Proceedings of the Nutrition Society (2004), **63**, 115–125 DOI:10.1079/PNS2003318

Dietary PUFA and the metabolic syndrome in Indian Asians living in the UK

Louise M. Brady*, Christine M. Williams and Julie A. Lovegrove
Hugh Sinclair Human Nutrition Unit, School of Food Biosciences, PO Box 226, University of Reading, White Knights, Reading RG6 6AP, UK

Indian Asians living in the UK have a 50% higher CHD mortality rate compared with the indigenous Caucasian population, which cannot be attributed to traditional risk factors. Instead, features of the metabolic syndrome, including raised plasma triacylglycerol, reduced HDL-cholesterol (HDL-C) and an increased proportion of small dense LDL particles, together with insulin resistance and central obesity, are prevalent among this population. The present review examines evidence to support the hypothesis that an imbalance in dietary PUFA intake, specifically a higher intake of *n*-6 PUFA in combination with a lower intake of the long-chain (LC) *n*-3 PUFA, plays an important role in the prevalence of the metabolic syndrome observed in Indian Asians. Data are presented to illustrate the impact of manipulation of the background *n*-6 PUFA intake (moderate or high *n*-6 PUFA) and the subsequent response to supplementation with LC *n*-3 PUFA on blood lipids and insulin action in a group of Indian Asian volunteers. The results demonstrate that supplementation with LC *n*-3 PUFA had no impact on insulin action in those subjects consuming either the moderate- or high-*n*-6 PUFA diet. In the postprandial phase reductions in plasma triacylglycerol concentrations were greater in those consuming the high-*n*-6 PUFA background diet subsequent to fish oil supplementation. The present study concludes that, contrary to the central hypothesis, the prevalence of metabolic abnormalities in Indian Asians compared with Caucasians may not be attributable to differences in intakes of *n*-6 and *n*-3 PUFA.

Indian Asians: Metabolic syndrome: PUFA: Fish oils: Insulin sensitivity

Indian Asians living in the UK have an approximately 50% higher CHD mortality rate compared with the native Caucasian population (British Heart Foundation, 2003), which is not attributable to an increased incidence of conventional risk factors such as cigarette smoking, hypertension or hypercholesterolaemia (Dhawan & Petkar, 1998; Chambers *et al*. 2000). However, evidence has confirmed that features of the metabolic syndrome have a higher prevalence among Indian Asians compared with Caucasians (Banerji *et al*. 1999; Zoratti *et al*. 2000). It has been hypothesised that the prevalence of metabolic abnormalities among this ethnic population may be related to an imbalance in dietary PUFA intake (Ghafoorunissa, 1998), specifically the higher intake of *n*-6 PUFA in combination with the lower intake of long-chain (LC) *n*-3 PUFA that has been reported previously in this group (Sevak *et al*. 1994). The aim of the present review is to summarise the evidence demonstrating that features of the metabolic syndrome are prevalent among UK Indian Asians and to examine evidence of the putative protective effects of LC *n*-3 PUFA supplementation against the development of risk factors for CHD. Finally, the hypothesis that an imbalance in dietary PUFA intake may modulate the putative beneficial effects of fish oil supplementation is tested in a dietary intervention study involving manipulation of background *n*-6 PUFA intake and subsequent observation of the effects of fish oil supplementation on CHD risk factors in Indian Asians.

Metabolic syndrome and Indian Asians

Features of the metabolic syndrome that have been reported among migrant Indian Asians include dyslipidaemia, comprising a raised plasma triacylglycerol (TAG) concentration (Chambers *et al*. 2000), reduced HDL-cholesterol (HDL-C) concentrations (McKeigue *et al*. 1991) and increased

Abbreviations: ALNA, α-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HDL-C, HDL-cholesterol; HOMA IR, homeostasis model for insulin resistance; LA, linoleic acid; LC, long chain; LDL3, small dense LDL particles; LDL-C, LDL-cholesterol; LPL, lipoprotein lipase; QUICKI, quantitative insulin-sensitivity check index; TAG, triacylglycerol.

***Corresponding author:** Ms Louise M. Brady, fax +44 118 931 0080, email l.brady@reading.ac.uk

circulating levels of the pro-atherogenic small dense LDL particles (LDL3; Kulkarni *et al*. 1999). Additional metabolic abnormalities typically observed among Indian Asians compared with Caucasians include central obesity (Banerji *et al*. 1999) and insulin resistance (Zoratti *et al*. 2000). Our research group has recently confirmed a prevalence of these metabolic abnormalities in a group of fifty-five Indian Asians compared with fifty-five Caucasian men (Lovegrove *et al*. 2003*a*).

The exact mechanisms linking features of the metabolic syndrome have yet to be elucidated. However, it is generally accepted that normal insulin-stimulated suppression of hormone-sensitive lipase-mediated release of NEFA from the adipocyte is defective in insulin-resistant individuals (Frayn *et al*. 1997). The resultant continuous delivery of NEFA to the liver is thought to have several negative effects, including enhanced gluconeogenesis, increasing hepatic glucose output and hence hyperglycaemia (Lam *et al*. 2003). Impaired binding and hepatic extraction of insulin from the circulation has also been previously reported among individuals presenting with elevated NEFA concentrations, further exacerbating hyperinsulinaemia (Wajchenberg, 2000). Finally, re-esterification of excess NEFA, in the liver, to TAG and subsequent incorporation into liver-derived VLDL increases circulating plasma TAG concentrations. In insulin-resistant individuals the lack of responsiveness to insulin-stimulated lipoprotein lipase (LPL)-mediated hydrolysis of TAG from TAG-rich lipoproteins leads to an increased period of residence for TAG in the circulation (Karpe, 1999). Elevated plasma TAG concentrations enable enhanced neutral-lipid exchange between TAG-rich lipoproteins and the cholesterol-containing lipoproteins, HDL and LDL, via cholesteryl ester transfer protein, leading to a preponderance of LDL3 and reduced HDL concentrations (Karpe, 1999). In summary, the adverse effects of abnormal responses to insulinaemia, including continuous lipolysis and subsequent delivery of NEFA to the liver, in combination with impaired fatty acid uptake into adipocytes, may together exacerbate the dyslipidaemia associated with the metabolic syndrome.

Assessment of insulin action

As insulin resistance is a major characteristic of the metabolic syndrome, its accurate assessment is important in facilitating the investigation of the links between insulin resistance and other features of the metabolic syndrome, as well as in the formulation of effective preventative strategies. The glucose-clamp technique (DeFronzo *et al*. 1979) is considered to be the gold standard for the measurement of insulin sensitivity, although the more recently developed minimal model provides a well-validated alternative and has been shown to have a significant association with the glucose clamp (r 0·55, $P < 0·001$; Saad *et al*. 1994). Both these procedures involve intervention with glucose and insulin to mimic normal body homeostasis. However, both techniques are time-consuming and invasive, rendering them unsuitable for use when determining the insulin status of large population groups. As a consequence, recent research has focused on the development of simpler less-invasive surrogate measures of insulin resistance and sensitivity, which can be easily calculated from fasting insulin and glucose concentrations.

Surrogate measures documented in the literature include the homeostasis model for insulin resistance (HOMA IR; Matthews *et al*. 1985), which is the most commonly employed surrogate measure and provides a reliable alternative to the more robust glucose clamp (r −0·82, $P < 0·0001$; Bonora *et al*. 2000) and minimal model techniques (r −0·51, $P < 0·00$; Fukushima *et al*. 2000). Other surrogate methods, including fasting insulin (Folsom *et al*. 1994), Bennetts Index (Anderson *et al*. 1995) and insulin : glucose (Caro, 1991), also demonstrate good agreement with insulin sensitivity obtained by the more-invasive techniques (Laasko, 1993; Ikeda *et al*. 2001; McAuley *et al*. 2001). The recently developed quantitative insulin-sensitivity check index (QUICKI; Katz *et al*. 2000), which takes the product of both the reciprocal and the logarithm of fasted insulin and glucose concentrations, gives values that correlate well with the glucose clamp (r 0·78, $P < 0·00$) and minimal model (r 0·52, $P < 0·00$) techniques (Katz *et al*. 2000). However, the revised version of QUICKI (revised QUICKI; Perseghin *et al*. 2001), which incorporates a value for fasting NEFA into the QUICKI formula, has a stronger relationship with the glucose clamp (r 0·51, $P < 0·05$) than QUICKI alone (Perseghin *et al*. 2001). As described earlier, there are strong metabolic justifications for the inclusion of NEFA in surrogate measures of insulin sensitivity. A recent study in our research group examined the relationships between surrogate measures of insulin action (Table 1) and insulin sensitivity from a frequently-sampled intravenous glucose tolerance test and subsequent minimal model analysis

