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n-3 Polyunsaturated fatty acids, inflammation and obesity-related disease

Lucy M. Browning
MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL, UK

Obese individuals are at increased risk from a range of metabolic diseases, including insulin resistance, dyslipidaemia and hypertension. Adipose tissue is an important endocrine organ, secreting a range of inflammatory mediators, including tumour necrosis factor α and interleukin 6. Circulating concentrations of these cytokines are increased in obesity and may contribute to the pathogenesis of metabolic diseases. The present review considers the evidence linking inflammation and obesity-related disease. The data show that an inflammatory phenotype, measured by serum sialic acid concentration, identifies individuals with insulin resistance, dyslipidaemia and hypertension. Serum sialic acid concentration increases progressively in obese individuals with none, one or multiple features of the metabolic syndrome, independent of BMI. Supplementation with long-chain *n*-3 polyunsaturated fatty acids has shown anti-inflammatory effects in studies of both healthy populations and in models of chronic inflammatory conditions. The effect on insulin sensitivity has been varied, with both positive and negative effects. This variability may relate to the metabolic characteristics of the study population; individuals with high background inflammation may derive greater benefits from *n*-3 polyunsaturated fatty acid supplements, suggesting a possible interaction between diet and phenotype. Future research is needed to fully evaluate the role of anti-inflammatory strategies in the dietary management of obesity.

***n*-3 Polyunsaturated fatty acids: Fish oil: Inflammation: Obesity**

The link between obesity and chronic disease is long established. Obese individuals are two to three times more likely to die prematurely than their lean counterparts (Calle *et al.* 1999), primarily due to the association between obesity and type 2 diabetes and CHD. BMI, a measure of obesity, is positively associated with the risk of developing insulin resistance in both men and women (Colditz *et al.* 1990; Chan *et al.* 1994). For an obese individual with a BMI of 30 kg/m^2 the risk of developing type 2 diabetes is increased thirteen times for men and twenty times for women relative to an individual with a BMI of 22 kg/m^2. Similarly, individuals with a BMI > 29 kg/m^2 have three times the risk of CHD relative to those with a BMI < 21 kg/m^2 (Manson *et al.* 1990). Central obesity, characterised by a preponderance of abdominal fat, is particularly associated with risk factors for CHD, such as hypertension, type 2 diabetes and dyslipidaemia (Alexander, 2001).

Even though the literature documenting an association between obesity and disease is extensive, the pathogenesis is not fully understood. A range of mechanisms have been hypothesised, including an increase in non-esterified fatty acids contributing to insulin resistance (Frayn, 2001), an increase in autonomic nervous system activity, which is associated with hypertension and dyslipidaemia (Landsberg, 1999), and dysregulation of the hypothalamic–pituitary–adrenal axis, which may be associated with each aspect of the metabolic syndrome (Andrews & Walker, 1999; Bjorntorp & Rosmond, 2000). Recently, it has been recognised that adipose tissue itself is an important endocrine organ and actively secretes many substances into the circulation, including complement factors, prostaglandins and soluble receptors (Fruhbeck *et al.* 2001). Many of these substances are involved in inflammation, and emerging evidence suggests that inflammation is an important modulator of obesity-related disease.

The role of inflammation

There is a positive association between adiposity and the levels of circulating cytokines. It has been shown in human subjects that plasma levels of the cytokine tumour necrosis factor (TNF) α are associated with obesity (Tsigos *et al.* 1999)

Abbreviations: CRP, C-reactive protein; CVD, cardiovascular disease; IL, interleukin; PUFA, polyunsaturated fatty acids; TNF, tumour necrosis factor.
Corresponding author: Lucy M. Browning, fax +44 1223 437515, email Lucy.Browning@mrc-hnr.cam.ac.uk

and decrease with weight loss (Kern *et al*. 1995; Dandona *et al*. 1998). Adipose tissue expression and secretion of TNF-α and interleukin (IL) 6 are also increased in obesity (Kern *et al*. 2001). This factor may explain the specific association between central fat and disease, since abdominal obesity is specifically associated with increases in cytokine concentrations in addition to the effects of body weight (Tsigos *et al*. 1999). It has been estimated that abdominal adipose tissue may produce up to three times as much IL-6 as subcutaneous adipose tissue (Fruhbeck *et al*. 2001).

