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Review

Dietary fats and insulin action

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Background

The history of research into the relationship between dietary fat intake and impaired insulin action has its origin in the work of Himsworth approximately 60 years ago [1, 2]. In a series of pioneering studies using crude indices of insulin action and limited subject numbers (only one in an often quoted paper!), Himsworth linked high levels of fat intake with insulin resistance, and conversely, improved insulin action with predominantly carbohydrate diet. However, the link was tenuous and really apparent only at the extreme ends of the dietary spectrum (< 20 or > 80 % of calories as fat).

These studies, flawed as they were, influenced the field enormously in the absence of any significant work in the area for a remarkable period of time. The conclusions reached by Himsworth were reinforced to some extent by misuse of glucose tolerance data that showed deteriorations following periods on diets extremely low in carbohydrate (< 50 g/day) and improvement on liquid formula diets providing a remarkably high percentage (75–85 %) of calories from carbohydrate [3–5]. It was really not until the 1980s that the development of acceptable techniques for the measurement of insulin action in vivo allowed the relationship between dietary fat intake and insulin action to be accurately assessed. These studies have essentially been confined to investigations in rodents and humans.

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Abbreviations: NIDDM, non-insulin-dependent diabetes mellitus; n-3 fatty acids, omega-3 fatty acids.

Dietary fat and insulin action in rodents

In rodent studies, the results are relatively straightforward. Initial studies investigated insulin action in vitro in adipocytes and isolated skeletal muscles [6–8]. High fat feeding consistently impaired insulin-stimulated glucose uptake in both fat and more importantly muscle, the major tissue determining insulin-stimulated glucose uptake. The mechanism(s) underlying these observations were not definitively elucidated, although a number of observations were made. For example, Felber and co-workers [9, 10] noted a major increase in stored triglyceride in muscle from high fat fed rats and increased lipid availability should, via the mechanism of the glucose/fatty acid cycle of Randle and co-workers [11, 12], impair glucose utilisation. Consistent with this, early reports [9] indicated impaired activation of the pyruvate dehydrogenase complex, thought to be rate-limiting for glucose oxidation. However, the results vary between tissues and there is a question regarding any impairment in skeletal muscle [13]. Other relevant observations were of decreased binding of insulin to its receptor and impairment of the ensuing activation of tyrosine kinase [14], although that effect may well be dietary fat-type specific [15]. Certainly there is evidence for impaired glucose transport [8] possibly due to reduced amounts of the insulin-regulable glucose transporter GLUT4 in very high fat diets ([16, 17]; cf. below); and reduced proportion of glycogen synthase in the I (or active) form in skeletal muscle [18] consistent with the observation of a defect in glucose storage as glycogen.

These largely in vitro studies were followed up in vivo using the hyperinsulinaemic, euglycaemic clamp technique. Initial studies compared high fat and high carbohydrate diets [19, 20]. Even when the diets were fed in equicaloric amounts, insulin action at the whole-body level deteriorated markedly within a

short (3–4 weeks) period on the high fat diet. Further, combining the clamp procedure with tracer doses of labelled glucose and the non-metabolisable glucose analogue 2-deoxy-D-glucose (which is taken up into tissues in competition with glucose, phosphorylated and trapped [21]) allowed insulin action to be assessed in individual tissues including liver and the important skeletal muscles. These studies showed the insulin resistance to occur firstly in the liver within a very short period of time (3 days; [22]) followed by widespread impairment in insulin action in a range of skeletal muscles and in white and brown adipose tissue by 3 weeks. These effects on insulin action occurred in combination with a relative reduction in metabolic rate and accumulation of body fat in the high fat fed rats despite equicaloric feeding [19].