Table 1. Surrogate measures of insulin action

	Definition	Reference
Insulin-sensitivity measures		
QUICKI	$1/\log(\text{glucose}_0\ (\text{mg/dl})) + \log(\text{insulin}_0\ (\mu\text{U/ml}))$	Katz *et al.* (2000)
Revised QUICKI	$1/\log(\text{glucose}_0\ (\text{mg/dl})) + \log(\text{insulin}_0\ (\mu\text{U/ml})) + \log(\text{NEFA}_0\ (\text{mmol/l}))$	Perseghin *et al.* (2001)
Insulin-resistance measures		
HOMA IR	$\text{Insulin}_0\ (\mu\text{U/ml}) \times \text{glucose}_0\ (\text{mmol/l})/22{\cdot}5$	Matthews *et al.* (1985)
FIRI	$\text{Insulin}_0\ (\mu\text{U/ml}) \times \text{glucose}_0\ (\text{mmol/l})/25$	Frost *et al.* (1998)
Bennetts index	$1/\log(\text{glucose}_0\ (\text{mmol/l})) \times \log(\text{insulin}_0\ (\mu\text{U/ml}))$	Anderson *et al.* (1995)
Fasting insulin	One fasting insulin (μU/ml)	Folsom *et al.* (1994)
Insulin : glucose	$\text{Insulin}_0\ (\mu\text{U/ml}) : \text{glucose}_0\ (\text{mmol/l})$	Caro (1991)

QUICKI, quantitative insulin sensitivity check index; HOMA IR, homeostasis model for insulin resistance; FIRI, fasting insulin resistance index.

https://doi.org/10.1079/PNS2003318 Published online by Cambridge University Press

(Haffner *et al*. 1996). Results demonstrated a strong significant positive relationship between revised QUICKI (insulin sensitivity) and the minimal model (r 0·67, $P < 0·000$; Brady *et al*. 2003*b*). A significant positive association was also observed between the minimal model and QUICKI alone (r 0·51, $P = 0·007$), and significant negative relationships were observed between several measures of insulin resistance and insulin sensitivity derived from the minimal model (HOMA IR r −0·50, $P = 0·009$; fasting insulin resistance index r −0·50, $P = 0·008$; fasting insulin r −0·44, $P < 0·05$; Brady *et al*. 2003*b*). Although in our study revised QUICKI emerged as the most useful surrogate estimate of insulin action, it did not distinguish between Indian Asians and Caucasians, probably because of the small numbers of subjects (n 13 and 14 respectively) However, in our previous study, which included fifty-five males in each study group, the revised QUICKI demonstrated significantly lower insulin sensitivity in Indian Asians than in Caucasians ($P < 0·05$; Lovegrove *et al*. 2003*a*). The revised QUICKI has also been reported to differentiate between normal individuals and offspring of diabetic parents (Perseghin *et al*. 2001). However, to date, this surrogate technique has not been applied widely in studies, and there is a need for its further validation in different population groups with varying extents of insulin resistance.

Dietary PUFA intake in Indian Asians

Few studies have investigated dietary fatty acid intake among Indian Asians living in the UK. However, a higher intake of *n*-6 PUFA, mainly from linoleic acid (LA), has been reported from both household inventories (McKeigue *et al*. 1985) and weighed dietary records (Miller *et al*. 1988) in Indian Asians compared with Caucasians. Additionally, lower intakes of the cardio-protective LC *n*-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been observed in Indian Asians compared with Europeans (Sevak *et al*. 1994). Previously, in our research group, a significantly higher *n*-6 PUFA intake, a significantly lower LC *n*-3 PUFA intake and a significantly higher *n*-6:*n*-3 dietary PUFA was observed among Indian Asians compared with Caucasians (*n*-6:*n*-3 PUFA 11·2 *v*. 6·7 respectively; $P < 0·001$) from analysis of 5 d estimated diet diaries (Lovegrove *et al*. 2003*b*). Although prospective dietary investigation tools are commonly used for assessing nutritional intake among the population, they are limited by the tendency for volunteers to modify their diet in order to avoid recording complex meals and food items (MacDiarmid & Blundell, 1997). Furthermore, the inherent limitations involved with the determination of nutritional intake by nutrient database, such as a lack of foods or insufficient nutritional information for some foods in the database, may limit the usefulness or accuracy of nutritional data, particularly when studying ethnic populations. To validate the nutrient database used in our studies a small substudy was conducted to investigate differences in dietary fatty acid intake between Indian Asians (n 9) and Caucasians (n 9) using 3 d duplicate diet food collection (Brady *et al*. 2003*a*). Duplicate diet food collection and subsequent chemical analysis is thought to provide a more accurate representation of nutritional intake compared with alternative methods (Mertz, 1992; Isaksson, 1993); mainly because it does not rely on memory, portion size estimates and nutrient database-derived nutritional information (Isaksson, 1993). Our study confirmed the previously reported higher *n*-6 PUFA intake, lower LC *n*-3 PUFA intake and a higher *n*-6:*n*-3 dietary PUFA among the Indian Asians compared with Caucasians, although values did not reach significance, probably because of the small numbers in the study groups (Fig. 1; Brady *et al*. 2002). This study also observed a significantly lower intake of saturated fatty acids in Indian Asians ($P < 0·05$), which has been reported previously by other authors and which may explain the lower LDL-cholesterol concentrations observed in this population group compared with Caucasians (Miller *et al*. 1988; McKeigue *et al*. 1989). Dietary differences between the two ethnic groups were also reflected in the composition of membrane and plasma phospholipids in Indian Asians compared with Caucasians, both in our study (Lovegrove *et al*. 2003*b*) and in other studies (Miller *et al*. 1988; Das *et al*. 1994).

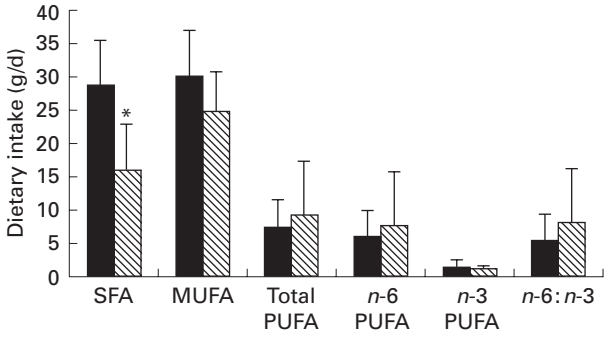

Fig. 1. Comparison of dietary fatty acid intake in Indian Asian Sikhs (▨; n 9) and Caucasians (■; n 9), determined by duplicate diet analysis of 3 d food collection. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; *n*-6:*n*-3, dietary *n*-6:*n*-3 PUFA. Values are means with their standard errors respresented by vertical bars. Mean value was significantly different from that for the Caucasians: *$P < 0·05$.

n-6 and *n*-3 PUFA

Man is unable to synthesise LA (18:2*n*-6) or α-linolenic acid (ALNA; 18:3*n*-3); thus, these fatty acids are known as essential fatty acids. ALNA is the parent of the LC *n*-3 PUFA EPA (20:5*n*-3) and DHA (22:6*n*-3). Dietary intake of ALNA is derived chiefly from vegetable oils, with rapeseed and soyabean oils being traditionally rich sources, although a large proportion of ALNA is lost during the hydrogenation stage of processing (Simopolous, 1991). The richest natural source of the LC *n*-3 PUFA EPA and DHA in the diet is oily fish such as mackerel and herring (British Nutrition Foundation, 1999). LA is the precursor for arachidonic acid (20:4*n*-6) and, in the diet, is predominantly derived from seed oils, e.g. mustard, maize and rapeseed oils (British Nutrition Foundation, 1999). LA and ALNA are converted to their LC PUFA metabolites through a series of enzymic reactions involving desaturation and chain elongation. Although the LC *n*-6 and *n*-3

https://doi.org/10.1079/PNS2003318 Published online by Cambridge University Press

PUFA are non-convertible, their pathways are in constant competition for the same enzymes, with the Δ6-desaturase enzyme having a preference for ALNA compared with LA. However, as the dietary intake of LA is considerably greater than that of ALNA, there is an increased production of arachidonic acid relative to the ALNA metabolites EPA and DHA (Horrobin, 1991; Emken *et al.* 1994). As the LC *n*-3 PUFA are cardio-protective, it has been proposed that the greater intake of *n*-6 PUFA relative to *n*-3 PUFA in the Indian Asian diet may be in part responsible for favouring a less-desirable atherogenic profile, contributing to the elevated CHD risk observed among the Indian Asian population (Ghafoorunissa, 1998).

