

Ageing alters the impact of nutrition on immune function

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Ageing alters the impact of nutrition on immune function

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Abbreviations used: AMPK, AMP-activated protein kinase; AA, arachidonic acid; CMV, cytomegalovirus; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; mTOR, mechanistic target of rapamycin; PBMC, peripheral blood mononuclear cells; PUFA, polyunsaturated fatty acid; TCR, T-cell receptor.

26 **Abstract**

27 Immunosenescence during ageing is a major challenge, weakening the ability of older
28 individuals to respond to infection or vaccination. There has been much interest in dietary
29 strategies to improve immunity in older people, but there is an assumption that modulation of
30 the immune response in older people will be based on the same principles as for younger
31 adults. Recent evidence suggests that ageing fundamentally alters the impact of nutrition on
32 immune function. As a result, interpretation of data from studies investigating the impact of
33 diet on immune function is highly dependent on subject age. Study design is critically
34 important when investigating the efficacy of dietary components, and most studies involving
35 older people include rigorous inclusion/exclusion criteria based on medical history,
36 laboratory tests, general health status, and often nutritional status. However, immunological
37 status is rarely accounted for, but can vary significantly, even amongst healthy older people.
38 There are several clear examples of age-related changes in immune cell composition,
39 phenotype and/or function, which can directly alter the outcome of an intervention. This
40 review uses two case studies to illustrate how the effects of n-3 polyunsaturated fatty acids
41 and probiotics differ markedly in young vs older subjects. Evidence from both suggests that
42 baseline differences in immunosenescence influence the outcome of an intervention,
43 highlighting the need for detailed immunological characterization of subjects prior to
44 interventions. Finally, future work elucidating alterations in metabolic regulation within cells
45 of the immune system as a result of ageing may be important in understanding the impact of
46 diet on immune function in older people.

47

48

49 **Introduction**

50 Nutritional status has a profound influence on resistance to infection, which is exemplified by
51 the vicious cycle between undernutrition and infection in developing countries ⁽¹⁾. However,
52 vulnerable groups in developed countries are also at risk of age- or disease-related
53 malnutrition, which can impact on the immune response to infection and to vaccination.
54 Thus, while decreased immune function due to malnutrition primarily affects children in
55 developing countries, in the developed world, it is mainly a problem for older people ⁽²⁾. By
56 2050, approximately 25% of the population will be older than 65 years ⁽³⁾ and the impact of
57 this on public health is a major global challenge. However, decreased immune function as a
58 result of malnutrition should not be confused with immunosenescence; an obvious difference
59 is that malnutrition and, to some extent its consequences, are treatable. Immunosenescence is
60 irreversible and describes the biological ageing of the immune system, which is associated
61 with a progressive decline in both innate and adaptive immunity, poor response to
62 vaccination and increased prevalence of cancer, infections and autoimmune and chronic
63 diseases. While nutritional interventions may delay this process, the evidence for this remains
64 controversial, particularly in terms of the nature and potency of immunomodulatory activity
65 and of translation into a corresponding change in clinical outcome ^(4; 5). Furthermore, there is
66 a fundamental lack of understanding as to how immunosenescence alters the response of cells
67 of the immune system to dietary components. Most studies examining the effects of diet on
68 immune function fail to adequately characterize target populations in terms of nutritional
69 status, health status, genetic background and few, if any, characterize them in terms of
70 immunological status. This review focuses on two case studies, which demonstrate that
71 failure to account for immunosenescence can significantly influence the outcome of a
72 nutritional intervention. It also explores proposed mechanisms by which ageing alters

73 metabolic regulation of immune cells and whether metabolic pathways could be targeted for
74 immunoregulation.

75

76 **Case study: ageing alters the immune response to n-3 polyunsaturated fatty acids**