As well as these dietary manipulations in adult rats, workers from Girard's group in Paris [23] investigated the transition from the high fat diet of suckling to a high carbohydrate diet of laboratory chow. Here the insulin-resistant suckling rat becomes insulin sensitive; however, if weaned on to a high fat diet, insulin resistance persists. These observations were straightforward. However, an interesting wrinkle appeared when attention was directed to the type of fat that made up the high fat diet [24, 25]. These studies were prompted by the observations of the hypotriglyceridaemic properties of omega-3 (ω-3 or n-3) fatty acids [26]. Given the excess accumulation of adipose tissue with high fat diets predominantly of the omega-6 (ω-6 or n-6) type [19], and the known glucose/lipid interactions embodied in the glucose-fatty acid cycle of Randle and co-workers [11, 12], it was proposed that inclusion of n-3 fatty acids in the high fat diet may be beneficial. Indeed that was the observation. Insulin resistance in both the liver and skeletal muscles was prevented; the beneficial effects in the latter tissues being an improvement in both oxidative and storage components of insulin-stimulated glucose disposal [24]. This latter observation was suggestive of either multiple defects or a single defect at an early, common point in the glucose metabolic pathway, perhaps at the membrane level.

Follow-up work was aimed at extending the in vitro work in terms of elucidating mechanisms. An extensive study was carried out comparing a number of high fat diets which were pair-fed and differed only in the fatty acid profile of the fat components [25]. The results demonstrated marked intergroup differences in insulin-stimulated glucose metabolism when assessed using the euglycaemic clamp. Some high fat fed groups progressed to major insulin resistance and others did not. The effects were particularly pronounced in skeletal muscle. Two variables were closely associated with development of muscle insulin resistance: 1) accumulation of storage lipid, consistent with the glucose/fatty acid cycle of Randle; and 2) a relative reduction in the percentage of highly unsaturated n-3 fatty acids in muscle membrane structural lipid (see Figs. 4 and 5 of [25]). In a further attempt to provide mechanistic information, GLUT4 mRNA levels were determined in skeletal muscle across the range of dietary groups [27]. While no differences were observed despite a fourfold range in insulin action, more recent data from transgenic mice indicate increases in GLUT4 levels can prevent the impairment in glycaemic control induced by a high fat (safflower oil) diet [28]. Taken together these latter two studies might suggest that feeding high levels of some, but not all, types of fats might impair either the translocation or intrinsic activity of glucose transporters [29].

Dietary fat and insulin action in humans

The work at that stage then prompted an assessment of how these results might relate to those available regarding the effects of diets relatively high in fat on whole-body, and in particular muscle, insulin action in humans. The influence of the fatty acid profile was also examined. While there is literature on diet and glucose tolerance, it is complicated by alterations in insulin secretion (particularly important when dietary fat subtypes are considered [30]) and analysis was restricted to studies which had employed currently acceptable techniques for the in vivo assessment of insulin action. The results were surprising [31]. Essentially, there were remarkably few studies which had actually compared high fat, low carbohydrate and lower fat, high carbohydrate (as per the current recommendations) diets. Even in the few studies we could identify [32–37], the results were equivocal with no clear conclusion possible. More recent studies again provide no clear direction, with one actually showing poorer whole-body insulin action with a high carbohydrate, compared to a higher fat, diet [38].

Short-term dietary interventions aimed at changing the type of fat, usually with fish oil supplements to increase the proportion of highly unsaturated n-3 fatty acids, have not shown as dramatic a result as might have been predicted from the rat studies. Although positive results have been obtained in two investigations using fish oil supplements [39, 40], there were relatively modest improvements in insulin action. Balanced against these results are two negative studies [41, 42] which showed no change at all in insulin action. One further positive study showed improved insulin action where the manipulation was an increase in the polyunsaturated to saturated fat ratio [43].

Overall, one would have to conclude that the evidence for changes in insulin action in humans with short-term manipulation of dietary fat amount and type is very limited and entirely inconclusive.

