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Symposium 5 (Joint Nutrition Society and BAPEN Symposium): Too many pies: metabolic competencies in obesity
The true cost of in-patient obesity: impact of obesity on inflammatory stress and morbidity

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The objective of the present review is to provide an overview of the metabolic effects of pro-inflammatory cytokine production during infection and injury; to highlight the disadvantages of pro-inflammatory cytokine production and inflammatory stress on morbidity and mortality of patients; to identify the influence of genetics and adiposity on inflammatory stress in patients and to indicate how nutrients may modulate the inflammatory response in patients. Recent research has shown clearly that adipose tissue actively secretes a wide range of pro- and anti-inflammatory cytokines. Paradoxically, although inflammation is an essential part of the response of the body to infection, surgery and trauma, it can adversely affect patient outcome. The metabolic effects of inflammation are mediated by pro-inflammatory cytokines. Metabolic effects include insulin insensitivity, hyperlipidaemia, muscle protein loss and oxidant stress. These effects, as well as being present during infective disease, are also present in diseases with a covert inflammatory basis. These latter diseases include obesity and type 2 diabetes mellitus. Inflammatory stress also increases during aging. The level of cytokine production, within individuals, is influenced by single nucleotide polymorphisms (SNP) in cytokine genes. The combination of SNP controls the relative level of inflammatory stress in both overt and covert inflammatory diseases. The impact of cytokine genotype on the intensity of inflammatory stress derived from an obese state is unknown. While studies remain to be done in the latter context, evidence shows that these genomic characteristics influence morbidity and mortality in infectious disease and diseases with an underlying inflammatory basis and thereby influence the cost of in-patient obesity. Antioxidants and *n*-3 PUFA alter the intensity of the inflammatory process. Recent studies show that genotypic factors influence the effectiveness of immunonutrients. A better understanding of this aspect of nutrient–gene interactions and of the genomic factors that influence the intensity of inflammation during disease will help in the more effective targeting of nutritional therapy.

Obesity: Inflammation: Genotype: Chronic disease: Immunonutrition

The immune response to infection injury and inflammatory agents

The immune system has evolved to combat micro-organisms and initiate repair of injured tissue. Its normal function is central to successful recovery of hospitalised patients. Likewise, in the community, the immune system supports a good quality of life and longevity.

The system has a large capability for immobilising invading microbes, creating a hostile environment for them

Abbreviations: LT-α, lymphotoxin α; SNP, single nucleotide polymorphism.

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and bringing about their destruction[1]. The system also becomes activated by stimuli and conditions that do not directly involve pathogens (burns, penetrating and blunt injury, the presence of tumour cells and the presence of chronic inflammatory diseases). The broad spectrum of patients entering hospital will be experiencing these events to different extents. The response of the immune system to these diverse factors outlined above contains common elements. The elements of the response include activation of lymphocytes and macrophages, the production of immunomodulatory proteins (cytokines), oxidant molecules (H_2O_2, superoxide, hypochlorous acid and NO), anti-inflammatory hormones (cortisol), natural antagonists (cytokine receptor antagonists), antioxidants (glutathione) and antioxidants enzymes (superoxide dismutase, catalase and glutathione peroxidase)[1].

The cellular part of the response sets in train a dichotomous response. In general, lymphocytes produce a specific acquired immune response and macrophages initiate an inflammatory response. This latter part of the response is exemplified by the symptoms of 'rubor, calor and dolor' (redness, heat and pain) as described by Paracelsus. However, the inflammatory process may exist over a wide range from symptomless intensities to high and life threatening intensity, as seen in sepsis.

Inflammation is an essential part of the response to infection, surgery and trauma. Its prime purpose is to kill pathogens by creating a hostile tissue environment through production of oxidant molecules and activation of T and B lymphocytes. Substrate is released, from endogenous sources, by the inflammatory process to support the activity of T and B lymphocytes, and to enhance antioxidant defences so that healthy tissue may be protected from the potent mediators released during inflammation. Three pro-inflammatory cytokines, IL-1β, IL-6 and TNFα, modulate these events. Under their influence blood lipids are elevated, muscle protein is lost, gluconeogenesis is enhanced, catabolic hormone production is increased and insulin insensitivity occurs. All of these cytokine-induced effects, however, may play a role in the pathology of a wide range of chronic diseases[2].

