Review article

Effect of the dietary fat quality on insulin sensitivity

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Recent evidence shows that specific fatty acids affect cell metabolism, modifying the balance between fatty acid oxidation and lipogenesis. These effects may have important implications in addressing the present epidemic of nutrition-related chronic disease. Intake of dietary saturated and *n*-6 PUFA have increased while *n*-3 fatty acid intake has decreased. Obesity, type 2 diabetes and insulin resistance are highly prevalent, and both are strongly related to disorders of lipid metabolism characterized by an increased plasma and intracellular fatty acid availability. Thus, it has been hypothesized that change in the quality of dietary fat supply is able to modify the degree of insulin sensitivity. Animal studies provide support for this notion. However, there is limited human data either from normal or diabetic subjects. This review aims to analyse human studies that address this question. To this purpose, the experimental design, dietary compliance, insulin-sensitivity method used and confounding variables are discussed in order to identify the role of dietary fat quality as a risk factor for insulin resistance. Most studies (twelve of fifteen) found no effect relating to fat quality on insulin sensitivity. However, multiple study design flaws limit the validity of this conclusion. In contrast, one of the better designed studies found that consumption of a high-saturated-fat diet decreased insulin sensitivity in comparison to a high-monoun-saturated-fat diet. We conclude that the role of dietary fat quality on insulin sensitivity in human subjects should be further studied, using experimental designs that address the limitations of existing data sets.

Insulin sensitivity: Fat quality: Lipid metabolism

Diet and physical activity patterns have changed drastically in both industrialized and developing countries together with a rapid increase in nutrition-related chronic diseases such as obesity, CVD and type 2 diabetes[1]. Physical activity has decreased while total energy, fat and refined carbohydrate consumption have increased in virtually all age groups[1]. By now most recognize that sustained changes in diet and physical activity, which promote better health, are difficult to achieve. Nutritionists have focused on dietary characteristics that could contribute to the adverse consequences of obesity, for instance fat quality that could be amenable to change, manipulating dietary ingredients or the fatty acid (FA) composition of the traditional fats consumed.

Fat ingested is a blend of different FA, no source is pure. The types of fat consumed are SFA, MUFA and PUFA. The latter are separated into two classes based on the presence of unsaturated bonds in positions *n*-6 or *n*-3. Simopoulos[2] has described major time-related trends in total and SFA intake over the past two centuries; an increase in the total and in the SFA consumed, while the proportion of *n*-3:*n*-6 PUFA has decreased significantly. These changes have proven to be important in the risk of CVD[3]. Convincing evidence exists to support the notion that higher consumption of linoleic, oleic, and *n*-3 PUFA (α-linolenic, EPA and DHA) FA and lower intake of SFA (myristic and palmitic acids) and *trans*-FA reduce the risk of CVD. Differential capacity to modify plasma LDL-cholesterol, blood pressure, cardiac function, endothelial function and vascular reactivity, as well as different influence on platelet aggregation and inflammation are the cellular basis to explain the well-documented relationship between dietary fat quality and CVD[4].

Incidence of type 2 diabetes also might be modified as a function of dietary FA quality, since several studies in animals have demonstrated a differential impairment in the degree of insulin sensitivity (IS) after feeding with SFA, *n*-6 PUFA or *n*-3 PUFA[5,6]. Impaired IS is a main risk factor for type 2 diabetes, hence strategies to prevent a reduction in IS may have a large impact on reducing populations affected with type 2 diabetes. In human subjects, several studies have been performed to evaluate the role of dietary FA quality on IS[7–10]

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although currently more published studies report no effect of dietary fat quality on this variable. This review analyses human studies where the effects of dietary fat quality on IS have been evaluated. Special attention was given to the methodological aspects of each study. We conducted a literature search in PubMed for randomized clinical trials in human subjects published until 31 August 2007. These articles were identified with the following key words: fatty acid type and insulin. Other articles not identified after this first search were found using the Related Articles option available in Medline. Bibliographies of primary references were used to identify additional relevant studies.