During acute infection or injury cytokines (IL-6 and TNF-α) are released from the site of tissue injury and promote an acute-phase response. The acute-phase response is characterised by production of a range of proteins, primarily from hepatocytes but also from other cells such as monocytes, fibroblasts and adipocytes. These proteins, including C-reactive protein (CRP) and orosomucoid are involved in tissue repair, clearance of cellular debris and killing of infectious agents and can be raised by up to 100-fold above normal levels. Chronic low-grade inflammation, characterised by a less extreme elevation of acute-phase proteins, has been associated with a number of obesity-related health risks: peripheral vascular disease (Ridker *et al*. 2001); myocardial infarction (Danesh, 1999); hypertension (Chae *et al*. 2001); insulin resistance (Yudkin *et al*. 1999); type 2 diabetes (Pradhan *et al*. 2001); dyslipidaemia (Ridker *et al*. 2000). A prospective 7-year follow-up study examined the development of type 2 diabetes in individuals who had baseline measures for the acute-phase protein markers sialic acid and orosomucoid. Those individuals with baseline inflammation levels above the median were approximately four times more likely to develop type 2 diabetes based on sialic acid and approximately eight times more likely to develop type 2 diabetes based on orosomucoid (Schmidt *et al*. 1999). There is also evidence for the role of CRP as a predictor of disease. For example, a 3-year follow-up study of apparently healthy women showed that those with CRP concentrations in the highest quartile had a 4·4-fold increased risk of having a cardiovascular event when compared with those with concentrations in the lowest quartile (Ridker *et al*. 2000).

To date, evidence for a direct pathogenic role for inflammation in disease is limited. The TNF-α-knockout mouse model showed improved insulin sensitivity relative to wild-type mice when both were placed on a high-fat diet to induce obesity (Uysal *et al*. 1997). Using the *ob/ob* mouse model, a TNF-α receptor-knockout mouse was also created. In this model of a more severe genetic form of obesity mice with no functional TNF-α had significantly higher ($P < 0·001$) insulin sensitivity relative to that of the control *ob/ob* mice, although the improvement was not to the same extent as in the previous study (Uysal *et al*. 1997). These studies suggest that, in mice, TNF-α is a key link between obesity and insulin resistance. This finding may suggest a role for inflammation, although TNF-α may also reduce non-esterified fatty acids, improve glucose transport by increasing glucose transporter 4 expression and improve insulin receptor signalling, which may contribute to the reduction in disease. In human subjects, however, anti-TNF-α therapy in patients with type 2 diabetes had no impact on insulin sensitivity (Ofei *et al*. 1996).

Table 1. Variability in acute-phase response markers measured on three to six occasions in fifteen overweight men and women and the correlation with serum sialic acid

Acute-phase response marker	*F* ratio	R^2 (with serum sialic acid)
Sialic acid	37·1***	
Orosomucoid	19·8***	0·716***
C-reactive protein	10·3***	0·672***
α_1-Antichymotrypsin	6·7***	0·308***

*** $P < 0·0001$.

In order to be able to understand the relationship between obesity and inflammation it is important to have a reliable measure of habitual inflammatory status. A number of markers of acute-phase response have been used as measures of inflammatory status, but the ability of a single measure to appropriately reflect habitual status has not been assessed. Sialic acid, CRP, orosomucoid and α_1-antichymotrypsin were measured on three to six occasions in fifteen overweight men and women to identify a marker with the least variability that would provide a useful measure of habitual status (Browning *et al*. 2001). Table 1 shows the *F* ratio for each marker and the correlation with sialic acid. This analysis demonstrates that sialic acid provides the most reliable measure of habitual status when comparing inter- and intra-individual variability in overweight subjects. Each of the other acute-phase proteins measured correlated well with sialic acid, indicating that it is representative of the overall acute-phase response. This result is not surprising, as sialic acid is not an acute-phase protein but the terminal glycoprotein found as part of many acute-phase proteins. Thus, measuring serum sialic acid provides a useful biological measure of the overall acute-phase response and may be useful when defining status.

It is noteworthy that sialic acid is more stable than CRP, which has been widely used in many epidemiological analyses. A number of studies have shown that CRP is an important predictor of cardiovascular disease (CVD; Ridker *et al*. 2000; Ridker, 2001) and type 2 diabetes (Festa *et al*. 2002). The greater day-to-day variability in CRP relative to sialic acid would tend to reduce the agreement with long-term health outcomes, suggesting that inflammation may be a more important determinant of disease than previously recognised. There is, however, much less data on sialic acid.