Beneficial effects of fish oils

Evidence of the unique properties of the LC *n*-3 PUFA in reducing CHD risk factors emerged initially from the identification of an inverse relationship between the traditional Inuit diet and mortality rates from myocardial infarction among Inuits (Dyerberg & Bang, 1982). These findings were attributed to the abundance of the LC *n*-3 PUFA EPA and DHA in the Inuit diet, arising from a high intake of seal, whale and fish (Kromhout *et al.* 1985). Since these earlier observations the cardio-protective effects of a daily LC *n*-3 PUFA supplement have been identified in several large-scale intervention studies (Burr *et al.* 1989; Singh *et al.* 1997; GISSI-Prevenzione Investigators, 1999).

The multi-faceted actions of fish oils include production of less-potent eicosanoids with anti-inflammatory and anti-thrombotic effects compared with those produced by *n*-6 PUFA. For example, LC *n*-3 PUFA reduces synthesis of thromboxane A2 from arachidonic acid, which causes platelet aggregation and vasoconstriction, and alternatively initiates production of the less-potent thromboxane A3 (Connor & Connor, 1997). There have been consistent reports of the hypotriacylglycerolaemic effects of a moderate daily fish oil supplement from cross-sectional and intervention studies (Connor & Connor, 1997). Other benefits include small reductions in blood pressure, platelet aggregation and cardiac arrthymias in animals subsequent to fish oil supplementation (Connor & Connor, 1997; O'Keefe & Harris, 2000).

PUFA and insulin action

Insulin action is known to vary according to dietary fatty acid composition, and studies in both animals and human subjects have determined a positive relationship between saturated fat intake and insulin resistance (Storlien *et al.* 2000; Rivellese, 2000). Furthermore, animal studies have shown that supplementation with LC *n*-3 PUFA can improve insulin action, reversing the adverse effects of saturated fatty acids (Storlien *et al.* 1996). However, Jucker *et al.* (1999) observed that feeding animals an *n*-6 PUFA-rich oil intensified insulin resistance when compared with feeding LC *n*-3 PUFA.

Prospective studies of human subjects report a protective effect of previous fish intake on the development of insulin resistance (Feskens *et al.* 1991, 1995). However, there are no consistent reports of improvements in insulin action in response to LC *n*-3 PUFA supplementation in dietary intervention studies. The majority of studies have been conducted in patients with diabetes, and it is generally accepted that LC *n*-3 PUFA supplementation does not have a negative effect (Storlien *et al.* 1996) and may improve insulin sensitivity in diabetics (Popp-Snijders *et al.* 1987) and in patients with impaired glucose tolerance (Fasching *et al.* 1991). Other studies have shown no effect of LC *n*-3 PUFA supplementation in hypertensive (Toft *et al.* 1995) or hypertriacylglycerolaemic (Eritsland *et al.* 1994) patients. However, few groups have investigated the impact of LC *n*-3 PUFA supplementation in normal healthy volunteers. One recent study reported an improvement in insulin sensitivity in 50% of the study population in response to a daily supplement of 1·8 g DHA (Denkins *et al.* 2002). Waldhausl *et al.* (1989) also reported improvements, while Rivellese (2000) observed no change in insulin action in response to a daily supplement of fish oil (3·6 g). Similarly, Lovegrove *et al.* (2003*b*) observed no change in insulin action (measured by HOMA IR) in healthy Caucasian or Indian Asian volunteers subsequent to a moderate daily fish oil supplement containing 2·5 g EPA + DHA. Overall, animal studies consistently suggest a beneficial impact of LC *n*-3 PUFA on insulin sensitivity, while evidence in human subjects is limited and largely inconclusive.

Hypotriacylglycerolaemic effects of long-chain *n*-3 PUFA supplementation

The hypotriacylglycerolaemic effects of LC *n*-3 PUFA supplementation have been investigated previously in a range of study populations and using a variety of doses. Observations have consistently reported reductions in TAG in both healthy (Schmidt *et al.* 1990) and hyperlipidaemic (Harris *et al.* 1991) patients at supplementation levels of 3–4 g LC *n*-3 PUFA/d. In addition to the effects on fasting TAG concentrations, the beneficial effects of LC *n*-3 PUFA have also been reported to elicit a decrease in the magnitude of the postprandial lipidaemic response in both normal (Brown & Roberts, 1991) and hypertriacylglycerolaemic (Zampelas *et al.* 1994) patients.

The majority of studies have been conducted in Caucasian groups, although some studies have investigated the effects of LC *n*-3 PUFA supplementation on blood lipids in Indian Asians. Our previous study demonstrated that a daily LC *n*-3 PUFA supplement (2·5 g EPA + DHA/d) over a 12-week period resulted in a significant reduction in fasting plasma TAG concentrations ($P = 0{\cdot}01$; Lovegrove *et al.* 2003*b*), while in comparison supplementation with DHA alone for a 6-week period had no impact on blood lipids (Conquer & Holub, 1998). Furthermore, Indu & Ghafoorunissa (1992) showed in a group of Indian Asians that daily supplementation with 1·4 g Max EPA/d over a 3-week period resulted in a reduction in fasting plasma TAG concentrations. Thus, the hypotriacylglycerolaemic effects of fish oil supplementation are similar to observations in Caucasian volunteers.

https://doi.org/10.1079/PNS2003318 Published online by Cambridge University Press

Mechanisms of action for *n*-6 and long-chain *n*-3 PUFA

Postulated mechanisms for the putative beneficial effects of LC *n*-3 PUFA on insulin action include beneficial alterations in the physical properties of the cellular membranes, such as fluidity (Lovejoy, 2002). Studies in Caucasians, by our research group (Lovegrove *et al*. 2003*b*) and by other groups (Boberg *et al*. 1986; Harris *et al*. 1991), have shown that supplementation with LC *n*-3 PUFA causes a marked increase in the EPA and DHA content of plasma and platelet phospholipids with a concomitant reduction in arachidonic acid. However, it has been suggested that increased dietary LC *n*-3 PUFA may alter the binding affinity of the insulin receptor and improve glucose transport into cells via glucose transporters (Vessby, 2000). The hypotriacylglycerolaemic effects of LC *n*-3 PUFA are believed to be the result of a combination of suppressed production and secretion of hepatically-derived TAG and an accelerated rate of TAG clearance from the circulation (Westphal *et al*. 2000). It has also been suggested that LC *n*-3 PUFA may markedly reduce plasma TAG by stimulating an increase in LPL activity. This reduction has been observed in patients consuming a LC *n*-3 PUFA-rich meal when compared with those receiving a saturated fatty acid-rich meal (Zampelas *et al*. 1994). Murphy *et al*. (1999) investigated the effects of fish oil on gene expression of LPL in human adipose tissue, and reported increased LPL mRNA expression following consumption of a LC *n*-3 PUFA-enriched diet when compared with a non-enriched diet. Increased fatty acid oxidation and reduced fatty acid synthesis decrease fatty acid availability for TAG synthesis and incorporation into lipoproteins, while increased LPL activity in response to fish oil treatment may accelerate clearance of TAG from the circulation (Weber & Raederstorff, 2000).

The involvement of a genetic component in the regulation of insulin action by dietary fatty acid intake has also been suggested. LC *n*-3 PUFA may elicit their hypotriacylglycerolaemic effects via activation of transcription factors, such as the PPAR family, for which they are known to be natural ligands and potent activators (Clarke, 2000). *n*-3 PUFA are thought to play a more beneficial role in preserving insulin sensitivity than *n*-6 PUFA, through their actions on gene expression of proteins involved in glucose and insulin action (Clarke, 2000), although this role requires further investigation.

To test the hypothesis that an imbalance in dietary *n*-6 and *n*-3 PUFA is related to the prevalence of metabolic risk factors observed among UK Indian Asians compared with Caucasians, the effects of consumption of either a moderate- or a high-*n*-6 PUFA diet and subsequent fish oil supplementation on fasting and postprandial TAG concentrations and insulin sensitivity were assessed in a group of Indian Asian volunteers.