77 Fatty acids play diverse roles in all cells, serving as an important source of energy, as
78 structural components of cell membranes, signaling molecules, bioactive mediators and
79 regulators of gene expression. Human immune cell phospholipids contain about 1%
80 eicosapentaenoic acid (EPA) and 2.5% docosahexaenoic acid (DHA) in addition to 20%
81 arachidonic acid (AA) ^(6;7). As the long chain n-3 polyunsaturated fatty acid (PUFA) content
82 of the diet increases, lymphocyte AA decreases in a curvilinear fashion. In human studies,
83 dietary n-3 PUFA never exceeds 3 % of total energy, whereas in animal studies, intake is
84 often considerably higher, and this is thought to explain the discrepancies that exist between
85 animal and human studies investigating the immunomodulatory effects of n-3 PUFA ⁽⁷⁾. As a
86 result, it remains unclear to what extent and at what dose n-3 PUFA have immunomodulatory
87 effects in humans. Nevertheless, the literature suggests that fish oil has a greater impact on
88 immune function in elderly compared with young subjects ^(8;9;10) and that this may be related
89 to the fact that older subjects appear to incorporate EPA into plasma and PBMC more readily
90 than younger subjects ⁽¹¹⁾ (**Figure 1**). EPA resulted in a dose-dependent decrease in
91 neutrophil respiratory burst in older, but not younger subjects ⁽¹¹⁾. However, PGE₂ production
92 by PBMC was decreased in both groups and phagocytosis and cytokine production were not
93 affected in either group ⁽¹¹⁾. This highlights the fact that age is likely to be an important factor
94 when considering the impact of n-3 PUFA on immunity, not only because of the influence of
95 immunosenescence, but also because immune cells from older subjects appear to be more
96 responsive to the availability of n-3 PUFA. Recent work suggests that the cholesterol content
97 of T lymphocytes from healthy elderly subjects is higher than that of young subjects, and that

98 membrane fluidity is subsequently decreased ⁽¹²⁾. Furthermore, the coalescence of lipid rafts
99 at the site of T cell receptor engagement is impaired in elderly subjects ^(12; 13). The impact of
100 ageing on lipid raft composition and function appears to be most evident in the CD4⁺ T cell
101 population and affects cytokine signaling ^(13; 14). Thus, the greater responsiveness of T cell
102 membranes to n-3 PUFA in older subjects could result in alteration of lipid raft structure, and
103 subsequently of cell function, effects which are absent in younger subjects.

104

105 **Case study: ageing alters the immune response to probiotics**

106 Influenza is a major cause of death in older people and while vaccination offers a
107 prophylactic solution for preventing infection and associated complications,
108 immunosenescence significantly impairs vaccine efficacy ⁽¹⁵⁾. Potential adjuvants and dietary
109 strategies to improve the immune response to influenza vaccines are therefore of interest,
110 particularly in older people. Emerging evidence suggests that the resident gut microbiota
111 plays an influential role in shaping antiviral defenses and modulating the outcome of viral
112 infections through inflammasome-mediated cytokine release ⁽¹⁶⁾, Antibiotic-treated mice have
113 reduced levels of interleukin-1 β (IL-1 β) secretion in the lung during influenza infection,
114 supporting the suggestion that gut-resident bacteria support cytokine production [16]. It has
115 been speculated that gut microbes release low levels of pattern recognition receptor ligands,
116 which provide signals for inflammasome-mediated cytokine release (for example, in the lung
117 during influenza infection). These in turn regulate the activity of respiratory dendritic cells
118 during activation of adaptive immunity against the virus [16], and together, this forms the
119 basis for the hypothesis that pre- and probiotics may modulate responses to infection or
120 vaccination.

121 Trials investigating the use of probiotics in prevention of common respiratory illnesses have
122 produced mixed results ⁽¹⁷⁾, although a recent systematic review concluded that they

123 significantly reduce episodes of acute upper respiratory tract infection and antibiotic usage in
124 infants and young to middle-aged adults ⁽¹⁸⁾. Response to vaccination is increasingly being
125 used as a surrogate for the response to infection ⁽¹⁹⁾. The majority of studies investigating the
126 impact of probiotics on responses to vaccination have been conducted in healthy adults, and
127 some show borderline effects of probiotics on serum or salivary IgA titres, although the
128 clinical relevance is not clear ⁽²⁰⁾. Studies in infants and in elderly subjects, particularly those
129 examining the response to influenza vaccination, are very limited, as are studies on the effects
130 of prebiotics on immune function ⁽²¹⁾ and vaccination ⁽²⁰⁾. Since ageing is associated with
131 reduced biodiversity and compromised stability of the gut microbiota ⁽²²⁾, as well as
132 immunosenescence, older individuals may derive particular benefit from intervention with
133 pre- and/or probiotics.