Effect of fatty acid quality on insulin sensitivity

In human subjects, multiple epidemiological studies and intervention trials have assessed the role of dietary fat quality on IS. However, evidence to support a contrasting effect on IS was judged only as possible by the FAO/WHO expert group in a recent report on *Diet, Nutrition and Prevention of Chronic Disease*[1]. Since the convincing and probable categories were required to establish a recommendation, the role of FA quality in ameliorating the risk of type 2 diabetes was not incorporated in the guidance. This section will review the descriptive and intervention human studies published to date and the multiple methodological issues that should be considered in the interpretation of the results.

Descriptive studies

In general, epidemiological studies report that dietary content of SFA is directly, and unsaturated fat is inversely, associated with the incidence of type 2 diabetes or impaired IS, respectively[8,9]. For example, in a 14-year follow-up study conducted in women[11], an increase by 5 % of total energy intake as SFA or MUFA did not modify the relative risk of type 2 diabetes; however, the same relative increase as PUFA significantly reduced the relative risk to 0·63 (95 % CI 0·53, 0·76) once adjusted by relevant confounders. On the other hand, a cross-sectional study found no association between dietary FA quality with IS (by Minimal Model) after adjusting for critical confounders[12].

Interpretation of these results is complicated further by the close correlation between dietary FA in specific foods. For example animal fat consumption increases both SFA and MUFA; even within the SFA the effect of palmitic and stearic acids may be different since the latter is rapidly converted to oleic acid[13]. Inaccurate dietary assessment methods and inadequate FA food composition provide added complexity to the analysis. This has led to the measurement of FA composition of tissues or plasma lipid fractions as potential improved markers of dietary exposure.

Folsom *et al.*[14] found that an increase in SFA in plasma phospholipids equivalent to the interquartile range elevated the odds for hyperinsulinaemia to 2·4-fold (95 % CI 1·7, 3·3), after adjusting for BMI and fasting glycaemia. On the other hand, Pelikánová *et al.*[15] found a higher proportion of arachidonic acid in plasma phospholipids from type 2 diabetic compared to healthy subjects. In addition, in healthy individuals an inverse correlation between insulin-stimulated glucose disposal rate and serum phospholipid SFA:linoleic acid ratio was observed, which explained about one-third of the IS variance in this group.

Information of greater relevance may be obtained from skeletal muscle given its critical role in insulin-stimulated glucose uptake[16]. Borkman *et al.*[17] found in healthy subjects that the proportion of PUFA with twenty to twenty-two carbons in skeletal muscle phospholipids was directly associated to IS. On the other hand, Manco *et al.*[18] observed in muscle TAG from obese individuals, a lower degree of unsaturation in comparison to lean subjects. In addition, the combination of increased muscle TAG and palmitate content were the main determinants of impaired IS. These studies suggest that dietary fat quality may be a relevant factor in pathogenesis of insulin resistance (impaired IS) and type 2 diabetes. Results from controlled and intervention studies permit a more specific assessment of the effect of FA quality on IS.

Controlled and intervention studies

Using the previously described search criteria, we identified forty-one articles assessing the role of dietary fat quality on glucose metabolism. In general, these studies did not show a differential effect of dietary FA quality on IS; however, several methodological flaws may explain this conclusion. In order to select those studies to be included in our analysis, we evaluated the quality of experimental designs based on the approach used to evaluate IS (dependent variable), control of dietary fat quality (independent variable) and other confounding variables.

Dependent variable: insulin sensitivity. A key factor affecting data quality is the selection of the IS marker. Surrogate measurements of IS based on fasting glycaemia and insulinaemia (i.e., homeostasis model assessment) are not recommended for physiological or clinical studies since they explain no more than 40 % of the IS variance observed in a population, and < 13 % of the IS variance in normal-weight subjects[19]. Reference methods such as the euglycaemic–hyperinsulinaemic clamp[20], insulin suppression test[21] and the frequently sampled intravenous glucose tolerance test with the Minimal Model[22] are considered reliable approaches to determine the degree of IS. Achievement of a fully suppressed hepatic glucose production is critical for interpretation of IS data using insulin infusion methods; otherwise the degree of IS will be underestimated unless hepatic glucose production is concomitantly measured. In lean, non-diabetic individuals hepatic glucose production is fully suppressed at plasma insulin concentration of about 60 mU/ml (about 40 mU insulin/m^2 per min or about 1 mU/kg body weight per min); however in type 2 diabetic subjects, the insulin dose needs to be increased at least 2-fold to observe a comparable effect[23,24].