Materials and methods

Study design

Twenty-nine healthy Indian Asian Sikh males were recruited from the Reading and Slough areas (mean age 48 (SE 2) years; BMI 25 (SE 1) kg/m^2; TAG 1·6 (SE 0·1) mmol/l; insulin 44 (SE 3) pmol/l) to participate in this randomised double-blind parallel-design study (Fig. 2). Experimental cooking oils and spreads formulated to provide either a moderate or a high *n*-6 PUFA content were used to alter the background dietary *n*-6 PUFA intake, to achieve either a moderate dietary *n*-6 : *n*-3 PUFA of 9 or a high dietary *n*-6 : *n*-3 PUFA of 16. Alteration of the *n*-6 PUFA content of the spreads and oils was achieved by varying the amounts of MUFA and *n*-6 PUFA, and included an olive oil-based spread and olive oil containing 710 and 790 g MUFA/kg and 60 and 60 g *n*-6 PUFA/kg respectively (moderate-*n*-6 : *n*-3 PUFA diet), or a maize oil-based spread and maize oil providing 300 and 320 g MUFA/kg and 460 and 530 g *n*-6 PUFA/kg respectively (high-*n*-6 : *n*-3 PUFA diet). The levels of total fat, saturated fatty acids, *trans*-fatty acids and *n*-3 PUFA in the two diets were comparable. The oils and spreads were provided by Van den Bergh Oils (Crawley, West Sussex, UK). At the start of the study volunteers were randomised to consume either the moderate (olive oil-based)- or the high (maize oil-based)-*n*-6 PUFA cooking oils and spreads for a

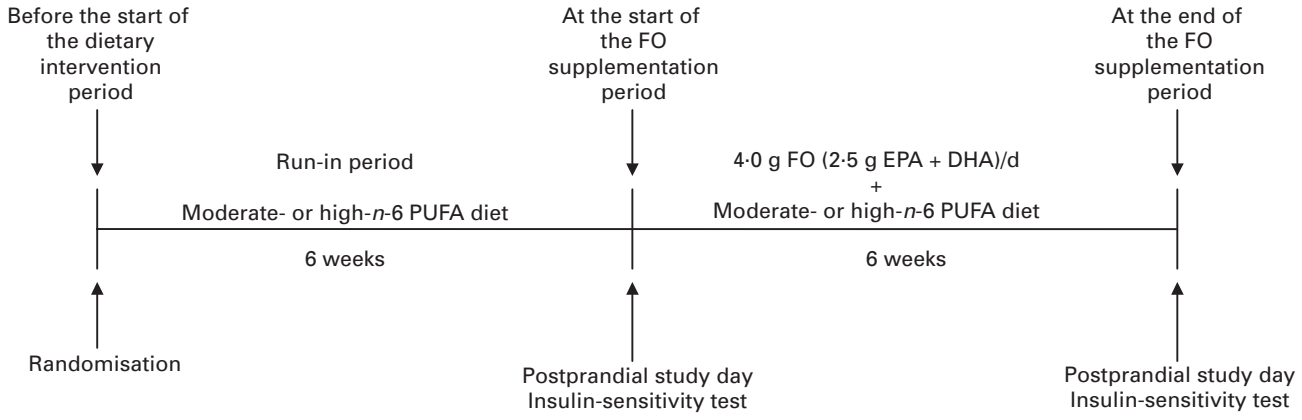

Fig. 2. Overview of the design of the study to assess the effects of consumption of either a moderate- or a high-*n*-6 PUFA diet and subsequent fish oil (FO) supplementation on fasting and postprandial TAG concentrations and insulin sensitivity in a group of Indian Asian volunteers. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

https://doi.org/10.1079/PNS2003318 Published online by Cambridge University Press

6-week run-in period (Fig. 2). In order to achieve the dietary targets for the background diet volunteers were requested to use the experimental oils and spreads during food preparation and cooking in place of their normal oils and spreads.

For the second 6 weeks of the study volunteers consumed a daily supplement of LC *n*-3 PUFA (4·0 g fish oil, 2·5 g EPA + DHA; EPAX 5500TG; Pronova, Lysaker, Norway) in combination with either the moderate- or the high-*n*-6 PUFA oils and spreads used for the run-in period. The EPA + DHA enrichment of fish oil supplement was approximately 60 g/100 g, providing 367 mg EPA and 251 mg DHA/g oil.

Before commencement of the 6-week run-in period, at the end of this 6-week run-in period and again at the end of the 6-week period of LC *n*-3 PUFA supplementation in combination with the *n*-6 PUFA dietary intervention, a blood sample was taken for the determination of the platelet phospholipid-fatty acid composition. At the start and end of the 6-week period of fish oil supplementation in combination with dietary treatment volunteers attended the Nutrition Unit, where fasting and postprandial blood lipids and insulin resistance (HOMA IR) and insulin sensitivity (revised QUICKI) were assessed using surrogate techniques. Insulin sensitivity was also measured in a subgroup of the study population (fourteen subjects; eight subjects from the moderate-*n*-6 PUFA dietary treatment group and six subjects from the high-*n*-6 PUFA dietary treatment group) using the more robust minimal model mathematical technique (Pacini & Bergman, 1986).

Postprandial evaluation

At the beginning and the end of the fish oil supplementation period a postprandial study using standard fat test meals was conducted on all volunteers following a 12 h overnight fast. On arrival at the Nutrition Unit a cannula was inserted into the antecubital vein of the forearm and two fasting blood samples were taken to assess TAG, total cholesterol, NEFA, insulin, glucose and LDL3 concentrations. ApoB48, C-reactive protein and α-tocopherol concentrations were also measured as part of the present study and this aspect of the study is described in detail elsewhere (Brady *et al.* 2003*c*). At 0 and 330 min respectively volunteers consumed a test breakfast (49 g fat) and a test lunch (31 g fat). Blood samples were collected at regular intervals throughout the day (0, 30, 60, 90, 150, 210, 270, 330, 360, 390, 420 and 480 min after consumption of the test breakfast) to assess the postprandial plasma TAG and NEFA responses.

Insulin sensitivity

Insulin sensitivity was assessed using an intravenous glucose tolerance test with frequent sampling, with minimal model analyses in a subgroup of volunteers (fourteen subjects; eight subjects from the moderate-*n*-6 PUFA dietary treatment group and six subjects from the high-*n*-6 PUFA dietary treatment group) within 3 d of the postprandial evaluation. This test has been described in more detail elsewhere (Brady *et al.* 2003*c*).

Biochemical analysis

Blood samples were collected into tubes containing 9 ml potassium EDTA on the postprandial study day and into tubes containing 5 ml potassium EDTA and tubes containing 1 ml fluoride oxalate (for the determination of blood glucose) on the insulin-sensitivity study day. Blood samples were centrifuged at 3000 rpm for 10 min. The plasma was stored at −20°C for later determination of plasma TAG, NEFA, insulin, total cholesterol, HDL-C and glucose. LDL-C was determined using the Friedewald formula (Friedewald & Levy, 1972). Plasma collected for the measurement of LDL subclass distribution was stored at 4°C and analysed within 24 h.

Plasma TAG, glucose, total cholesterol and HDL-C concentrations (using test kits supplied by Instrumentation Laboratories UK Ltd, Warrington, Ches., UK) and NEFA (using Wako NEFA C kit; Alpha Laboratories Ltd, Eastleigh, Hants., UK) were determined using the Monarch Automatic Analyzer and ILAB 600 (Instrumentation Laboratories UK Ltd). Insulin was measured using a specific commercial monoclonal ELISA kit (DAKO Ltd, Ely, Cambs., UK). LDL3 were determined by density-gradient ultracentrifugation (Griffin *et al.* 1990). Platelet-membrane phospholipid-fatty acid composition was determined by lipid extraction with subsequent quantification of fatty acid methyl esters by GC (Indu & Ghafoorunissa, 1992). The postprandial TAG response was expressed as area under the curve (0–480 min) and incremental area under the curve (0–480 min), calculated using the trapezoidal rule.

Statistical analysis

All statistical analyses were performed using SPSS (version 10.0; SPSS, Chicago, IL, USA) and $P < 0{\cdot}05$ was considered statistically significant. Before statistical analysis all data were examined for normality using the Shapiro-Wilks test and log transformed where necessary. Differences in the percentage and absolute changes in insulin action, platelet-membrane phospholipid-fatty acid composition and fasting and postprandial TAG area under the curve and incremental area under the curve over the 6-week fish oil supplementation period were determined using paired *t* tests. Differences between the dietary treatment groups were analysed using independent *t* tests. Results are presented as group means with their standard errors.