134 Previous studies investigating the effects of probiotics on the response to vaccination have
135 mainly focused on antibody production. While some studies have reported a modest effect of
136 probiotics on the antibody response to vaccination in adults, trials in older subjects are largely
137 inconsistent and data are limited ⁽²⁰⁾. In a recent study (the PRObiotics, IMMunity and
138 AGEing; PRIMAGE trial), we demonstrated that while there was marked impairment of the
139 antibody response to influenza vaccination in older subjects, intervention with a novel
140 synbiotic, *Bifidobacterium longum* bv. *infantis* CCUG 52486 combined with gluco-
141 oligosaccharide (*B. longum* + GI-OS) failed to reverse this impairment ⁽²³⁾. Although there is
142 general consensus that ageing impairs the response to influenza vaccination ⁽²⁴⁾, there are very
143 few robust studies specifically comparing responses of young and older subjects, and there
144 are no other studies directly comparing the efficacy of pre- and probiotics on the immune
145 response of young and older subjects to vaccination. In the PRIMAGE trial, the response of
146 the young and older subjects to the intervention differed to some degree. In older subjects
147 consuming the synbiotic, there was a trend for reduced seroconversion to the Brisbane

148 subunit of the vaccine, whereas in the young subjects, there were trends for enhanced
149 production of vaccine-specific IgM and, to some extent, IgG⁽²³⁾. Increased production of
150 vaccine-specific IgM and IgG following intervention with probiotics has been reported in
151 several other studies^(25; 26; 27; 28; 29). The possibility that there is a differential immune
152 response to probiotics in young vs older subjects has also been demonstrated in *in vitro*
153 studies. You *et al.*⁽³⁰⁾ demonstrated that peripheral blood mononuclear cells (PBMC) from
154 older subjects (60-85y) were more responsive to the immunoregulatory effects (IL-10
155 induction) of two strains of bifidobacteria than young subjects (18-30y), whereas PBMC
156 from young subjects were more responsive to the immunostimulatory effects (IL-12
157 induction) of two strains of lactobacilli. Further studies demonstrated that probiotics
158 increased the responsiveness of DCs in older subjects to a greater degree than young subjects,
159 but this was not sufficient to overcome the impact of immunosenescence in a mixed
160 leukocyte reaction⁽³¹⁾. The choice of probiotic, particularly for older individuals, is a matter
161 of debate and it has been suggested that ‘successfully aged’ donors of probiotic strains might
162 survive better in an older host and achieve a more suitable equilibrium with the resident
163 microbiota⁽³²⁾. *Bifidobacterium longum* *bv. infantis* CCUG 52486 is an example of a strain
164 present in particularly healthy subjects aged >90y⁽³³⁾. It has subsequently been demonstrated
165 to have particular ecological fitness and anti-pathogenic effects *in vitro*⁽³⁴⁾ and, as described
166 above, immunomodulatory effects which are strongly influenced by the age of the host^(30; 31).
167 Further immunological characterization in the PRIMAGE trial revealed that B and T cell
168 profiles differed markedly between young and older subjects, and that vaccination increased
169 numbers of specific memory subsets in young subjects, but failed to do so in older subjects
170 (Enani *et al.*, unpublished data). A key finding was the observation that there was a greater
171 degree of immunosenescence at baseline in older subjects randomized to the synbiotic, which
172 occurred entirely by chance, but could explain the particularly poor response of these subjects

173 to the vaccination ⁽²³⁾. T cells are particularly susceptible to senescence, resulting in loss of
174 CD28; repeated antigenic exposure, for example to cytomegalovirus (CMV), is suggested to
175 play a major role in this ^(35; 36). Latent infection with CMV has been demonstrated to result in
176 a poor response to infection and vaccination ⁽³⁶⁾. In the PRIMAGE trial, not only did older
177 subjects randomized to the synbiotic have a significantly higher number of senescent (CD28⁻
178 CD57⁺) helper T cells at baseline compared with those randomized to the placebo, they also
179 had significantly higher plasma levels of anti-CMV IgG and a greater tendency for CMV
180 seropositivity. Moreover, higher numbers of CD28⁻CD57⁺ helper T cells were associated with
181 failure to seroconvert to the Brisbane subunit of the vaccine, strongly suggesting that the
182 subjects randomized to the synbiotic were already at a significant disadvantage in terms of
183 likely ability to respond to the vaccine compared with those randomized to the placebo and
184 that differences in immunosenescence between the randomized groups at baseline may have
185 influenced the outcome of the intervention (**Figure 2**). Future work therefore needs to
186 consider prospective randomization of subjects based on robust immunological markers; this
187 is challenging given the wide range of potential markers and uncertainty regarding their
188 predictive value.