To date, sialic acid has been shown by multiple regression analysis to be independently related to individual features of the metabolic syndrome: blood pressure; plasma insulin; serum cholesterol (Crook *et al*. 1998). The incremental association between sialic acid and multiple features of the metabolic syndrome was examined in a community-based study of 263 overweight women (Krebs *et al*. 2003). Subjects were excluded if they had symptoms of intercurrent infection, known diabetes, treated dyslipidaemia, a chronic inflammatory condition, liver disease, malignancy, smoked more than twenty-five cigarettes per day or had an alcohol intake of more than twenty-five units per week. Individuals were grouped based on the presence or absence of three additional features of the metabolic syndrome: insulin resistance (defined as a homeostasis model

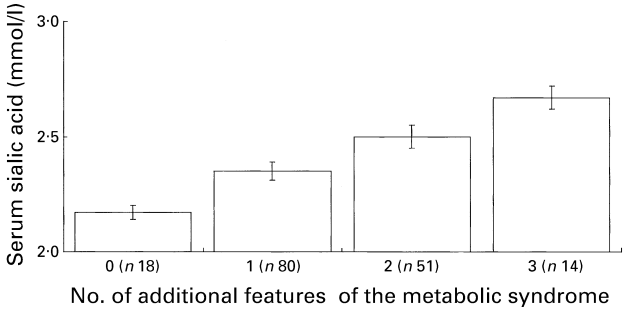

Fig. 1. Serum sialic acid concentration in 263 overweight women grouped according to the absence (0) or presence of additional features of the metabolic syndrome: insulin resistance; dyslipidaemia; hypertension (1–3). Values are means with their standard errors represented by vertical bars.

assessment value in the top tertile for the group); dyslipidaemia (defined as a fasting triacylglycerol level of $>1{\cdot}7$ mmol/l); hypertension (defined as systolic and diastolic readings of >160 and >90 mmHg respectively). The mean sialic acid concentration for each group was calculated. The number of subjects and the mean sialic acid concentrations for each of the groups are shown in Fig. 1. Linear regression analysis demonstrated a strong positive association between sialic acid and body weight, fatness, insulin resistance, dyslipidaemia and hypertension ($P<0{\cdot}0001$). Additionally, from an ordinal logistic regression model, there was a highly significant ($P<0{\cdot}001$) incremental association with none, one or multiple features of the metabolic syndrome. The odds ratio for multiple features of the metabolic syndrome compared with one or no feature was 2·7 times when sialic acid concentrations increased by 1 SD or 0·34 mmol/l (the model was adjusted for age, cigarette smoking status, alcohol consumption status, menopausal status, oral contraceptive and hormone-replacement therapy use). When further adjusted for BMI, the trend was attenuated but remained highly significant, with the odds ratio being 2·3 times for a 1 SD increase in sialic acid.

Although further data are needed in large cohorts, with hard end points, these preliminary data suggest that inflammation is an important modulator of obesity-related disease. This finding raises the possibility that dietary strategies can be used to decrease inflammation and improve disease outcome, independent of body weight.

Polyunsaturated fatty acids and obesity-related disease

In recent years there has been a shift in the relative proportions of different fatty acids in the diet. Reductions in saturated fat have been counterbalanced, in part, by increases in *n*-6 polyunsaturated fatty acid (PUFA). The value for *n*-6:*n*-3 PUFA has increased from historical estimates of approximately 4:1 to current estimates of between 10:1 and 20:1 (Simopoulos, 1999). The *n*-6:*n*-3 PUFA value is of metabolic interest, as the two groups of fatty acids compete directly with one another for enzymes for elongation and desaturation and for incorporation into cells, where they are able to influence cell functions such as signalling pathways and receptor function.

There are various dietary sources of *n*-3 and *n*-6 PUFA. α-Linolenic acid (a precursor *n*-3 PUFA) is found in green leafy vegetables, linseed and rapeseed oils, walnuts and Brazil nuts, while long-chain *n*-3 PUFA, especially eicosapentaenoic acid and docosahexaenoic acid, are found in marine or fish oils such as salmon, mackerel and herring. α-Linolenic acid may exert its biological effects directly or through conversion to long-chain *n*-3 PUFA such as eicosapentaenoic acid and docosahexaenoic acid. *n*-6 PUFA occur mostly in the diet as linoleic acid and can be found in sunflower, safflower and maize oils and many fruits, vegetables, nuts, grains and seeds.