Function of pro-inflammatory cytokines during the normal response to infection and injury

IL-1β, IL-6 and TNFα have widespread metabolic effects. Signs and symptoms experienced after infection and injury, such as fever, loss of appetite, weight loss, negative nitrogen, sulphur and mineral balance, and lethargy are caused directly or indirectly by pro-inflammatory cytokines. Indirect effects of cytokines are mediated by neural actions upon the adrenal glands and endocrine pancreas resulting in increased secretion of the catabolic hormones adrenalin, nor-adrenalin, glucococorticoids and glucagon. Insulin insensitivity occurs in addition to this 'catabolic state'.

The biochemistry of an infected individual is thus fundamentally changed to ensure that the immune system receives nutrients from within the body (Fig. 1). Muscle protein is catabolised to provide amino acids for synthesising new cells, glutathione and proteins for executing

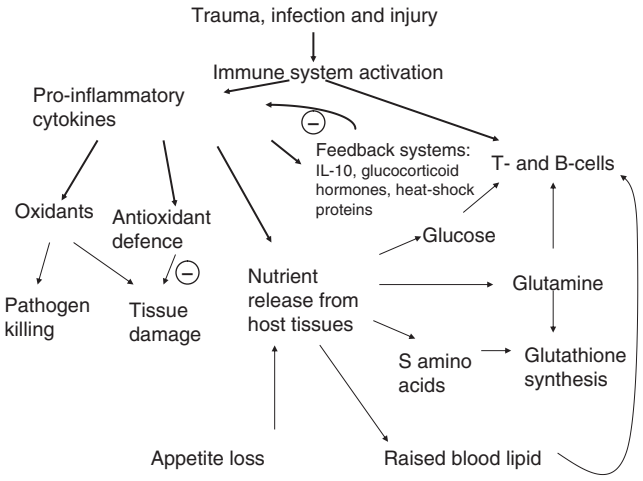

Fig. 1. The metabolic effects of pro-inflammatory cytokines. ⊖, response inhibited

and controlling the immune response. Amino acids are also converted to glucose (a preferred fuel, together with glutamine, for the immune system[1]). The extent of the rearrangement in protein metabolism is evident from changes in urinary nitrogen and sulphur following infection and injury and the rearrangement of lipid and glucose metabolism by elevation of plasma lipids[1].

Adverse effects of pro-inflammatory cytokines and inflammatory stress

Paradoxically pro-inflammatory cytokines, although essential for normal immune function, play a major role in tissue damage during inflammatory disease and may increase mortality from infections and mediate loss of muscle mass, following injury and surgery, in a wide range of infections. In conditions such as sepsis, pro-inflammatory cytokines are produced in excessive amounts and are an important factor in increased mortality[2]. Low-level inflammation has also been closely linked with poor clinical outcome and shortened lifespan. In 1989, data from the British Regional Heart Study showed that mortality rates from CVD and all causes were inversely related to serum albumin concentrations[3]. As albumin is a negative acute phase protein and is lowered during inflammation, the finding suggested that low-intensity, chronic inflammatory stress is inimical with health and avoidance of morbidity and mortality[2]. Subsequent studies clearly showed that atheromatous plaque growth and instability were due to pro-inflammatory cytokine production and inflammation within the plaque lumen. Thus the focus on the mechanistic basis of atherosclerosis shifted from one totally associated with aberrant cholesterol and TAG metabolism to a more complex scenario involving inflammatory stress.

Influence of adipose tissue mass on inflammatory stress

It is well known that obesity and smoking are strong risk factors in atherosclerosis and that obesity, insulin

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Table 1. Single nucleotide polymorphisms (SNP) in cytokine genes associated with altered levels of cytokine production

Gene and location of polymorphism in the promoter region	Genotype associated with raised or lowered cytokine production and/or altered outcome to inflammation*
Pro-inflammatory SNP	
TNFα – 308	TNF2 (A) allele
LT-β + 252	LT-β + 252 AA (TNFβ2:2)
IL-1β – 511	CT or TT
IL-6 – 174	G allele
Anti-inflammatory SNP	
IL-10 – 1082†	GG
TGF-1β + 915 (Arg-25-Pro)†	GG

LT, lymphotoxin; TGF, transforming growth factor.
*Poor outcome for pro-inflammatory cytokines.
†Improved outcome for anti-inflammatory cytokines.

insensitivity and diabetes mellitus form a triumvirate of disease. The recent finding that adipose tissue is an active endocrine organ and produces several inflammatory mediators provided a unifying mechanism for the linkage between the incidences of chronic diseases. The most abundant protein in adipose tissue is adiponectin, which stimulates immune cells to produce anti-inflammatory cytokines and may explain disturbed immune function in severely obese individuals[4,5]; adipose tissue has also been shown to overproduce TNFα and IL-6 in obesity[6–9]. Obesity is associated with a steady infiltration of macrophages into adipose tissue such that in grossly obese individuals, macrophages constitute up to 40% of the cellular population of the tissue[10]. Apart from the relevance of these findings for the pathogenesis of the metabolic syndrome, the inflammatory state related to obesity may also interfere with recovery of injury[11]. Similarly, dyslipidaemia, often encountered in obesity and an essential part of the metabolic syndrome, is also known to be an independent risk factor for the development of sepsis and increased mortality[12]. Indeed the risk of death from multi-organ failure was shown to be greater in obese patients than in patients with normal weight[13,14].