Results

There were no significant differences in anthropometric or biochemical characteristics between the moderate- and the high-*n*-6 PUFA dietary treatment groups following randomisation.

Platelet-membrane phospholipid-fatty acid composition

There were no significant differences between the moderate- and the high-*n*-6 PUFA dietary treatment groups at the start of the study (before commencement of the dietary intervention period), with the exception of EPA, which

https://doi.org/10.1079/PNS2003318 Published online by Cambridge University Press

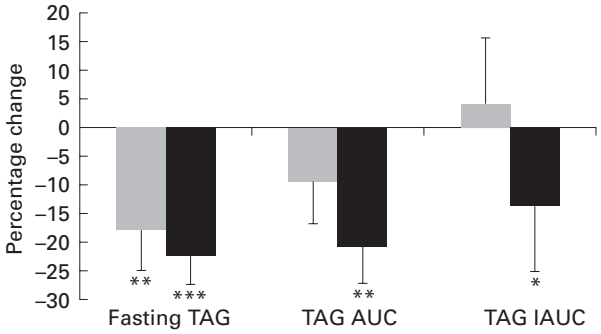

Fig. 3. Percentage change in fasting and postprandial plasma triacylglycerol (TAG) concentrations in Indian Asian volunteers (*n* 29) receiving either the moderate (□; *n* 15)- or the high (■; *n* 14)-*n*-6 PUFA diets in combination with fish oil supplementation for 6 weeks. For details of diets and procedures, see p. 119. AUC, area under the curve; IAUC, incremental area under the curve. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from baseline values: $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$.

was significantly higher in the moderate-*n*-6 PUFA treatment group ($P < 0{\cdot}05$). Over the 6-week fish oil supplementation period both the moderate- and high-*n*-6 PUFA treatment groups showed significant enrichment of the membrane phospholipids with EPA and DHA and a significant decrease in total *n*-6 PUFA and arachidonic acid ($P < 0{\cdot}05$ in all cases). There was a significant decrease in the membrane phospholipid *n*-6:*n*-3 PUFA in both dietary treatment groups over the 6-week fish oil supplementation period (−36 and −51% respectively in the moderate- and high-*n*-6 PUFA treatment groups; $P < 0{\cdot}05$).

Plasma lipids

Fasting plasma TAG showed a significant reduction in both the moderate- and high-*n*-6 PUFA dietary intervention groups after the 6-week fish oil supplementation period (−18 and −22% respectively; $P < 0{\cdot}01$ and $P < 0{\cdot}001$ respectively; Fig. 3). Postprandial TAG area under the curve and TAG incremental area under the curve were significantly reduced in the high-*n*-6 PUFA dietary group following fish oil supplementation ($P < 0{\cdot}01$ and $P < 0{\cdot}05$ respectively), with reductions in the moderate-*n*-6 PUFA dietary group failing to reach significance (Fig. 3). There were no significant differences between the moderate- and high-*n*-6 PUFA dietary treatment groups for either fasting or postprandial TAG concentrations.

The percentage change in fasting NEFA concentrations and the percentage NEFA suppression at 90 min postprandially showed no significant differences within or between the dietary treatment groups. Similar findings were obtained for total cholesterol, LDL-C and HDL-C. There was a significant reduction in the percentage LDL present as LDL3 in the high-*n*-6 PUFA dietary treatment group during fish oil treatment ($P < 0{\cdot}05$). At baseline 50% of the LDL was present as LDL3, which significantly reduced to 39% by the end of the study ($P < 0{\cdot}05$). No significant changes in the percentage LDL3 were observed in the moderate-*n*-6 PUFA dietary treatment group. There were no significant differences between the moderate- and the high-*n*-6 PUFA dietary treatment groups in the changes in LDL3 during the 6-week fish oil supplementation period.

Insulin sensitivity and resistance

Insulin resistance (HOMA IR) and insulin sensitivity (revised QUICKI) did not show any significant changes within the moderate- and the high-*n*-6 dietary PUFA groups during the period of fish oil treatment. In the insulin sensitivity substudy (fourteen subjects; eight subjects, from the moderate-*n*-6 PUFA dietary treatment group and six subjects from the high-*n*-6 PUFA dietary treatment group) no significant change was observed in insulin sensitivity (measured by the minimal model) over the 6-week period of fish oil supplementation either within or between the groups consuming the moderate- or the high-*n*-6 PUFA background diet.

These results are described in more detail elsewhere (Brady *et al.* 2003*c*).

Discussion

The present study investigated the hypothesis that the prevalence of the features of the metabolic syndrome observed among Indian Asians living in the UK may be related to an imbalance in dietary PUFA intake in this population group, specifically a higher intake of *n*-6 PUFA in combination with a lower intake of LC *n*-3 PUFA. This hypothesis was tested by dietary modification of the background *n*-6 PUFA intake to create either a moderate or a high *n*-6:*n*-3 PUFA, designed to represent the intake of a typical Caucasian or Indian Asian population respectively. Contrary to the hypothesis, the results demonstrated that the background dietary *n*-6:*n*-3 PUFA did not modulate the hypotriacylglycerolaemic effects of LC *n*-3 PUFA supplementation (2·5 g/d), as reductions in fasting plasma TAG concentrations were observed in both the moderate- and the high-*n*-6 PUFA dietary treatment groups after fish oil supplementation. In addition, postprandially, a greater hypotriacylglycerolaemic effect was observed in the high-*n*-6 PUFA treatment group. Furthermore, contrary to the hypothesis, the present study did not observe any effect of a moderate daily fish oil supplement on insulin action when given with either a moderate- or a high-*n*-6 PUFA background diet.

Evidence has previously demonstrated that ALNA, a plant-derived *n*-3 PUFA, can decrease the risk of myocardial infarction and death (Dolecek, 1992; de Lorgeril *et al.* 1994; Ascherio *et al.* 1996). However, when intake of LA, an *n*-6 PUFA, is high it is thought to reduce conversion of ALNA to its longer-chain derivatives EPA and DHA, via competition for the Δ6-desaturase enzyme (Garg *et al.* 1989). This effect was demonstrated previously by Emken *et al.* (1994), who studied the conversion rate of the parent precursor fatty acids (LA and ALNA) to their longer-chain metabolites using isotopically-labelled fatty acids. The authors reported that the conversion of ALNA to EPA and DHA was halved

https://doi.org/10.1079/PNS2003318 Published online by Cambridge University Press

when the dietary intake of LA was doubled, suggesting that higher dietary intakes of *n*-6 PUFA inhibit efficient conversion of ALNA to LC *n*-3 PUFA. Previous investigations of dietary fatty acid consumption have reported similar intakes of total *n*-3 PUFA for Indian Asians and Caucasians (Sevak *et al*. 1994; Lovegrove *et al*. 2003*b*). Thus, it is likely that the higher intake of LA consistently reported for Indian Asians (Miller *et al*. 1988; Sevak *et al*. 1994; Lovegrove *et al*. 2003*b*) operates to reduce the conversion of ALNA to EPA and DHA, and may have an impact on the increased rate of CHD observed among this population group. Furthermore, several studies have reported a lower dietary intake of the marine-derived LC *n*-3 PUFA and limited EPA and DHA in the membrane phospholipids of Indian Asians compared with Caucasians (Das *et al*. 1994; Sevak *et al*. 1994; Lovegrove *et al*. 2003*b*). However, it is well known that direct dietary consumption of EPA and DHA has a more potent impact on reductions in CHD risk factors than ALNA intake alone (DeDeckere *et al*. 1998), potentially via beneficial alterations in plasma membrane characteristics or activation of transcription factors (Rustan *et al*. 1997; Clarke, 2000).