It has been suggested that changes in *n*-6:*n*-3 PUFA and the absolute amounts of both these types of fatty acids may be linked to the risk of many chronic diseases, e.g. CVD and inflammatory disorders such as rheumatoid arthritis, asthma and Crohn's disease (Department of Health, 1994; Grimble, 1998). Interest in both the total amount and the type of fat in the diet is now the focus of much research. Absolute increases in *n*-3 PUFA have been suggested to be beneficial for health (Simopoulos, 1999). There is substantial epidemiological evidence for a protective or beneficial effect of fish. Greenland Inuits, who have a high total fat intake, have surprisingly low rates of CVD and type 2 diabetes (Dyerberg & Bang, 1979; Bjerregaard *et al.* 2000). However, evidence from human intervention studies for beneficial effects of long-chain *n*-3 PUFA is more limited. A recent meta-analysis examined the association between *n*-3 PUFA intake and risk of CHD. It was concluded that *n*-3 PUFA intake decreased overall mortality, deaths due to myocardial infarction and sudden death in patients with CHD (Bucher *et al.* 2002).

The potential mechanisms for the beneficial effects of *n*-3 PUFA on CVD risk relate to their hypotriacylglycerolaemic (Eritsland *et al.* 1994), anti-thrombotic (Vognild *et al.* 1998) and anti-fibrinolytic (Saynor & Gillott, 1992) effects. It is also hypothesised that they have anti-inflammatory effects. In animal studies the anti-inflammatory effects of *n*-3 PUFA have been demonstrated by the measurement of the cytokines TNF-α and IL-6 (Mulrooney & Grimble, 1993; Sadeghi *et al.* 1999). Human *n*-3 PUFA intervention studies have shown anti-inflammatory effects in patients with chronic inflammatory conditions such as rheumatoid arthritis (Geusens *et al.* 1994), asthma (Broughton *et al.* 1997), Crohn's disease (Belluzzi *et al.* 1996) and psoriasis (Mayser *et al.* 1998), and *n*-3 PUFA have been shown to alleviate symptoms of each disease. In healthy subjects decreases in pro-inflammatory cytokines such as TNF-α and IL-1β have been shown in both plasma (Caughey *et al.* 1996) and isolated cells (Endres *et al.* 1989). The anti-inflammatory effects of *n*-3 PUFA extend to specific functions of monocytes (Hughes, 1998) and neutrophils (Sperling, 1998). The anti-inflammatory actions of *n*-3 PUFA may be direct, such as their actions on transcription factors to influence gene expression, or mediated through eicosanoids. PUFA are incorporated into phospholipids and used to form eicosanoids, which have diverse biological functions in the coordination of immune and inflammatory processes. The eicosanoid formed is specific to the fatty

acid from which it is derived, eicosapentaenoic acid forms prostaglandins of the 3 series and leukotrienes of the 5 series, while the *n*-6 PUFA arachidonic acid forms 2 series prostaglandins and 4 series leukotrienes. *n*-3 PUFA form weaker pro-inflammatory eicosanoids than *n*-6 PUFA, and altering the relative proportions of *n*-3 and *n*-6 PUFA in the diet therefore modulates the overall inflammatory environment (Lee *et al*. 1985).

If the link between obesity and CVD is modulated by inflammation, *n*-3 PUFA may offer a useful anti-inflammatory dietary strategy to decrease obesity-related disease. This possibility has been tested in a controlled dietary intervention study by examining the differential impact of *n*-3 PUFA on CVD risk in groups with different inflammatory status (Browning *et al*. 2002). The subjects were female, premenopausal and non-diabetic, aged 19–51 years (mean 39 (SD 7) years) and had a BMI in the range 24–44 kg/m^2 (mean 30·8 (SD 5·1) kg/m^2). Fasting blood samples were collected and serum sialic acid was measured in sixty-three women in order to determine their inflammatory status. Serum sialic acid concentrations ranged from 1·61 to 2·70 mmol/l. Women in the top and bottom thirds of this population (see Fig. 2) were recruited to the randomised crossover study, representing groups with high and low inflammatory status respectively. Participants received five capsules daily providing either *n*-3 PUFA (1·3 g eicosapentaenoic acid and 2·9 g docosahexaenoic acid/d) or a placebo (2·8 g linoleic and 1·4 g oleic acid/d) for a period of 12 weeks for each treatment, with a 4-week washout between treatments. Detailed measures of CVD risk were made before and after each treatment period, including a five-point oral glucose tolerance test.