A study on obese women clearly showed that a reduction in adipose tissue mass, achieved by consuming 2520 kJ/day for 10 weeks, substantially reduced the ability of adipose tissue to produce TNFα, IL-6, IL-8 and leptin[15].

Influence of genotype on inflammation and disease

The explosion of new knowledge that followed the decoding of the human genome is helping to unify the understanding on the pathology of chronic disease.

Since the 1990s it has become clear that small, naturally occurring, variations (single nucleotide polymorphisms (SNP)), mostly in the promoter region of genes, influence the amount/bioactivity of product produced when the genes are activated. A large body of research has indicated that SNP occur in the upstream regulatory (promoter) regions of many pro- and anti-inflammatory cytokine genes that influence the level of cytokine production[16,17]. Recent findings have also suggested that SNP modify the responsiveness of individuals to changes in nutrient intake. For example, SNP influence the lipaemic response to dietary lipids[18], alter the interrelationship between plasma vitamin B12, folate and homocysteine[19] and modulate the ability of fish oil to reduce TNFα production[20].

Table 2. Influence of TNFα – 308 polymorphism and gender on the inflammatory response to surgery in patients with gastrointestinal cancer
(Mean values and standard deviations for *n* assays)

	Males			Females		
	Mean	SD	*n*	Mean	SD	*n*
Duration of operation (min)	214	125	65	172	76	56
Blood loss (ml)	473	521	65	258	348	54
Peak CRP concentration*						
TNF308 without allele 2	132	46	33	128	57	25
With allele 2	193‡	116	12	121	37	13
Peak IL-6 concentration†						
TNF308 without allele 2	439	402	24	362	376	15
With allele 2	676‡	544	7	315	147	5

CRP, C-reactive protein.
*mg/ml 2 d post-operatively.
†pg/ml 1 d post-operatively.
‡Significantly different from females with the same genotype by multivariate analysis allowing for longer operation time and greater blood loss $P = 0{\cdot}013$ and $P = 0{\cdot}027$ for CRP and IL-6, respectively.

Genetic effects on the intensity of the inflammatory process

SNP in the genes responsible for molecules involved in the inflammatory process modulate the intensity of inflammation. *In vitro* production of TNFα, by peripheral blood mononuclear cells from healthy and diseased subjects, stimulated with inflammatory agents, shows remarkable constancy in males and post-menopausal females[21]. This constancy suggests that genetic factors exert a strong influence. SNP in the promoter regions for the TNFα and lymphotoxin-α (LT-α) genes are associated with differential TNF production[16,22]. In addition to modifying the expression of LT-α itself, the TNFβ2 (A) alleles are linked to high TNF production, particularly in homozygous individuals. The TNFα – 308 (A) allele is associated with enhanced TNFα expression in a number of studies[16,22]. A number of SNP that have been implicated in the outcome to inflammatory stress are shown in Table 1.

Induction of oxidant molecules follows from activation of the immune system. NF-κB is activated by oxidants and switches on many of the genes involved in the inflammatory response (cytokines, adhesion molecules and acute phase proteins)[23]. Genomic factors influence the level of production of oxidants and NF-κB activation). Natural resistance associated macrophage protein 1 has pleiotropic effects on macrophage functions, including TNFα production and activation of inducible nitric oxide synthase, which occurs by cooperation between the natural resistance associated macrophage protein 1 and TNFα genes[24]. There are four variations in the natural resistance associated macrophage protein 1 gene, resulting in different basal

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Table 3. Influence of genotype and gender on length of stay in hospital and survival in geriatric care patients*
(Mean values and standard deviations for *n* patients)