Epidemiological, prospective and cross-sectional studies in humans

In contrast to these relatively short-term intervention studies, there is now accumulating evidence from cross-sectional and epidemiological studies which appear to link a higher fat, and particularly saturated fat, intake with insulin resistance (albeit measured indirectly) and progression to glucose intolerance and diabetes. A study from Lovejoy and DiGirolamo [44] used the frequently sampled intravenous glucose tolerance test (FSIGT) to calculate an insulin sensitivity index (S_i) in 45 lean and obese glucose-tolerant subjects. A food frequency questionnaire assessing habitual food intake was used. They were able then to show a significant ($r = -0.41$, $p = 0.01$) relationship between log S_i and percentage total dietary fat (no better relationships being observed with any subtype of fat). However, when S_i was adjusted for body mass index (BMI), then the percentage dietary fat was no longer a significant, independent predictor.

The other studies available relating dietary fat and insulin action have relied on more indirect measures of insulin action. Parker and co-workers [45] studied a large group of middle aged to elderly men and found significant positive correlations between percentage of total fat and percentage of saturated fat with both fasting and prandial insulin levels. These relationships remained significant even after adjustment for measures of adiposity. These results were similar to those found by Mayer and colleagues [46] in the Kaiser Permanente Women Twins Study. Again there was a significant positive relationship between both percentage of total and saturated fats and fasting insulin. In this study percentage of monounsaturated *and* percentage of polyunsaturated were also significantly related to fasting insulin, although all fat subtypes dropped out after adjustment for total fat. Interestingly in the monozygotic twins, total dietary fat was not significantly related to fasting insulin if adjustment was made for measures of adiposity [46] although this may reflect relatively small numbers of subjects. Finally, in this study it is important to note the authors' comment that, "the positive association of fasting insulin concentrations with dietary fat was significantly attenuated among relatively active women compared with sedentary women". The ability of physical activity to ameliorate high fat feeding induced insulin resistance has been noted previously in animal studies [47] and it will be important to understand these interactions in humans.

Investigators in two major epidemiological studies have reported on the relationships between measures of insulin resistance and dietary fat. Marshall, reporting for the San Luis Valley study [48], has shown that percentage of dietary fat intake was higher in glucose intolerant and non-insulin-dependent diabetic (NIDDM) individuals compared to BMI-matched control subjects. Further, in an interesting variation [49], Marshall followed 123 glucose-intolerant individuals for 1 to 3 years. In that time 60 subjects reverted to normal glucose tolerance, 43 remained glucose intolerant and 20 converted to overt NIDDM. Intake of total fat, saturated fat, and monounsaturated fats (but not polyunsaturated fat) was associated with an increasingly poor outcome (i.e. remaining glucose intolerant or, worse still, progressing to NIDDM) even when adjusted for age, sex and ethnicity.

The other major epidemiological study is the Zutphen Elderly Study. Feskens and co-workers initially reported that the percentage of saturated fat intake was positively related to glucose intolerance and fasting glucose levels [50]. This group has now investigated relationships between dietary variables and indices of hyperinsulinaemia including fasting C-peptide levels and the area under the insulin curve following an oral glucose load. It is particularly interesting that they have found a significant relationship between measures of percentage of total dietary fat and fasting C-peptide. Further, the percentage of saturated dietary fat was even more highly related to both C-peptide levels and to the area under the insulin curve. In contrast, there was a *negative* relationship between percent polyunsaturated dietary fat intake and the area under the insulin curve (i.e. the more polyunsaturates, the lower insulin levels in response to a glucose load). In this study, the polyunsaturated fats were not subdivided into n-6 and n-3s but this research group has previously shown a protective effect of fish intake, and thus presumably elevated n-3 levels in the diet, against the tendency to develop glucose intolerance [51].

Finally, in an interesting study, the fatty acid profile of serum cholesterol esters was related to risk of developing NIDDM at a 10-year follow-up [52]. Cholesterol ester fatty acid profile is thought to reflect dietary fatty acid profile over a relatively long period (at least weeks). This study showed increases in saturated fatty acids and a decrease in linoleic fatty acid in the serum cholesterol esters of those who developed NIDDM, compared to those who did not. The authors suggest that the lower level of linoleic fatty acid may reflect lower vegetable fat intake. In this regard the study by Colditz et al. [53] also showed a modest, but significant, relationship between increased vegetable fat intake (by dietary analysis) and development of NIDDM in a 6-year follow-up of over 80 000 women.