Another relevant issue to assess dietary fat quality effect on IS relates to the reproducibility of the IS measurement and sample size required to demonstrate a significant effect. All reference methods for assessing IS have an intra-individual variation of about 15 %[21,25,26], thus an average change in response to the dietary intervention equal or higher than this value (1 SD) might be considered as biologically relevant. Therefore, the sample size required to detect a difference (by at least 1 SD) between interventions is sixteen subjects, considering a type I error and power of 5 % and 80 %, respectively. Studies aiming to detect a difference between treatments lower than 1 SD will need higher sample size.

Independent variable: quality of the fat intake. Considering the potential interaction among various dietary components, studies which modify dietary fat quality as the single variable of interest are required to assess the effect of fat quality on IS. This is more feasible in short-term (1–7 d) studies; however, given that the magnitude of FA effects on IS may be dependent on duration of exposure, the need for appropriate assessment of dietary compliance is increased. This limitation is usually ignored or underestimated in most studies. In order to assure dietary compliance in free-living people the following conditions should be met: (i) maintain stable energy balance; (ii) maintain a fixed macronutrient composition of dietary energy intake; (iii) monitor the achievement of the target fat intake of a given composition. A simple strategy to evaluate energy balance is the recording of body weight. This should not fluctuate if energy intake is matched by energy expenditure. However, some studies do not even report this simple body weight information. With respect to macronutrient distribution of energy and dietary fat intake, instructions are usually given to subjects on how to incorporate the dietary changes. However, instructions are often difficult to follow and in many cases total energy and/or macronutrient intake may change since subjects tend to maintain their *ad libitum* diet. For example, Summers *et al.* [27] provided dietary instructions to modify SFA and PUFA intake for 5 weeks, reinforcing participants on an individual basis over the study period. Upon analysis of food consumption records and body weight changes the data were highly suggestive of a systematic underreporting of energy intake by an average of 1674 kJ/d and of total fat intake by 40 g/d.

Some studies provide key food ingredients (main fat sources) or provide scales to quantify consumption of specific foods. These attempts to control dietary compliance are insufficient. Controlled food consumption under direct supervision in an outpatient setting is the preferred method to assure compliance.

In order to objectively verify dietary compliance in terms of FA quality, changes in plasma or blood cell FA composition have been used. To interpret results from this evaluation, the following considerations should be kept in mind. First, changes in tissue FA profile can occur within a range of dietary FA intakes. Therefore, at best this is qualitative indicator of changes in dietary fat. Second, FA compositional changes in blood may not fully reflect the relevant cellular or subcellular pools. Third, the change for serum and tissue phospholipids subclasses, TAG or cholesterol esters are time dependent according to turnover rates of the specific tissue pool and the metabolism of the given FA[28–30]. Finally, we need to consider how the changes in FA profile expressed; in most cases they are measured as percentage of total FA injected in the gas chromatographer and not per unit of tissue weight or plasma volume. Since the dietary intervention may modify the total circulating or tissue fat content this may wrongly estimate the true change.

Confounding variables. Multiple confounders may obscure a true effect of FA on IS; these require a strict control, particularly when dietary changes are subtle or in small magnitude, as is often the case in long-term studies. Full control of multiple confounders is nearly impossible but we consider that the following factors are essential for a good study. Overall health and nutritional status, age, customary dietary patterns, alcohol consumption, physical activity level, gender, use of contraceptives, menstrual status and phase of the cycle when measurements are collected are the main confounding variables. Sub-clinical conditions such prediabetic state and hyperlipidaemia should also be assessed. Actually, several studies show an association between duration and quality of sleep and metabolic indices[31]. In addition, IS changes are highly variable between subjects in response to the same nutritional stimulus (i.e., fat overload). Emerging evidence suggests that genetic polymorphisms are able to account in part for this variability[32]. Efforts to adequately account for the confounding effects should provide stronger data where more conclusive statements can arise.

