrations in the absence of any illness symptoms; the current finding of lack of effect of Wellmune on plasma cytokines and chemokines in older subjects is consistent with this. In the earlier study, Wellmune resulted in lower plasma MCP-1 concentration during infection [16], i.e., in the presence of an immune/inflammatory stimulus. Therefore it is interesting that, in the current study, Wellmune did promote an increase in LPS-stimulated IFN-γ production (at day 90) and smaller time-dependent decreases in LPS-stimulated MCP-1 (trend at day 90) and MIG (at both day 45 and 90) than seen in the placebo group. These observations suggest priming of innate immune cells to an inflammatory stimulus by Wellmune; the stimulus was infection

in vivo in the study in young adults [16] and ex vivo exposure to LPS in the current study. There is evidence that Wellmune can prime innate immune cells to a subsequent immune stimulus [10-15]. Such an effect may underlie the trends to an effect seen on URTI incidence and duration in the current study. It is likely that the main responder cell type in LPS-stimulated whole blood cultures is the monocyte. We did not assess the effects of Wellmune on isolated innate immune cells or on innate immune responses other than peptide mediator production, such as phagocytosis and natural killer cell activity. These immune outcomes should be assessed in future studies.

Conclusion

Daily supplementation with Wellmune is well tolerated in older community dwelling subjects and may have a role in the prevention and faster resolution of URTI. This effect may be related to differences in innate immune responses in subjects consuming Wellmune. Larger studies seem warranted to explore the role of Wellmune for the prevention and control of common infections.

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Author contributions

RF and PCC designed the study. RF recruited the subjects, carried out the intervention and collected saliva and blood samples under the supervision of MVM and GL. RVO, HLF and PSN conducted the laboratory analysis under the supervision of PCC. RF and BLS conducted the statistical analysis. RF and PCC drafted the manuscript. All authors had input into the manuscript and approved the final version.

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Figure captions

Figure 1. Consort diagram showing the flow of participants through the study.

Table 1

Characteristics of participants at study entry according to treatment group

Variables	Wellmune group (n=50)	Placebo group (n=50)
Age (y)	*58.9 ± 5.6 Range: 50-68‡	*59.16 ± 5.5 Range: 50-68‡
Sex: Male Female	 26 (52%) 24 (48%)	 28 (56%) 22 (44%)
Height (m)	*1.7 ± 9.4	*1.7 ± 0.9
Weight (kg)	*78.5± 18.1	*78.8 ± 15.6
Body mass index (kg/m^2)	*26.8 ± 4.3	*26.8 ± 4.1
Current cigarette smoker	3 (6%)	7 (14%)
Seasonal influenza vaccination within past 12 months	18 (36%)	23 (46%)

*Values are mean ± standard deviation;

‡n = 29 aged 50 to 60 y and n = 21 aged > 60 y in each group

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Table 2

Summary of self-reported side effects according to treatment group

Placebo group	Wellmune group
Indigestion (mild) n=1	Bloating (mild) n=1
Generalised itch (moderate) n=1	Headaches (moderate) n=1
Constipation (severe) n=1* Constipation (mild) n=1	
Nausea (mild) n=1	
Tiredness (mild) n=1	

*Withdrew from the study

Table 3

A comparison of the number of participants experiencing URTI episodes by treatment group

	No episodes	1 episode	2 episodes
Placebo group	28 (56%)	16 (32%)	6 (12%)
Wellmune group	35 (70%)	13 (26%)	2 (4%)