It is noteworthy that in a number of the studies just discussed, a higher percentage of monounsaturated fats was associated with increasing adiposity and indices of insulin resistance. Focus in the diabetes literature has been on major beneficial effects in short-term trials [54, 55] and these results are consistent with the low rates of diabetes in areas with a high

intake of olive oil. However, the epidemiological and cross-sectional literature from North America and Europe show positive relationships between the percentage of monounsaturated fat intake and both measures of insulin resistance [56] and worsening diabetes outcome [49]. In the geographical areas of these studies the dietary intake of monounsaturated fats is largely in association with saturated fats (e.g. meat and dairy products) and this may account for the apparent inconsistencies in the effects of monounsaturates.

The results of the cross-sectional and epidemiological studies, while often demonstrating only modest relationships, are important. Use of the doubly labelled water technique (see [57, 58]) has, for the first time, allowed accurate measurement of 10–14 day total energy expenditure in freely living individuals. This has allowed some validation (at least for total energy intake) of the various methods used to collect dietary intake data. The results are discouraging. There is wide variation between reported and actual intakes (see [59, 60]). While it is possible that spurious relationships have arisen due to selective underreporting of particular macronutrient subclasses, it is perhaps even more significant that relationships between dietary fat/fat subtypes and measures of insulin action can be established at all in the face of such methodological adversity.

Muscle insulin resistance and membrane lipid subtypes

The contrasts are therefore established. In rats, a relatively short-term increase in the percentage of dietary fat, even without an increase in total caloric intake, leads to insulin resistance, whilst in humans the data are equivocal. In contrast, evidence has emerged over the past 3 to 4 years that longer-term exposure to a higher fat diet in humans does lead to insulin resistance, measured either directly or indirectly, and predisposes to the development of glucose intolerance and diabetes.

In parallel with these observations, short-term manipulation of the dietary fatty acid profile (i.e. changing the fat subtypes) in rats produces major effects on insulin action. In humans there is a modest, if any, change. However, there is again some evidence from analysis of longer-term dietary patterns in humans of a beneficial effect of increased n-3 in the diet with a relative reduction in the incidence of glucose intolerance [51].

These contrasts, and the observation in rats of the close relationship between the presence of highly unsaturated fats in muscle membranes and insulin action as noted earlier [25], led to cross-sectional studies in humans. The hypothesis was that habitual dietary fatty acid profile over a long period of time would be reflected in the fatty acid profile of the

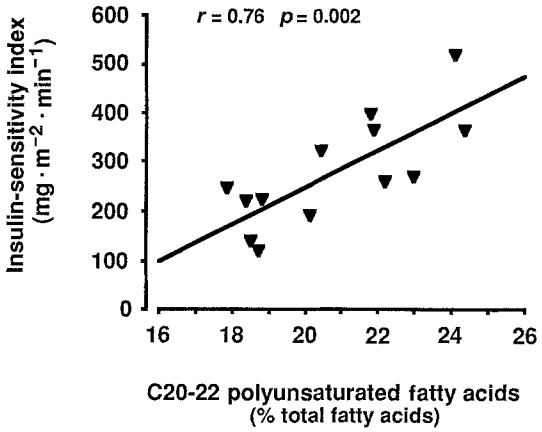

Fig. 1. Relationship between the percentage of long-chain polyunsaturated fatty acids in the phospholipid of skeletal muscle (vastus lateralis) and whole-body insulin sensitivity determined by the hyperinsulinaemic, euglycaemic clamp technique. Reproduced from [61] with permission