In the present study supplementation of moderate- or high-*n*-6 PUFA background diets with EPA and DHA, resulted in significant enrichment of phospholipid membranes in both dietary treatment groups ($P < 0{\cdot}05$ in both cases). The hypotriacylglycerolaemic effects of LC *n*-3 PUFA supplementation on fasting plasma TAG concentrations, which are reported consistently with daily doses of >1·5 g (Schmidt *et al*. 1990; Harris *et al*. 1991), were also observed in the present study in both the moderate- and the high-*n*-6 PUFA dietary treatment groups. In a previous investigation the effects of fish oil supplementation in combination with both high- and low-*n*-6 PUFA diets were studied (Gronn *et al*. 1991). The authors observed reductions in fasting TAG concentrations in both dietary groups, which is consistent with our findings. However, our observations that the postprandial hypotriacylglycerolaemic effects were greater in the high-*n*-6 PUFA dietary treatment group are novel and are inconsistent with our hypothesis that a high dietary *n*-6:*n*-3 PUFA may operate to reduce the beneficial effects of LC *n*-3 PUFA supplementation.

Plasma TAG concentrations are an important determinant of circulating LDL3 concentrations (Griffin, 1999). In the metabolic syndrome reduced LPL-mediated hydrolysis of TAG from TAG-rich lipoproteins allows for an extended period of residence for TAG in the circulation, which can directly affect the compositional characteristics of LDL. Increased transfer of TAG to LDL via cholesteryl ester transfer protein during neutral-lipid exchange and subsequent hydrolysis of LDL-TAG by lipases results in a preponderance of circulating LDL3 (Griffin, 1999). Indian Asians are typically reported to have a prevalence of the denser proatherogenic LDL3 when compared with Caucasians (Kulkarni *et al*. 1999; Lovegrove *et al*. 2003*b*), consistent with the higher circulating plasma TAG concentrations reported in the Indian Asian group (Miller *et al*. 1988; Chambers *et al*. 2000). In the present study the postprandial reduction in plasma TAG concentrations may be directly related to the reduction in LDL3 concentrations observed in the high-*n*-6 PUFA dietary treatment group. However, because before fish oil supplementation the high-*n*-6 PUFA dietary treatment group had a greater percentage of LDL3 than the moderate-*n*-6 PUFA dietary treatment group (50 *v*. 38) these findings may reflect regression towards the mean over time.

The present study also tested the hypothesis that LC *n*-3 PUFA supplementation may have a beneficial impact on insulin action in Indian Asians. Although animal studies have clearly identified a positive association between increased dietary LC *n*-3 PUFA and improvements in insulin action (Storlien *et al*. 1987; Behme, 1996; Somova *et al*. 1999), evidence in the literature for the putative beneficial effects of LC *n*-3 PUFA treatment on insulin action in human subjects is limited. Findings from the current study indicate that moderate LC *n*-3 PUFA supplementation over a 6-week period had little or no effect on insulin sensitivity in normoglycaemic Indian Asian subjects. Although significant ($P < 0{\cdot}05$) changes were observed in the fatty acid composition of platelet-membrane phospholipids over the 6-week intervention period in the present study, this period of supplementation may not have been sufficient to substantially alter skeletal and adipocyte membrane phospholipids, which directly impact on insulin action. The observed lack of effect of LC *n*-3 PUFA supplementation on changes in insulin action may also be attributed to small subject numbers, as the present study was powered to measure blood lipid responses rather than changes in insulin action.

In conclusion the present study showed that the dietary *n*-6 PUFA intake does not modulate the beneficial impact of fish oil supplementation on fasting and postprandial blood lipids in Indian Asians. In addition, LC *n*-3 PUFA supplementation had no impact on insulin action, which was assessed using comprehensive (minimal model) or surrogate measures (HOMA IR and revised QUICKI) for evaluating insulin action in this normal healthy subject group. However, the effects of fish oil supplementation were assessed over a relatively short period and in a small number of volunteers, and further studies of longer duration in normal healthy individuals are justified. In addition, contrary to the original hypothesis that a high-*n*-6 PUFA dietary intake could attenuate the beneficial effects of fish oil supplementation on blood lipids, a significant ($P < 0{\cdot}05$) reduction in postprandial plasma TAG concentrations was observed in the high-*n*-6 PUFA group, a response that was not observed in those consuming the moderate-*n*-6 PUFA diet. Thus, the combination of high-*n*-6 PUFA and LC *n*-3 PUFA may operate to produce a hypotriacylglycerolaemic effect that is more cardioprotective.

Acknowledgements

This work was supported by funding from the Food Standards Agency. The authors thank Dr John Wright, clinical consultant (School of Food Biosciences, University of Reading, UK) for undertaking the cannulation and the insulin infusions for the postprandial and insulin-sensitivity study days, Dr Barbara Gower (University of Alabama, Birmingham, AL, USA) for the determination of insulin

sensitivity from the minimal model, Van den Bergh Oils for providing the oils and spreads for the study and Pronova for providing the fish oil capsules. The authors would also like to thank other members of their research group (Mr Sean Lovegrove, Mrs Stephanie Lesauvage, Mrs Jan Luff and Mr William Roberts). Finally, they would like to thank all the volunteers for their time and cooperation in participating in this study.

References

Anderson RL, Hamman RF, Savage PJ, Saad MF, Laws A, Kades WW, Sands RE & Cefalu W (1995) Exploration of simple insulin sensitivity measures derived from frequently sampled intravenous glucose tolerance (FSIGT) tests. *American Journal of Epidemiology* **142**, 724–732.

Ascherio A, Rimm EB, Giovannucci EL, Spiegelman D, Stampfer MJ & Willett WC (1996) Dietary fat and risk of coronary heart disease in men: cohort follow-up study in the United States. *British Medical Journal* **313**, 84–90.

Banerji MA, Faridi N, Atluri R, Chaiken RL & Lebovitz HE (1999) Body composition, visceral fat, leptin and insulin resistance in Asian Indian men. *Journal of Clinical Endocrinology and Metabolism* **84**, 137–144.

Behme MT (1996) Dietary fish oil enhances insulin sensitivity in miniature pigs. *Journal of Nutrition* **126**, 1549–1553.

Boberg M, Vessby B & Selinus I (1986) Effects of dietary supplementation with n-6 and n-3 long-chain polyunsaturated fatty acids on serum lipoproteins and platelet function in hypertriglyceridaemic patients. *Acta Medica Scandinavica* **220**, 153–160.

Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, Monauni T & Muggeo M (2000) Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity. *Diabetes Care* **23**, 57–63.

Brady LM, Lesauvage SVM, Saini N, Williams CM & Lovegrove JA (2003*a*) Comparison of dietary fat and fatty acid intake estimated by the duplicate diet collection technique and estimated dietary records. *Journal of Human Nutrition and Dietetics* **16**, 393–401.

Brady LM, Lesauvage SVM, Williams CM & Lovegrove JA (2002) Duplicate diet comparison of total fat and fatty acid intake between Caucasians and Sikhs living in the UK. *Proceedings of the Nutrition Society* **61**, 53A.

Brady LM, Lovegrove SS, Gower BA, Williams CM & Lovegrove JA (2003*b*) Comparison of surrogate measures of insulin sensitivity and resistance with the minimal model in Indian Asians and Caucasians. *Proceedings of the Nutrition Society* **62**, 50A.

Brady LM, Lovegrove SS, Lesauvage SVM, Gower BA, Minihane AM, Williams CM & Lovegrove JA (2003*c*) Increased n-6 PUFA does not attenuate effects of dietary long chain n-3 PUFA on insulin sensitivity or triacylglycerol reduction in Indian Asians. *American Journal of Clinical Nutrition* (In the Press).

British Heart Foundation (2003) *Coronary Heart Disease Statistics*. London: British Heart Foundation.

British Nutrition Foundation (1999) *n-3 Fatty Acids and Health. The Report of the British Nutrition Foundation's Task Force*. London: Chapman and Hall.

Brown AJ & Roberts DC (1991) Moderate fish oil intake improves lipemic response to a standard fat meal. A study in 25 healthy men. *Arteriosclerosis and Thrombosis* **11**, 457–466.

Burr MI, Gilbert JF, Holliday RM, Elwood PC, Fehily AM, Rogers S, Sweetnam PM & Deadman NM (1989) Effects of changes of fat, fish and fibre intakes on death and myocardial reinfarction: Diet and Reinfarction Trial (DART). *Lancet* **ii**, 757–761.

Caro J (1991) Insulin resistance in obese and nonobese man. *Journal of Clinical Endocrinology and Metabolism* **73**, 691–695.

Chambers JC, Obeid OA, Refsum H, Uciand P, Hackett D & Hooper J (2000) Plasma homocysteine concentrations and risk of coronary heart disease in UK Indian Asian and European men. *Lancet* **355**, 523–527.