	Males			Females		
	Mean	SD	*n*	Mean	SD	*n*
Length of stay in hospital (days)						
Patients with IL-1 −511 CC genotype	9	11	9	15	7	26
Patients with IL-1 −511 CT or TT genotype	14†	6	13	14	12	28
Survival post-hospitalisation (months)						
Patients with LT-α+252 GG or AG genotype	21	12	11	21	15	19
Patients with LT-α+252 AA genotype	10†	12	10	22	15	28
Patients with IL-1 −511 CC genotype	27	13	9	19	15	26
Patients with IL-1 −511 CT or TT genotype	14†	13	16	25	14	28

LT, lymphotoxin.
*Mean age (SD 83) 7 years.
†Significantly different from the value for the same sex possessing the other genotype. $P<0{\cdot}05$ Mann–Whitney test.

levels of activity and differential sensitivity to stimulation by inflammatory agents. Alleles 1, 2 and 4 are poor promoters, while allele 3 causes high gene expression. A number of molecules suppress the production of pro-inflammatory cytokines and exert an anti-inflammatory influence. These include antioxidant defences and IL-10[25]. There are at least three polymorphic sites (−1082, −819, −592) in the IL-10 promoter which influence production[26]. Enhancement of antioxidant defences is important, in protecting healthy tissues and in preventing excessive activation of NF-κB by the oxidative cellular environment, during inflammation. SNP also occur in genes encoding enzymic components of antioxidant defences, such as catalase, superoxide dismutase and glutathione peroxidase, which influence levels of activity[27–29].

Thus each individual possesses combinations of SNP in the genes associated with inflammation, which will bestow on them 'inflammatory drives' of differing intensities. At an individual level this may express itself as differing degrees of morbidity and mortality. Circumstantial evidence of this phenomenon has been reported in a number of studies. The strength of the genomic influence on the inflammatory process may affect the chances of an individual developing inflammatory disease, particularly if their antioxidant defences are poor. In intensive care patients, the 1082 G high-producing allele, for IL-10, was present in those who developed multi-organ failure, with a frequency of only one fifth of that of the normal population[30]. In sepsis, patients with the TNFα −308 A allele had a 3·7-fold risk of death than those without the allele and patients who were homozygous for the LT-α A allele had twice the mortality rate and higher peak plasma TNFα concentrations than heterozygotic individuals[31,32]. An unresolved issue is whether the strength and outcome of low-intensity inflammation and its sequelae are influenced by SNP.

Gender gene effects

In general, males are more sensitive to the genomic influences on the strength of the inflammatory process than females[33]. In a study on LT-α+252 genotype and mortality from sepsis, it was found that males with an AA genotype had a mortality of 72% compared with men who were GG who had a 42% mortality rate. In female patients the mortalities for the two genotypes were 53 and 33%, respectively[34]. In a study on patients undergoing surgery for gastrointestinal cancer, it was found that post-operative C-reactive protein and IL-6 concentrations were higher in men than women and that in multivariate analysis, in which greater operation duration and blood loss in males was allowed for, males possessing the TNFα − 308 A allele had greater responses than men without this allele. The genomic influence was not seen in females (Table 2)[35]. In a study on hospitalised geriatric care patients, men possessing the 'less inflammatory' LT-α+252 AA or IL-1 −511 CT or TT genotype had a shorter 3-year survival rate than men possessing the LT-α+252 GG or AG, or IL-1 −511 CC genotype. Furthermore, possession of the IL-1 −511 T allele was associated with a 48% greater length of stay in hospital in men (Table 3)[36,37]. Women were unaffected by these genetic influences.

Genotype insulin sensitivity and body fat mass and distribution

Paradoxically, insulin insensitivity may, at first, exert a beneficial effect on the response to infection and injury, but has an adverse influence on chronic disease processes. Glucose and glutamine are major fuels for cells of the immune system. An insulin-insensitive state will reduce glucose uptake by tissues in which the process is insulin dependent (muscle) thereby increasing availability for tissues in which the process is not insulin dependent (immune tissue). During inflammation, secretion of catabolic hormones, which enhances muscle protein breakdown and glutamine release, will, as a secondary effect, oppose insulin action.