structural membrane lipids in skeletal muscle. In turn this fatty acid profile, as in the rat studies, would relate to insulin action. Two studies were initially carried out. The first was an investigation in normoglycaemic cardiovascular surgery patients in which the fasting insulin level was used as a crude index of insulin action and a muscle biopsy was obtained at surgery. The follow-up study in healthy volunteers employed the hyperinsulinaemic, euglycaemic clamp to assess insulin action directly along with a percutaneous biopsy of vastus lateralis muscle. In each of these studies the phospholipid fatty acid composition of the biopsied muscle was then determined [61]. Phospholipids are structural lipids essentially confined to membranes. They form a major lipid component of the membrane bilayer: from the proportionally largest component of plasma membrane to almost all the lipid in mitochondrial membrane. These studies demonstrated clear and consistent relationships between the percentage of highly unsaturated lipids in the muscle structural membrane lipids and insulin action ([61]; Fig. 1). Conversely, the more saturated the muscle membrane phospholipids, the more insulin resistant the individual.

This work has been recently followed-up in disparate populations from the United States and Sweden. In the diabetes- and obesity-prone Pima Indians results consistent with the original observations were obtained, but with two major advances [62]. Close relationships were established between relative leanness and increased muscle membrane lipid unsaturation (conversely, the greater the obesity, the more saturated the muscle membrane). Secondly, the Pima Indians had a much lower level (< 40 %) of the highly unsaturated n-3 fatty acids than our Australian, largely Caucasian, population. In fact it was striking that, despite the overall effect of polyunsaturated fats being positive for insulin action, there was a clear effect

of the n-6 to n-3 ratio (being the two major classes of polyunsaturated fats) in both our Australian and the Pima study groups. When this ratio is plotted for both groups together the results show that the higher the n-6/n-3 ratio, the higher the fasting insulin (a measure of insulin resistance in these normoglycaemic populations), and the higher the relative body weight [63]. This is consistent with work from Raheja et al. [64] showing a beneficial effect on glycaemic control in NIDDM subjects as a result of reducing the dietary n-6/n-3 ratio. In the past 30–40 years western countries have been undergoing a major dietary experiment of high n-6 polyunsaturated fat intake, largely driven by the cardiovascular literature. It would be ironic if at the same time, this experiment has had deleterious effects on diabetes and obesity.

In addition to the studies in Australians and native Americans, a similar analysis has been carried out by Vessby and colleagues on a Swedish population [65]. Again highly significant relationships have been established between muscle phospholipid fatty acid composition and insulin action. However, differences between the Swedish and the Australian and Pima Indian results did occur. First, in the Swedish study the highest correlation occurred with saturated fatty acids: the greater the proportion of saturated fats in the muscle membranes, the more insulin resistant the individual. Relationships with the highly unsaturated fatty acids were not, as in the other studies, significant. The second major difference, which may account for the first, is that the percentage of n-3 fatty acids in the Swedish population was much higher than in the Australian, and of course very much higher than in Pima subjects. These observations presumably reflect, at least in part, a difference in the dietary intake of n-3 fatty acids. However, there is also very likely to be a genetic predisposition to incorporation of specific fatty acids into membranes. In the studies of Borkman et al. [61] dietary analysis of a limited number of subjects showed that relative amounts of, for example n-3 fatty acids, did not relate at all well to apparent dietary intake. It will be interesting to determine if the low level of n-3 fatty acids in the Pima Indian muscle membranes is more closely related to low dietary intake or reflects a genetic predisposition. In this regard it is interesting that an intestinal fatty acid binding protein on chromosome 4q has been linked with insulin resistance in this population [66].