Clarke SD (2000) Polyunsaturated fatty acid regulation of gene transcription: a mechanism to improve energy balance and insulin resistance. *British Journal of Nutrition* **83**, S59–S66.

Connor SL & Connor WE (1997) Are fish oils beneficial in the prevention and treatment of coronary artery disease? *American Journal of Clinical Nutrition* **66**, Suppl., 1020S–1031S.

Conquer JA & Holub BJ (1998) Effect of supplementation with different doses of DHA on the levels of circulating DHA as non-esterified fatty acid in subjects of Asian Indian background. *Journal of Lipid Research* **39**, 286–292.

Das UN, Kumar KV & Ramesh G (1994) Essential fatty acid metabolism in South Indians. *Prostaglandins Leukotrienes and Essential Fatty Acids* **50**, 253–255.

DeDeckere EAM, Korver O, Verschuren PM & Katan MB (1998) Health aspects of fish and n-3 polyunsaturated fatty acids from plant and marine origin. *European Journal of Clinical Nutrition* **52**, 749–753.

DeFronzo RA, Toin JD & Andres R (1979) Glucose clamp technique: A method for quantifying insulin secretion and resistance. *American Journal of Physiology* **237**, 214–223.

de Lorgeril M, Renaud S, Mamelle N, Salen P, Martin JL, Monjaud I, Guidollet J, Touboul P & Delaye J (1994) Mediterranean alpha linolenic acid rich diet in secondary prevention of coronary heart disease. *Lancet* **343**, 1454–1459.

Denkins YM, Lovejoy JC & Smith SR (2002) Omega 3 PUFA supplementation and insulin sensitivity. *FASEB* **16**, A24.

Dhawan J & Petkar S (1998) Epidemiology of coronary heart disease in South Asians. *Current Science in India* **74**, 1060–1063.

Dolecek TA (1992) Epidemiological evidence of relationships between dietary polyunsaturated fatty acids and mortality in the multiple risk factor intervention trial. *Proceedings of the Society for Experimental Biology and Medicine* **200**, 177–182.

Dyerberg J & Bang HO (1982) A hypothesis on the development of acute myocardial infarction in Greenlanders. *Scandinavian Journal of Clincal and Laboratory Investigation* **161**, Suppl., 7–13.

Emken EA, Adlof RO & Gulley RM (1994) Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic acid and linolenic acids in young adult males. *Biochimica et Biophysica Acta* **1213**, 277–288.

Eritsland J, Seljeflot I, Abdelnoor M, Arnesen H & Torjesen PA (1994) Long term metabolic effects of n-3 fatty acids on serum lipids and glycaemic control. *Scandinavian Journal of Clinical and Laboratory Investigation* **54**, 273–280.

Fasching P, Ratheiser K, Waldhausl W, Rohac M, Osterrode W, Nowotny P & Vierhapper H (1991) Metabolic effects of fish oil supplementation in patients with impaired glucose tolerance. *Diabetes* **40**, 583–589.

Feskens EJM, Bowles CH & Kromhout D (1991) Inverse association between fish intake and risk of glucose intolerance in normoglycaemic elderly men and women. *Diabetes Care* **14**, 935–941.

Feskens EJM, Virtanen SM, Rasanen L, Tuomilehto J, Stengard J, Pekkanen J, Nissinen A & Kromhout D (1995) Dietary factors determining diabetes and impaired glucose

tolerance – A 20 year follow up of the Finnish and Dutch cohorts of the 7 Countries Study. *Diabetes Care* **18**, 1104–1112.

Folsom AR, Eckfeldt JH, Weitzman S, Ma J, Chambless LE, Barnes RW, Cram KB & Hutchinson RG (1994) Relation of carotid wall thickness to diabetes mellitus, fasting glucose and insulin, body size and physical activity. Atherosclerosis Risk in Communities (ARIC) Study Investigators. *Stroke* **25**, 66–73.

Frayn KN, Summers LKM & Fielding BA (1997) Regulation of the plasma non-esterified fatty acid composition in the postprandial state. *Proceedings of the Nutrition Society* **56**, 713–721.

Friedewald WT & Levy RI (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry* **18**, 499–502.

Frost GS, Keogh BE, Smith D, Leeds AR & Dornhurst A (1998) Reduced adipocyte insulin sensitivity in Caucasians and Asian subjects with coronary heart disease. *Diabetic Medicine* **15**, 1003–1009.

Fukushima M, Taniguchi A, Sakai M, Doi K, Nagata I, Nagasaka S, Tokuyama K & Nakai Y (2000) Assessment of insulin sensitivity from a single sample. *Diabetes Care* **23**, 1434–1435.

Garg ML, Wierzbicki AA, Thompson ABR & Clandinin MT (1989) Dietary saturated fat level alters the competition between alpha linolenic acid and linoleic acid. *Lipids* **24**, 334–339.

Ghafoorunissa (1998) Requirements of dietary fats to meet nutritional needs and prevent the risk of atherosclerosis – An Indian perspective. *Indian Journal of Medical Research* **108**, 191–202.

GISSI-Prevenzione Investigators (1999) Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione Trial. *Lancet* **354**, 447–455.

Griffin BA (1999) Lipoprotein atherogenicity: an overview of current mechanisms. *Proceedings of the Nutrition Society* **58**, 163–169.

Griffin BA, Caslake MJ, Yip B, Tait GW, Packard CJ & Shepherd J (1990) Rapid isolation of low density lipoprotein (LDL) subfractions from plasma by density gradient ultracentrifugation. *Atherosclerosis* **83**, 59–67.

Gronn M, Gorbitz C, Christensen E, Levorsen A, Ose L, Hagve TA & Christopherson BO (1991) Dietary n-6 fatty acids inhibit the incorporation of dietary n-3 fatty acids in thrombocyte and serum phospholipids in humans: a controlled dietetic study. *Scandinavian Journal of Clinical and Laboratory Investigation* **51**, 255–263.

Haffner SM, D'Agostino R, Saad MF, Rewers M, Mykkanen L, Selby J, Howard G, Savage PJ, Hamman RF & Wagenknecht LE (1996) Increased insulin resistance and insulin secretion in nondiabetic African-Americans and Hispanics compared with non-Hispanic whites: The insulin resistance atherosclerosis study. *Diabetes* **45**, 742–748.

Harris WS, Windsor SL & Dujovne CA (1991) Effects of four doses of n-3 fatty acids given to hyperlipidaemic patients for six months. *Journal of the American College of Nutrition* **10**, 220–227.

Horrobin DF (1991) Interaction between n-3 and n-6 essential fatty acids in regulation of cardiovascular disorders and inflammation. *Prostaglandins Leukotrienes and Essential Fatty Acids* **44**, 127–131.

Ikeda Y, Suehiro T, Nakamura T, Kumon Y & Hashimoto K (2001) Clinical significance of the insulin resistance index as assessed by the homeostasis model assessment. *Endocrinology Journal* **48**, 81–86.

Indu M & Ghafoorunissa (1992) n-3 fatty acids in Indian diets – Comparison of the effects of precursor (alpha-linolenic acid) vs product (long chain n-3 polyunsaturated fatty acids). *Nutrition Research* **12**, 569–582.

Isaksson B (1993) A critical evaluation of the duplicate portion technique in dietary surveys. *European Journal of Clinical Nutrition* **47**, 457–460.

Jucker BM, Cline GW, Barucci M & Shulman GI (1999) Differential effects of safflower oil versus fish oil feeding on insulin stimulated glycogen synthesis, glycolysis and pyruvate dehydrogenase flux in skeletal muscle. *Diabetes* **48**, 134–140.

Karpe F (1999) Postprandial lipoprotein metabolism and atherosclerosis. *Journal of Internal Medicine* **246**, 341–355.

Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G & Quon MJ (2000) Quantitative insulin sensitivity check index: A simple accurate method for assessing insulin sensitivity in humans. *Journal of Clinical Endocrinology and Metabolism* **85**, 2402–2410.

Kromhout D, Bosschieter EB & de Lezenne Coulander C (1985) The inverse relation between fish consumption and 20 year mortality from coronary heart disease. *New England Journal of Medicine* **312**, 1205–1209.

Kulkarni K, Markovitz J, Nanda NC & Segrest JP (1999) Increased prevalence of smaller and denser LDL particles in Asian Indians. *Arteriosclerosis, Thrombosis and Vascular Biology* **19**, 2749–2755.

Laasko M (1993) How good a marker is insulin level for insulin resistance. *American Journal of Epidemiology* **137**, 959–964.