Many studies, conducted on large uninfected populations, have shown a clear link between obesity, oxidant stress and inflammation[38]. As indicated above, the link lies in the ability of adipose tissue to produce pro-inflammatory cytokines. There is also a positive relationship between adiposity and TNF production. A positive correlation has been noted between serum TNFα, TNFα production and BMI in non-insulin dependent diabetes

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mellitus patients and healthy women[39,40]. Thus plasma TAG, body fat mass and inflammation may be loosely associated because of these endocrine relationships. We investigated cytokine production in 139 healthy males and found that while there were no statistically significant relationships between BMI, plasma fasting TAG and the ability of peripheral blood mononuclear cells to produce TNFα in the study population as a whole, individuals with the LT-α+252 AA genotype (associated with raised TNF production) showed significant positive relationships between TNF production, BMI and fasting TAG[41]. Thus, although the study population was composed of healthy subjects, within that population were individuals with a genotype that resulted in an 'aged' phenotype as far as plasma lipids, BMI and inflammation were concerned. Furthermore, individuals with the 'aged' phenotype may be disadvantaged should they become hospitalised. It has become clear recently that adipose tissue at different sites around the body has differing propensity for inflammatory mediator production. Visceral adipose tissue has a greater potential for production of these molecules than subcutaneous adipose tissue. This difference in capacity explains the adverse influence of visceral obesity on CHD and insulin insensitivity[42,43].

Influence of genotype on anti-inflammatory responses to nutrients

As can be seen from the earlier sections of this paper, oxidant stress and genetic factors are potent determinants of pro-inflammatory cytokine production. A reduction in inflammatory stress can be achieved by feeding nutrients that either suppress pro-inflammatory cytokine production or act as antioxidants. Fish oil is in the first category and vitamin E and N-acetyl cysteine are in the second category. Rheumatoid arthritis and inflammatory bowel disease have been most successfully treated with fish oil[44]. The anti-inflammatory mechanism may be by means of suppression of pro-inflammatory cytokine production. Endres *et al.* showed that a large dose (15 g/d for 6 weeks) of the oil, in nine healthy volunteers, gave a small reduction in TNFα and IL-1β production from peripheral blood mononuclear cells[45]. Subsequently, less than half of 11 similar small intervention studies were unable to demonstrate a statistically significant reduction in cytokine production[20,46]. We have shown, however, that healthy subjects with the LT-α+252 A allele and IL-6 – 174 GG genotype responded to fish oil with a decrease in TNFα. Likewise phenotype influences responsiveness. A BMI >25 kg/m^2 bestows sensitivity to the anti-inflammatory effects of fish oil. Clearly, while the level of inflammation determines whether fish oil will exert an anti-inflammatory influence or not, and is influenced by both LT-α+252 and IL-6 – 174 G alleles, the precise genomic mechanism for an anti-inflammatory effect is unclear at present[2].

Antioxidant intake also modifies cytokine production. In a study on healthy men and women and smokers, dietary supplementation with α-tocopherol (600 IU/d) for 1 month suppressed the ability of PBMC to produce TNFα. Production was reduced by 22 and 33% in non-smokers and smokers, respectively[47]. In a dietary intervention study on normolipaemic and hypertriglyceridaemic subjects given 600 IU α-tocopherol/d for 6 weeks, reduced TNFα, IL-1-β and IL-8 production by lipopolysaccharide-stimulated blood mononuclear cells occurred[47,48]. A similar effect of α-tocopherol was noted in a study on normal subjects and type 2 diabetics[49]. However, there were large standard deviations in the data from these studies, indicating major intra-individual variability in the ability of vitamin E (and antioxidant status) to suppress the production of the cytokine. This phenomenon suggests a significant genomic influence.

While a number of studies have shown that α-tocopherol suppresses superoxide production, the situation with regard to nitric oxide is less clear[47,48]. At present, it is not known whether antioxidants interact with SNP in the genes associated with oxidant stress and inflammation in a differential manner as may occur with the other anti-inflammatory nutrient, *n*-3 PUFA[2].

Conclusions

Inflammation is both an essential process for human survival and one that plays a disadvantageous role in a wide range of diseases. Many of these diseases are common associates with the current epidemic of obesity that is assailing both industrialised and non-industrialised countries. Furthermore, the biological cost of this interrelationship is impacting adversely on health budgets. In addition to infective agents, inflammation can be induced by oxidant stress and obesity. The pro- and anti-inflammatory cytokines, nuclear transcription factors and antioxidant defences influence the intensity of this latter response. The recent insights from the characterisation of the human genome have revealed individual differences in the degree to which the key proteins in this physiological matrix are expressed. The variability in protein expression induced by SNP in the genes associated with the inflammatory process is being shown to be an important determinant of the strength of, and outcome from, the inflammatory process.

A number of studies have shown that these genomic factors impinge on a broad range of diseases. Studies are starting to show that individual responsiveness to nutrient therapy may be influenced by genomic factors. Thus individual responsiveness, to nutrients that can or might modulate inflammation, now has to be considered within the genomic framework that is currently unfolding during the post-genomic era. In this way the 'cost' of in-patient obesity may be reduced.

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