189

190 **Ageing alters metabolic regulation of T cells**

191 Over the past decade, our understanding of T cell activation has extended to exploration of
192 integration between canonical T cell signalling pathways and metabolic signalling
193 programmes ⁽³⁷⁾, and it has been proposed that immunosenescence is linked to alterations or
194 defects in that integration ⁽³⁸⁾. Although several transcription factors and serine/threonine
195 kinases are central to the integration of immunological and metabolic pathways ⁽³⁷⁾, the
196 energy sensor, AMPK, is of particular interest in the context of ageing. AMPK is a central
197 regulator of metabolic stress and is activated by an increase in the AMP/ATP ratio, as well as

198 by T cell receptor (TCR) engagement. In fact, it has been suggested that AMPK activation in
199 response to antigen anticipates ATP depletion even in the presence of adequate nutrients ⁽²³⁾.
200 In AMPK deficient T cells, metabolic stress due to glucose deprivation induces enhanced cell
201 death. Senescent T cells demonstrate spontaneous phosphorylation- and therefore activation-
202 of AMP ⁽³⁸⁾. However, contrary to expectation, senescent cells did not contains low levels of
203 ATP ^(8; 38). Instead, it is suggested that AMPK activation triggered by glucose deprivation
204 results in activation of the p38 pathway, which leads to DNA damage and immunosenescence
205 ⁽³⁸⁾. Conversely, AMPK silencing restores proliferation ⁽³⁷⁾. This is a previously unrecognized
206 mode of activation for p38 in T cells and the first demonstration of a pathway which
207 integrates low nutrient sensing with DNA damage and senescence. The observation that
208 nutrient deprivation triggers pathways linked with immunosenescence seems to contradict the
209 widely-held belief that caloric restriction enhances life span, but data on caloric restriction
210 and infections is not clear cut and this remains an important area for future work.

211 Transcription factors and signalling proteins involved in regulatory and metabolic pathways
212 represent novel targets for immune modulation. Indeed, it has been suggested that targeting
213 AMPK and mTOR may be a strategy for suppressing immune responses and treating
214 inflammatory diseases ⁽³⁷⁾. However, the suggestion that this may allow more selective
215 regulation of immune responses than ubiquitous signalling pathways should be interpreted
216 with caution as there is no clear reason to believe that this is the case.

217

218 **Concluding remarks**

219 Ageing alters the immune response to dietary interventions; specific examples described in
220 this review demonstrate that young and older subjects respond differently to interventions
221 involving dietary fatty acids and probiotics. It is critical that baseline differences in
222 immunosenescence in dietary studies involving older subjects are accounted for as they can

223 directly influence the outcome of the intervention. Ageing also alters metabolic regulation of
224 T cells; elucidation of alterations in metabolic regulation in ageing T cells may prove to be
225 important in understanding the impact of diet on immune function in older people.