Preliminary analysis of this dataset has focused on insulin sensitivity. At baseline there were significant differences between the two groups, the group with the raised inflammatory status having significantly higher sialic acid, (2·19 (SD 0·25) *v*. 1·82 (SD 0·15) mmol/l; $P<0·001$), BMI (32·0 (SD 6·7) *v*. 28·0 (SD 5·7) kg/m^2; $P<0·05$) and area under the insulin curve, calculated from the oral glucose tolerance test (35 663 (SD 30 784) *v*. 22 796 (SD 10 091) pmol·min/l; $P<0·01$), but no significant difference in fasting insulin, glucose, homeostasis model assessment or area under the glucose curve (Browning *et al*. 2002). These findings support previous evidence of the association between obesity-related disease and inflammatory status. Fig. 3 provides evidence of an interaction between inflammatory status and insulin sensitivity. *n*-3 PUFA supplementation was associated with a significant improvement in area under the insulin curve in the group with the raised inflammatory status ($P<0·05$), whilst there was no significant improvement with the placebo treatment. There were no changes with *n*-3 PUFA or placebo in the low inflammatory status group. When comparing the effect of *n*-3 PUFA with that of the placebo treatment directly there was a significant difference between the final time points for each supplementation period, again only in the high inflammatory status group ($P<0·05$). There were no significant differences in weight, fasting glucose or insulin or area under the glucose or insulin curves during either the *n*-3 PUFA or placebo treatment individually or when comparing the treatment end points directly. The present study will go on to examine other aspects of CVD risk.

Previous studies have shown variable results when reporting effects of *n*-3 PUFA on insulin sensitivity. Animal studies have demonstrated protective effects of *n*-3 PUFA. For example, rats fed high-fat diets developed insulin resistance, while those fed high-fat diets also high in *n*-3 PUFA remain insulin sensitive (Storlien *et al*. 1987). In human studies no effect of *n*-3 PUFA on insulin sensitivity was shown in hypertensive subjects (Mori *et al*. 1999) but

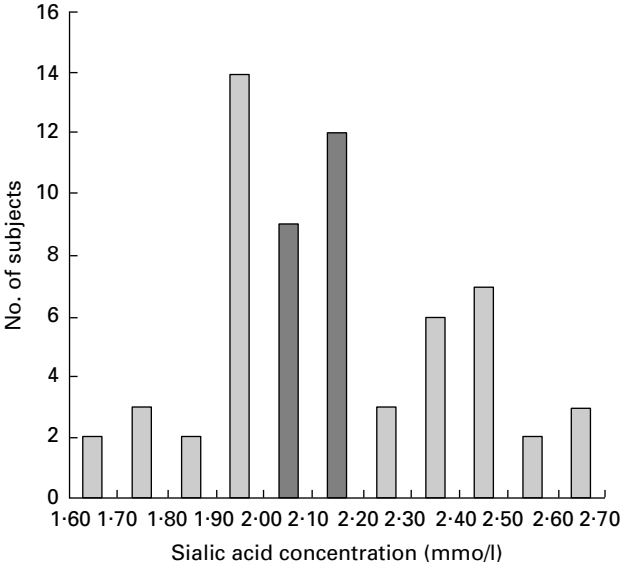

Fig. 2. Distribution of serum sialic acid concentrations in sixty-three women screened for participation in the *n*-3 PUFA intervention study, grouped into thirds. (▒), Women not recruited to the study.

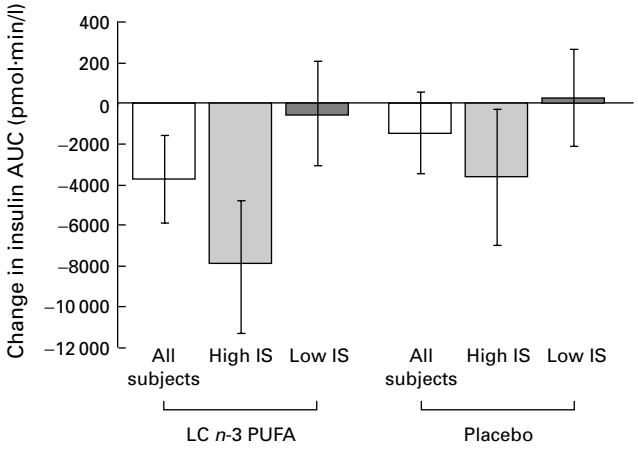

Fig. 3. Change in area under the curve (AUC) for insulin (final – baseline) with *n*-3 polyunsaturated fatty acid (PUFA) and placebo supplementation for premenopausal non-diabetic female subjects aged 19–51 years, with BMI in the range 24–44 kg/m^2 who were grouped according to inflammatory status (IS; based on serum sialic acid (mmol/l); low IS <2·00, high IS > 2·20). LC, long-chain. Values are means with their standard errors represented by vertical bars. Mean value was significantly different from that for the placebo treatment: $^*P<0·05$.