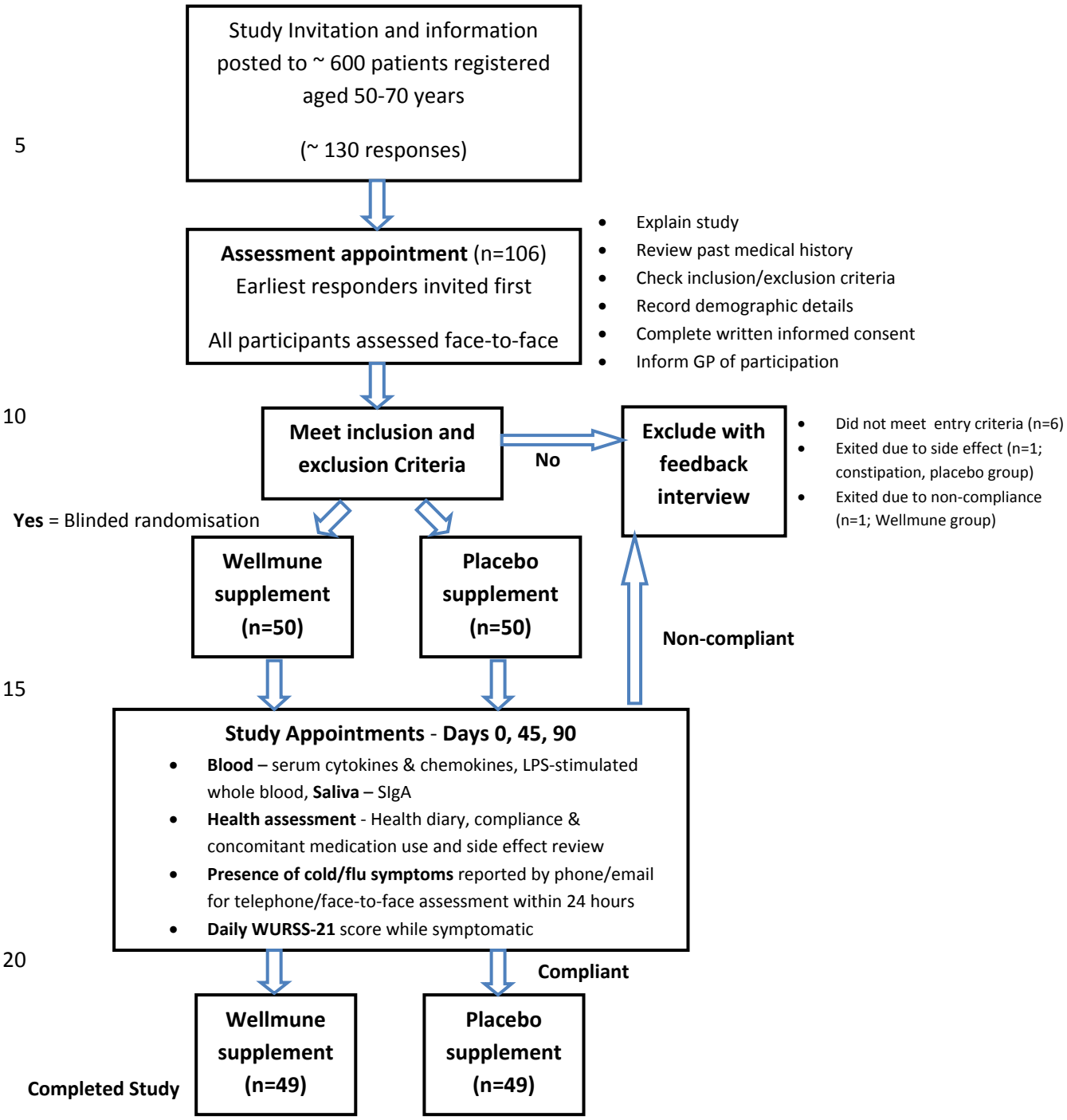
Table 4

Within and between-group differences in the change from baseline at days 45 and 90 in selected cytokines and chemokines in LPS-stimulated blood samples

	Placebo group day 0	Wellmune group day 0	Placebo group difference: Day 0-45	Wellmune group difference: Day 0-45	Between groups P	Placebo group difference: Day 0-90	Wellmune group difference: Day 0-90	Between groups P
IFN-γ	139.4 (0.8, 859.5)	107.8 (0.8, 637.3)	4.8 (-48.5, 432.8)	-49.1 (-639.7, 73.8)	0.016	15.5 (-172.1, 319.5)	0.0 (15.2, 372.8)	0.758
MCP-1	2335.0 (1277.6, 2911.8)	1411.1 (986.5, 2744.6)	477.9 (-323.7, 1997.9)	77.7 (-729.0, 919.9)	0.102	1666.6 (507.3, 2400.7)	796.7 (347.1, 1492.0)	0.069
MIG	104.6 (37.8, 205.7)	58.2 (17.7, 144.5)	79.7 (0.0, 187.3)	22.6 (-38.2, 131.5)	0.032	104.0 (38.7, 202.2)	48.4 (2.3, 102.4)	0.046

Data are median and 25th and 75th percentiles. IFN-γ, interferon-gamma; MCP-1, monocyte chemoattractant protein-1; MIG, monocyte induced by interferon-gamma

Figure 1


Study Invitation and information posted to ~ 600 patients registered aged 50-70 years
(~ 130 responses)
Assessment appointment (n=106)
Earliest responders invited first
All participants assessed face-to-face
Explain study
Review past medical history
Check inclusion/exclusion criteria
Record demographic details
Complete written informed consent
Inform GP of participation
Meet inclusion and exclusion Criteria
No
Exclude with feedback interview
Did not meet entry criteria (n=6)
Exited due to side effect (n=1; constipation, placebo group)
Exited due to non-compliance (n=1; Wellmune group)
Yes = Blinded randomisation
Wellmune supplement (n=50)
Placebo supplement (n=50)
Non-compliant
Study Appointments - Days 0, 45, 90
Blood – serum cytokines & chemokines, LPS-stimulated whole blood, Saliva – SIgA
Health assessment - Health diary, compliance & concomitant medication use and side effect review
Presence of cold/flu symptoms reported by phone/email for telephone/face-to-face assessment within 24 hours
Daily WURSS-21 score while symptomatic
Compliant
Completed Study
Wellmune supplement (n=49)
Placebo supplement (n=49)