Identifying the FA effect on IS may also depend on the appropriateness of the data analysis and statistical testing for covariates. For instance, as suggested by the KANWU multicenter study[33], after splitting the group by the median energy fat percentage, significant differences in IS according to fat quality (SFA *v.* MUFA) were observed. The net result for the MUFA effect was that the sub-group with low-fat intake had a 20 % enhanced IS relative to a SFA diet, whereas no MUFA-induced changes in IS were observed in the high-fat intake subgroup. Unfortunately, lack of inclusion of other relevant confounders (age, gender, nutritional status, degree of IS, menopausal status, geographic location) in the statistical model limit the application of this finding.

Study selection for analysis of effect of dietary fatty acid quality on insulin sensitivity in human subjects. Based on what has been presented in the previous section we attempted to categorize and select studies according to the quality of the experimental design. The criteria considered for study selection are shown in Table 1. Two authors (J. G. and E. D.) independently applied the criteria for study selection. Additional analysis included evaluation of strength and weakness of each study. We considered as points of strength well-powered studies (at least sixteen subjects in each group or intervention period), with a crossover design and inclusion of washout period; providing evidence of good or excellent dietary compliance, body weight stability and control for menstrual

Table 1. Criteria for selection of studies evaluating effect of dietary fatty acid quality on insulin sensitivity in human subjects

Criteria	Detail
Fatty acid quality as unique independent variable	Diets with similar energy and macronutrient content supplied according a random, crossover/parallel design
Dietary compliance at least fairly controlled	No changes in body weight indicating energy balance in equilibrium
	Controlled by at least one of these strategies: diets prepared in metabolic kitchen and consumed under supervision; provision of the main fat sources with defined nutrient and lipid composition; continuous dietary instructions and food records/capsule counting supervised by dietitians; blood/tissue fatty acid profile determination
Insulin sensitivity assessed using reference methods	Evaluated with euglycaemic–hyperinsulinaemic clamp, insulin suppression test or frequently sampled intravascular glucose tolerance test with Minimal Model

cycle as appropriate. Glucose disposal rate corrected for hepatic glucose production was considered as additional strength in studies in type 2 diabetic subjects.

From the total number of identified studies (*n* 41), fifteen matched the proposed quality criteria. These studies are summarized separately for non-diabetic subjects[30,33–40] (Table 2) and type 2 diabetic subjects[27,41–45] (Table 3). Those studies not selected for the analysis and reason for exclusion are shown as supplementary material. Three out of the fifteen studies reported a differential effect on IS, showing decreased IS after SFA *v.* MUFA[33] or PUFA[27] diets; whereas increased insulin resistance after fish oil supplementation was observed in type 2 diabetic individuals[45]. Also, we observed that according to the strength and weakness analysis (Table shown as supplementary material), the best rated study is by Vessby *et al.*[33]. This study reported a significant decrease in IS of 10 % after consuming a SFA-enriched diet for 12 weeks, whereas no change in IS was observed with the MUFA-enriched diet. Fish oil *v.* olive oil supplementation were additionally compared in this study; however, no differential effect on IS was found.

Potential mechanisms involved in fatty acid quality-dependent insulin resistance

In human subjects, using vegetable fat (safflower or soya-bean) emulsions infused intravenously[46–48] or hyperenergetic, high-SFA diets it is possible to impair IS within few days or even hours[49,50]. In both conditions, intramyocellular lipid content is increased which is causally related to impaired IS[51,52], since specific lipid species (i.e., diacylglycerols or ceramides) can activate specific serine kinases[53–56]. These kinases increase serine-phosphorylation of critical insulin signaling proteins (i.e., insulin receptor substrate 1) which reduce insulin-dependent GLUT-4 translocation and finally glucose uptake[52]. Specific FA probably induce these changes at different levels of cell function. Change in FA cell membrane composition is one of the most recurrent mechanisms. FA can modify membrane function by changing overall membrane fluidity, affecting membrane thickness/volume, modifying lipid phase properties, inducing changes in the membrane microenvironment, or by interactions of specific lipid components with membrane proteins[57,58]. However, the limited evidence to support this idea is based on weak associations between specific membrane FA profile and the IS data derived from cross-sectional studies in subjects with different metabolic states and poorly characterized diets[17,59–61]. On the other hand, FA may affect intracellular lipid balance based on its differential individual FA oxidation rate[62–65] and ability to modify the binding of the regulatory proteins (i.e., PPAR) to DNA response elements involved in lipid metabolism[66–68]. In animals, Pan *et al.*[69] demonstrated differential 24 h [1-^{14}C]α-linolenic oxidation rate and ^{14}C muscle incorporation in rats fed for 1 month with SFA, MUFA, and *n*-6 PUFA diets. The highest 24 h $^{14}CO_2$ recovery was found in the safflower-fed group, followed by the olive oil and lard diet; whereas skeletal muscle ^{14}C incorporation followed the inverse order. Finally, FA may differentially affect inflammatory pathways[70], which are tightly related to impaired IS. For example, toll-like receptor (TLR)-4-deficient mice (TLR-4