Lam TKT, Carpentier A, Lewis GF, van de Werve G, Fantus IG & Giacca A (2003) Mechanism of the free fatty acid induced increase in hepatic glucose production. *American Journal of Physiology* **284**, E863–E873.

Lovegrove JA, Brady LM, Lesauvage SVM, Lovegrove SS, Minihane AM & Williams CM (2003*a*) Lack of association between central adiposity and lipaemia in UK Sikh men. *International Journal of Obesity* **27**, 1373–1382.

Lovegrove JA, Lovegrove SS, Lesauvage SVM, Brady LM, Minihane AM, Saini N & Williams CM (2003*b*) Moderate fish oil supplementation reverses low platelet membrane long chain PUFA status and reduces plasma triacylglycerol status in British Indian Asians. *American Journal of Clinical Nutrition* (In the Press).

Lovejoy JC (2002) The influence of dietary fat on insulin resistance. *Current Diabetic Reports* **2**, 435–440.

McAuley KA, Williams SM, Mann JI, Walker RJ, Lewis-Barned NJ, Temple LA & Duncan AW (2001) Diagnosing insulin resistance in the general population. *Diabetes Care* **24**, 460–464.

MacDiarmid JI & Blundell JE (1997) Dietary under reporting: what people say about recording their food intake. *European Journal of Clinical Nutrition* **51**, 199–200.

McKeigue PM, Marmot MG, Adelstein AM, Hunt SP, Shipley MJ, Butler SM, Riemersma RA & Turner PR (1985) Diet and risk factors for coronary heart disease in Asians in North-West London. *Lancet* **ii**, 1086–1090.

McKeigue PM, Miller GJ & Marmot MG (1989) Coronary heart disease in South Asians overseas: a review. *Journal of Clinical Epidemiology* **42**, 597–609.

McKeigue PM, Shah B & Marmot MG (1991) Relation of central obesity and insulin resistance with high diabetes prevalence and cardiovascular risk in South Asians. *Lancet* **337**, 382–386.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF & Turner RC (1985) Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentration in man. *Diabetologia* **28**, 412–419.

Mertz W (1992) Food intake measurements: Is there a gold standard. *Journal of the American Dietetic Association* **92**, 1463–1465.

Miller GJ, Kotecha S, Wilkinson WH, Wilkes H, Stirling Y, Sanders TA, Broadhurst A, Allison J & Meade TW (1988) Dietary and other characteristics relevant for coronary heart disease in men of Indian, West Indian and European descent in London. *Atherosclerosis* **70**, 63–72.

Murphy MC, Brooks CN, Rockett JC, Chapman C, Lovegrove JA, Gould BJ, Wright JW & Williams CM (1999) The quantitation of lipoprotein lipase mRNA in biopsies of human adipose tissue, using polymerase chain reaction and the effect of increased consumption on n-3 polyunsaturated fatty acids. *European Journal of Clinical Nutrition* **53**, 441–447.

O'Keefe JH & Harris WS (2000) From Inuit to implementation: Omega-3 fatty acids come of age. *Mayo Foundation for Medical Education and Research* **75**, 607–614.

Pacini G & Bergman RN (1986) MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test. *Computing Methods and Programs in Biomedicine* **23**, 113–122.

Perseghin G, Caumo A, Caloni M, Testolin G & Luzi L (2001) Incorporation of the fasting plasma FFA concentration into QUICKI improves its association with insulin sensitivity in nonobese individuals. *Journal of Clinical Endocrinology and Metabolism* **86**, 4776–4781.

Popp-Snijders C, Schouten JA, Heine RJ, van der Meer J & van der Veen EA (1987) Dietary supplementation of omega 3 polyunsaturated fatty acids improves insulin sensitivity in non insulin dependant diabetes. *Diabetes Research* **4**, 141–147.

Rivellese AA (2000) Effects of n-3 fatty acids on carbohydrate and lipid metabolism in healthy people: The Kanwu Study. *Diabetologia* **43**, 11S.

Rustan AC, Nenester MS & Drevon CA (1997) Omega-3 and omega-6 fatty acids in the insulin resistance syndrome. Lipid and lipoprotein metabolism and atherosclerosis. *Annals of the New York Academy of Sciences* **827**, 310–326.

Saad MF, Anderson RL, Laws A, Watanabe RM, Kades WW, Chen I, Evan-Sands R, Pei D, Savage PJ & Bergman RN (1994) A comparison between the minimal model and the glucose clamp in the assessment of insulin sensitivity across the spectrum of glucose tolerance. *Diabetes* **43**, 1114–1121.

Schmidt EB, Varming K, Ernst E, Madsen P & Dyerberg J (1990) Dose response studies on the effect of n-3 polyunsaturated fatty acids on lipids and hemostasis. *Thrombosis and Hemostasis* **63**, 1–5.

Sevak L, McKeigue P & Marmot MG (1994) Relationship of hyperinsulinaemia to dietary intake in South Asian and European men. *American Journal of Clinical Nutrition* **59**, 1069–1074.

Simopolous AP (1991) Omega 3 fatty acids in health and disease and in growth and development. *American Journal of Clinical Nutrition* **54**, 438–463.

Singh RB, Niaz MA, Sharma JP, Kumar R, Rastogi V & Moshiri M (1997) Randomized, double-blind, placebo controlled trial of fish oil and mustard oil in patients with suspected acute myocardial infarction: the Indian experiment of infarct survival. *Cardiovascular Drugs Therapy* **11**, 485–491.

Somova L, Moodley K, Channa MC & Nada A (1999) Dose dependent effect of dietary fish oil (n-3) polyunsaturated fatty acids on in vivo insulin sensitivity in rat. *Methods and Findings in Experimental and Clinical Pharmacology* **21**, 275–278.

Storlien LH, Higgins JA, Thomas TC, Brown MA, Wang HQ, Huang XF & Else PL (2000) Diet composition and insulin action in animal models. *British Journal of Nutrition* **83**, Suppl. 1, S85–S90.

Storlien LH, Kraegen EW, Chisholm DJ, Ford GL, Bruce DG & Pascoe WS (1987) Fish oil prevents insulin resistance induced by high fat feeding in rats. *Science* **237**, 885–888.

Storlien LH, Pan DA, Kriketos AD, O'Connor J, Caterson ID, Cooney GJ, Jenkins AB & Baur LA (1996) Skeletal muscle membrane lipids and insulin resistance. *Lipids* **31**, 261S–265S.

Toft I, Bonaa KH, Ingebretsen OC, Nordoy A & Jenssen T (1995) Effects of n-3 polyunsaturated fatty acids on glucose homeostasis and blood pressure in essential hypertension: a randomised controlled trial. *Annals of Internal Medicine* **123**, 911–918.

Vessby B (2000) Dietary fat and insulin action in humans. *British Journal of Nutrition* **83**, Suppl. 1, S91–S96.

Wajchenberg BL (2000) Subcutaneous and visceral adipose tissue and their relation to the metabolic syndrome. *Endocrine Reviews* **21**, 697–738.

Waldhausl W, Ratheiser K, Komjati P, Nowotny P, Pirich K & Vierhapper H (1989) In *Health Effects of Fish and Fish Oils*, pp. 171–187 [RK Chandra, editor]. St Johns, Nfld: ARTS Biomedical Publishers.

Weber P & Raederstorff D (2000) Triglyceride lowering effect of omega 3 LC n-3 polyunsaturated fatty acids – A review. *Nutrition Metabolism and Cardiovascular Diseases* **10**, 28–37.

Westphal S, Orth M, Ambrosch A, Osmundsen K & Luley C (2000) Postprandial chylomicrons and VLDLs in severe hypertriacylglycerolemia are lowered more effectively than are chylomicron remnants after treatment with n-3 fatty acids. *American Journal of Clinical Nutrition* **74**, 914–920.

Zampelas A, Williams CM, Morgan LM, Wright J & Quinlan PT (1994) The effect of triacylglycerol fatty acid positional distribution on postprandial plasma metabolite and hormone responses in normal adult men. *British Journal of Nutrition* **71**, 401–410.

Zoratti R, Godsland I, Chaturvedi N, Crook D, Stevenson JC & McKeigue P (2000) Relation of plasma lipids to insulin resistance, non esterified fatty acid levels and body fat in men from three different ethnic groups: Relevance to variation in risk of diabetes and coronary disease. *Metabolism* **49**, 245–252.