226

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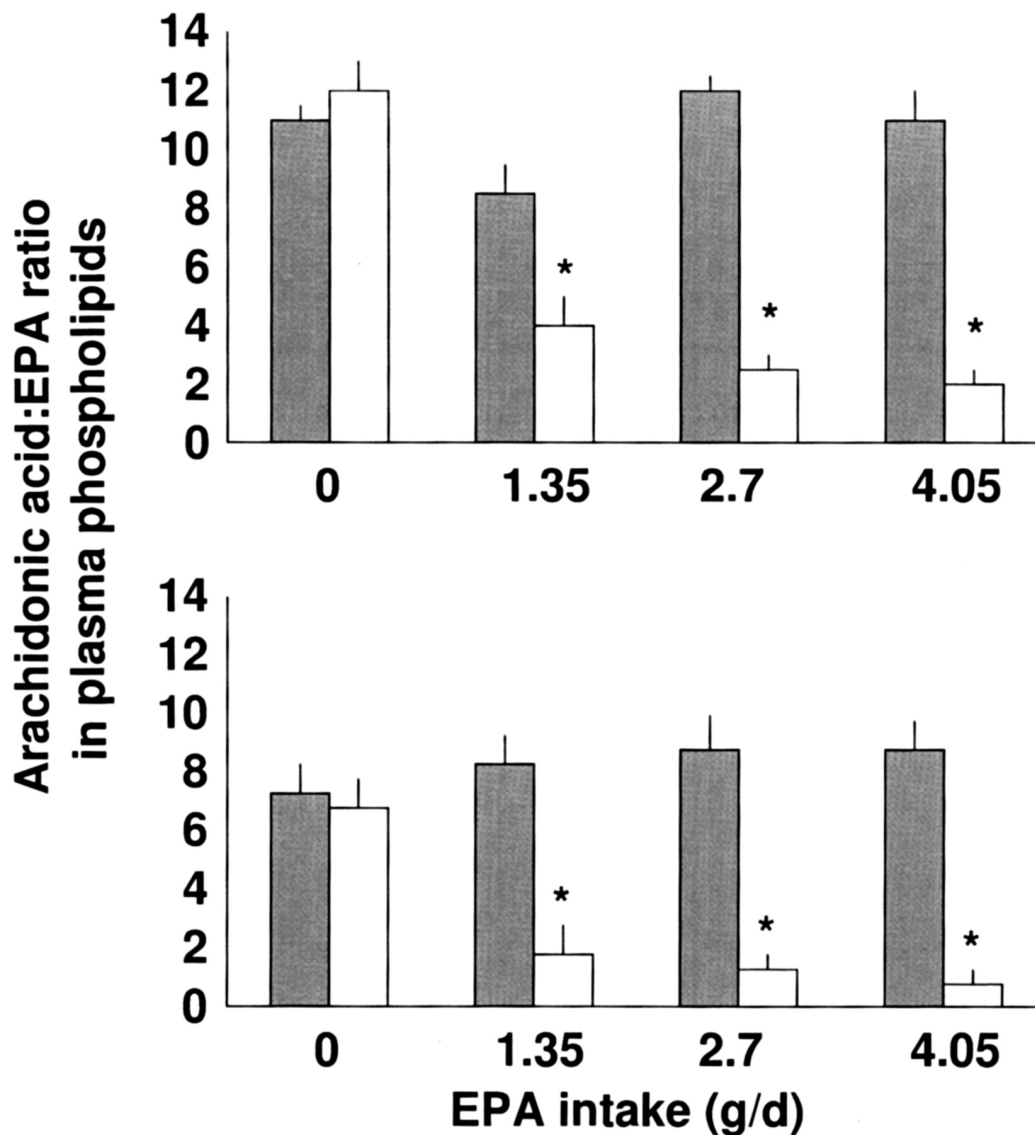
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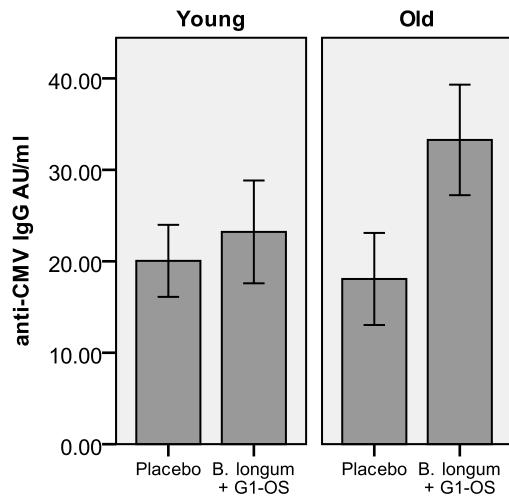
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322

323 **Figure 1. Arachidonic acid to eicosapentaenoic acid ratio in plasma phospholipids from**
 324 **young and older subjects.** Mean (\pm SEM) ratios of arachidonic acid to eicosapentaenoic acid
 325 (EPA) in plasma phospholipids before (gray bars) and after (white bars) supplementation
 326 with placebo (0 g EPA) or low (1.35 g/d), moderate (2.7 g/d), or high (4.05 g/d) doses of an
 327 EPA-rich oil for 12 wk in the young (upper panel) and older (lower panel) subjects. $n = 24,$
 328 $23, 23,$ and 23 for the young subjects in the placebo, low-EPA, moderate-EPA, and high-EPA
 329 groups, respectively. $n = 16, 16, 15,$ and 15 for the older subjects in the placebo, low-EPA,
 330 moderate-EPA, and high-EPA groups, respectively. At baseline there was a significant effect
 331 of age ($P < 0.001$) but not of treatment group (ie, EPA dose) and no age \times treatment group
 332 interaction. At baseline the ratio was significantly higher in the young than in the older
 333 subjects ($P < 0.05$). Two-factor ANOVA showed a significant effect of treatment group ($P <$
 334 0.001) but not of age and no age \times treatment group interaction for the change in the ratio of
 335 arachidonic acid to EPA. *Significantly different from baseline, $P < 0.001$ (paired Student's t
 336 test). Figure taken from ⁽¹¹⁾, with permission.

337



339

340 **Figure 2. Baseline levels of anti-CMV IgG differ in older subjects randomized to *B.***
 341 ***longum* + GI-OS and placebo.** Data are anti-CMV IgG (AU/ml) \pm 2SEM for n=45 young
 342 and n=45 older subjects randomized to *B. longum* + GI-OS or placebo. Data were analysed
 343 using Student's independent t-tests for differences between young and older subjects. *
 344 Denotes significant difference between treatment groups within age cohort ($p < 0.05$). The
 345 difference in CMV status between the cohorts may have influenced the outcome of the
 346 subsequent intervention. Figure taken from ⁽²³⁾, published by Springer.

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348