is important for mediating innate immune response to bacterial pathogens) have a much lower reduction of IS after infusing a lipid emulsion compared to wild-type animals[71]. Interestingly, increased TLR-4-mediated cytokine generation was observed after SFA (palmitic acid), but not *n*-3 PUFA supplementation.

Discussion and conclusions

Human studies have failed to demonstrate a consistent differential effect of dietary fat quality on IS, which contrasts with observation from animal studies, where *n*-6 PUFA in comparison to *n*-3 PUFA decrease IS[5,6]. Several factors might explain this disparate finding. Perhaps the simplest explanation involves the differences in the fish oil and *n*-6 PUFA dose used in animals as compared to human studies. In human subjects, the maximal fish oil dose studied does not exceed 20 g/d (about 0·25 g/kg body weight per d)[45] whereas studies in rats used 3·4 g[5] and 20 g[6] fish oil/kg body weight per d. After correcting these doses for differences in metabolic rate, the fish oil administered to rats is between three and twenty times greater than the maximal dose used in human subjects. This is obtained after applying a scaling factor of 0·75; acceptable for comparisons among mammalian species with different body mass[72]. Then, for an 80 kg human subject an approximately 4-fold lower metabolic rate than that of a 0·35 kg rat on a per kg basis is calculated; the equivalent dose of 0·25 g fish oil/kg per d in a human subject corresponds to 1 g/kg per d for the rat. When the same estimation is done for *n*-6 PUFA, the doses used in human subjects and rats are much closer, since large amount of *n*-6 PUFA are present in most Western diets.

In addition, the effect of fish oil on IS has been mainly assessed in individuals with type 2 diabetes (Table 3). Perhaps at this stage of metabolic impairment, glucose homeostasis may not be capable of an improvement even when a large dose of fish oil is provided. In non-diabetic individuals the effects of fish oil on IS has been scarcely evaluated. In a well-powered long-term study, diets enriched in SFA or MUFA were compared[33]. Additionally, each diet was supplemented with fish oil or olive oil (placebo). As commented before, significant differences in IS between SFA and MUFA diets were found; however fish oil supplementation did not modify IS (see Table 2).

The potential protective effect of *n*-3 PUFA on IS in human subjects might require higher fish-oil doses associated with lower *n*-6 PUFA content. Studies in a healthy and diabetic population using diets with low absolute amounts of *n*-6 PUFA should be preferred in order to adequately test the potential effect of fish oil on IS. Fat sources such as linseed oil, which are rich in α-linolenic acid (>55 % of total FA) can be used to reduce amount of *n*-6 FA with the aim to achieve a dietary *n*-6 : *n*-3 PUFA ratio lower than 4:1 but as close to 1:1 as possible. This *n*-6:*n*-3 PUFA ratio is similar to that observed in traditional Asian diets considering the use of both marine and land sources of *n*-3 PUFA.