positive effects have been reported in hypertriacylglycerolaemic subjects (Eritsland *et al*. 1994). Results for diabetic subjects have been mixed, with both positive (McManus *et al*. 1996) and negative (Popp-Snijders *et al*. 1987; Vessby & Boberg, 1990) effects. Our findings provide evidence to support a role for *n*-3 PUFA in improving insulin sensitivity. The design of the study, separating individuals by inflammatory status, also identifies a possible reason why some previous studies may not have shown marked improvements in insulin sensitivity with *n*-3 PUFA. It suggests that individuals with an inflammatory phenotype respond best to this type of strategy to improve the risk of obesity-related disease, and this factor may have important implications for the management of obese individuals.

The inflammatory phenotype identified in the present study, in terms of habitual serum sialic acid, presumably reflects an underlying inflammatory genotype. Several candidate genes that may influence inflammatory status have already been identified, for example, in the cytokines IL-6 and TNF-α. These genotypes have not only been associated with chronic inflammatory conditions but also risk factors for CHD (Humphries *et al*. 2001) and insulin resistance (Dalziel *et al*. 2002). More recently, inflammatory genotypes have been shown to be associated with habitual inflammatory status, measured by serum CRP (Vickers *et al*. 2002).

Conclusions

Inflammation may be an important modulator of the relationship between obesity and the metabolic syndrome. Inflammatory status, identified using serum sialic acid, also shows a significant ($P < 0{\cdot}001$) incremental association with the presence of none, one or multiple additional features of the metabolic syndrome, suggesting that it may be a useful predictor of disease and premature mortality. Sialic acid is a more stable marker of the acute-phase response than the more commonly used high-sensitivity CRP.

The mechanisms through which obesity is associated with insulin resistance and CVD may in part be mediated through inflammation. There is now some preliminary evidence that dietary *n*-3 PUFA can modulate at least some aspects of disease risk. Further research into the mechanism of action of *n*-3 PUFA is required, but emerging data raise the possibility that the effect of *n*-3 PUFA is achieved in part through an anti-inflammatory mechanism. This integrated hypothesis is shown in Fig. 4 and has potentially important implications for the dietary management of obesity.

Although *n*-3 PUFA are probably the most extensively studied nutrients with anti-inflammatory actions, there is limited evidence that a number of other dietary components may also have similar effects, which may in part explain their beneficial effects on obesity-related disease. For example, monounsaturated fatty acids have been shown to improve insulin sensitivity (Vessby *et al*. 2001) and have anti-inflammatory effects (Yaqoob *et al*. 1998), and glycaemic index has been correlated with serum CRP in a cross-sectional study (Liu *et al*. 2002) and positively related to CHD risk in a 10-year follow-up (Liu *et al*. 2000). This area is an exciting one for future nutrition research.

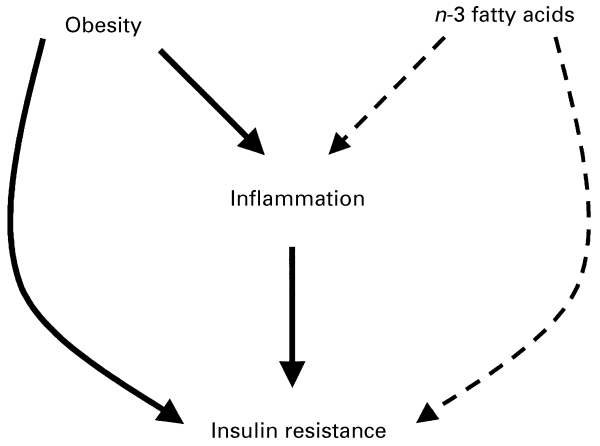

Fig. 4. The role of inflammation in obesity-related disease. Positive relationships (——); inverse relationships (– – – –).

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