There is also a need to distinguish between fish oil and highly-unsaturated *n*-3 FA (EPA and DHA), since the amount of EPA and DHA commonly present in fish oil is about one third of the total FA content and the relative balance between EPA and DHA may also vary. To the best of our

Table 2. Effect of dietary fat quality intervention on insulin sensitivity in non-type 2 diabetic subjects

Study	Subjects (n for IS data)	Design	Time	Dietary intervention	IS method	Outcome for IS*
Schwab et al. (1995)[34]	Healthy, young, non-obese women (n 11)	Crossover, random. 2 week diet control before interventions	4 weeks	Fat % 38. Diets high in lauric (5 %) or palmitic (12 %) acids	FSIVGTT with MinMod program	No change Lauric: 4·5 (SD 0·5) Palmitic: 4·8 (SD 0·6)
Fasching et al. (1996)[35]	Healthy, young, non-obese men (n 8)	Crossover, random. Washout period for 2 weeks	1 week	Fat % 55. Diets high in SFA (30 %) or PUFA (26 %)	Euglycemic clamp (190 mU/m² per min)	No change SFA: 9·7 (SD 0·8) PUFA: 9·8 (SD 0·9)
Louheranta et al. (1998)[36]	Healthy, young, non-obese women (n 14)	Crossover, random. 2 week basal diet before each intervention	4 weeks	Fat % 40. Diets high in oleic (19 %) or stearic (19 %) acids	FSIVGTT with MinMod program	No change Oleic: 5·2 (SD 1·6) Stearic: 5·4 (SD 1·9)
Louheranta et al. (1999)[37]	Healthy, young, non-obese women (n 14)	Crossover, random. 2 week basal diet prior to each period	4 weeks	Fat % 37. Diets high in trans- (5 %) or cis- (19 %) MUFA	FSIVGTT with MinMod program	No change trans-MUFA: 5·1 (SD 0·4) cis-MUFA: 4·8 (SD 0·4)
Lovejoy et al. (2002)[38]	Healthy, young, non-obese subjects both sexes (n 25)	Crossover, random, double blind. Washout period for 2 weeks	4 weeks	Fat % 27. Diets high in trans- (7 %), cis- (15 %) MUFA or SFA (11 %)	FSIVGTT with MinMod program	No change trans-MUFA: 3·4 (SD 0·3) cis-MUFA: 3·4 (SD 0·3) SFA: 3·2 (SD 0·3)
Andersson et al. (2002)[30]	Non-diabetic subjects both sexes (n 32)	Parallel, random, single blind	3 months	Fat % 37. Diets high in MUFA (21 %) or SFA (18 %). Each group supplemented with 3·6 g/d n-3 FA (2·4 g EPA + DHA) or olive oil	FSIVGTT with MinMod program	No change (values not reported)
Brady et al. (2004)[39]	Healthy, lean and obese men (n 14)	Parallel, random, double blind	12 weeks	Fat % 37. Diets high in MUFA (15 %) or n-6 PUFA (10 %)	FSIVGTT with MinMod program	No change MUFA: 2·8 (SD 0·7) n-6 PUFA: 2·0 (SD 0·4)
Vessby et al. (2001)[33]	Non-diabetic subjects both sexes (n 162)	Multicenter study. Parallel, random, single blind	3 months	Fat % 37. Diets high in MUFA (21 %) or SFA (18 %). Each group supplemented with 3·6 g/d n-3 FA (2·4 g EPA + DHA) or olive oil	FSIVGTT with MinMod program	Lower after SFA (P=0·03) No change with fish oil MUFA: 4·9; SFA: 3·7 (SD not reported)
Toft et al. (1995)[40]	Non-treated hypertensive, non-diabetic subjects both sexes (78)	Parallel, random, double blind	16 weeks	Fat % not reported. Supplementation with 4 g/d fish oil (3·4 g EPA + DHA) or corn oil	Euglycemic clamp (40 mU/m² per min)	No change Fish: 0·07; Placebo: 0·07 (SD not reported)

IS, insulin sensitivity; FSIVGTT with MinMod, frequently sampled intravascular glucose tolerance test with Minimal Model.
* IS units are different in each study and these have been not included in the table.

Table 3. Effect of dietary fat quality intervention on insulin sensitivity in type 2 diabetic subjects

Study	Subjects (n for IS data)	Design	Time	Dietary intervention	IS method	Outcome for IS*
Summers et al. (2002)[27]	Non-obese, obese and type 2 diabetic (n 17)	Crossover, random. No washout period included	5 weeks	Fat % 42 in SFA diet and 34 % in PUFA diet. Diets enriched in SFA (21 %) or PUFA (9 %)	Euglycemic clamp (40 mU/m² per min)	Higher after PUFA SFA: 0·51 (SD 0·08) PUFA: 0·64 (SD 0·10) (P=0·02)
Borkman et al. (1989)[41]	Type 2 diabetic subjects both sexes (n 10)	Crossover, random, double blind. Washout period for 3 weeks	3 weeks	Fat % 37. Supplementation with 10 g/d fish oil (3 g EPA + DHA) or safflower oil	Euglycemic clamp (1·1 mU/kg per min) with HGP correction	No change Fish: 21·5 (SD 3·5) Placebo: 21·2 (SD 3·8)
Luo et al. (1998)[42]	Type 2 diabetic, hypertriacylglycerolaemic men (n 10)	Crossover, random, double blind. Washout period for 2 months	8 weeks	Fat % 44. Supplementation with 6 g/d fish oil (1·8 g EPA + DHA) or sunflower oil	Euglycemic clamp with 2 insulin doses (40 and 250 mU/m² per min) and HGP correction	No change Low-insulin: Fish 3·3 (SD 0·2); Placebo 3·8 (SD 0·5) High-insulin: Fish 8·1 (SD 0·9); Placebo 8·8 (SD 0·8)
Boberg et al. (1992)[43]	Type 2 diabetic both sexes (n 14)	Crossover, random, double blind. No washout period included	8 weeks	Dietary fat % not indicated. Supplementation with 10 g/d fish oil (3 g EPA + DHA) or olive oil	Euglycemic clamp (not indicated insulin dose)	No change Fish 4·7; Placebo 4·9 (SD not reported)
Rivellese et al. (1996)[44]	Type 2 diabetic both sexes (n 16)	Parallel, random, double blind	6 months	Fat % 30. Supplementation with fish oil (first 2 mo 3·2 g/d (2·6 g EPA + DHA) and last 4 mo 2·1 g/d (1·7 g EPA + DHA)) or olive oil	Euglycemic clamp (2 mU/kg per min)	No change Fish 4·0 (SD 0·5); Placebo 4·1 (SD 0·5)
Mostad et al. (2006)[45]	Type 2 diabetic both sexes (n 26)	Parallel, random, double blind	9 weeks	Fat % 38. Supplementation with 18 ml/d fish oil (5 g EPA + DHA) or corn oil	Euglycemic clamp (40 mU/m² per min)	Lower after fish oil (values not reported) (P=0·049)

IS, insulin sensitivity; HGP, hepatic glucose production.
* IS units are different in each study and these have been not included in the table.

knowledge only one study has assessed the effect of EPA and DHA on IS separately[73]; however, the lack of a reliable marker of IS in this study does not permit an adequate conclusion.

The time to respond in terms of IS to the intervention may also be critical in explaining the lack of differential effect of fat quality on IS in human subjects. From the available data it is unclear what is the required time to increase the chances of finding a differential effect on IS relating to the FA quality. At present, well-supported effects have been demonstrated after 12 weeks of intervention[33], suggesting that changes in plasma and/or sub-cellular FA composition might be critical. On the other hand, it is well known that even after short-time periods, SFA-enriched diets (days)[49,50] or vegetable fat-based lipid infusions (hours)[46–48] are able to reduce insulin action in human subjects. To date, only two studies have assessed the short-term effect of FA quality on IS; however, small sample sizes[35] or inappropriate assessment of IS[74] do not permit firm conclusions to be drawn. Thus, a well-powered, controlled short-term intervention should not be excluded as a suitable approach to assess dietary fat quality effect on IS in human subjects.

Finally, the realization that not all fats are the same is by no means new; however, we are just beginning to unravel the physiologic and possibly therapeutic effects of specific FA. These nutrients should no longer be considered solely as a source of energy but should rather be acknowledged as potent regulators of intermediary metabolism, and possible contributors to the regulation of glucose homeostasis in both type 2 diabetic and non-diabetic individuals.

Acknowledgements

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