Finally, an interesting aspect of the Borkman study [61] and the follow-up in Pima Indians [62] was the strong relationship between insulin action and indices of the Δ5 desaturase activity. This enzyme is necessary in production of the most unsaturated fatty acids in both the n-6 and n-3 series of essential polyunsaturated fats. It is clearly true that the Unsaturation Index (the number of double bonds per fatty acyl group times 100 – a convenient measure of overall lipid unsaturation) of the average 'western' diet is much less (UI equals ≈ 80 – Australian dietary survey, National Health and Medical Research Council, 1991) than the UI of skeletal muscle phospholipid which averages 160–170 in the above studies. With the exception of certain marine oils, the major dietary fatty acids must all be substantially elongated and desaturated to be transformed into the fatty acids that our results have shown to be associated with insulin sensitivity. The elongase and desaturase enzymes are crucial rate limiting elements in this transformation. Activity of these enzymes can be inferred from product/precursor ratios (for example the ratio of 20:4n-6 to 20:3n-6 gives an activity index of the Δ5 desaturase enzyme). While not an optimal method for measuring enzyme activity, our results in humans, both in an Australian largely Caucasian sample [61] and in Pima Indians [62], have shown remarkably strong relationships between reduced Δ5 desaturase activity (20:4 n-6/20:3 n-6) and both insulin resistance and obesity. In this regard, agents that influence the activity of desaturase enzymes may play a central role in development of disease. It is interesting in this regard to note that it has been suggested that trans fatty acids may play a role in the development of obesity and insulin resistance [67] and that this subtype of fatty acid has also been shown to inhibit the activity of desaturase enzymes (see [68]).

Taken together these studies [61, 62, 65, 69] and the previous rat studies [24, 25] suggest a powerful role for the fatty acid composition of membrane lipids in determining insulin action.

In summary, a good deal of evidence has now accumulated to show that the fatty acid profile of the diet is important. The evidence has ranged from early in vitro evidence of differences in insulin binding and glucose transport in cells incubated with different types of fat [70, 71] or in animals fed different fats [24, 25] to cross-sectional relationships between membrane structural lipid fatty acid profile and insulin resistance in humans [61, 65, 69] and finally to cross-sectional and epidemiological evidence linking particularly high saturated fat intake [45, 48–50, 56, 72] with hyperinsulinaemia and increased risk of diabetes. This contrasts with the lack of relationship, or even possible protective effect, of polyunsaturated fats on insulinaemia. In particular habitual increased n-3 polyunsaturated dietary fat intake (as fish fats) [51, 73, 74] would appear to be protective against development of glucose intolerance.

Dietary fats and energy balance

It has recently been argued that the single variable most predictive of insulin resistance is adiposity and, in particular, central adiposity [45, 75, 76]. So what is the evidence linking fat intake with obesity and is it

the case that the sole effect of dietary fat on insulin action is via an increase in adiposity?

From a theoretical perspective there is certainly reason to argue that fat is stored more efficiently as fat than is carbohydrate (energy wastage on the fat to fat conversion is theoretically only some 3 % while wastage on the dietary carbohydrate to stored fat conversion is over 20 % (see [77]). Further, while not an unequivocal observation [78], there is some evidence that an increase in caloric intake as fat does not provoke a rise in fat oxidation [79]. This latter finding would seem to be particularly true in the post-obese subjects studied by Astrup et al. [80] where increasing fat intake would certainly be a powerful provocation to weight regain.

The experimental animal literature would suggest a link between dietary fat intake and obesity but with some caveats. Certainly the straight comparison between a high carbohydrate diet and a high fat diet, fed equicalorically, leads to the conclusion that the high fat diet is handled more efficiently and results in a greater accumulation of adiposity in rats [19]. However, the type of fat is important. In the short term the relative propensity of various fats to be oxidised or stored is related to their unsaturation, the position of the double bond(s), and the carbon backbone length [81, 82]. Translating these studies into the longer term, again the predisposition to obesity following exposure to any particular high fat diet appears to be markedly influenced by the fatty acid profile of that diet [83–85] (see [86] for a recent review). The more unsaturated the fatty acid profile of the diet, the more resistant the animals to obesity – a general pattern consistent with the above results of Leyton et al. [81].

Human studies on this topic have not been consistent. In a reanalysed study [87], with strict control under hospital controlled conditions in normal weight individuals, the conclusion was that equal intakes of a high carbohydrate or a high fat diet led to equal weight gain – although the actual adiposity was not measured and may conceivably have been greater (as in the equivalent animal study [19]). The conclusions of Leibel and co-workers [87] are nicely congruent with those of Hill et al. [88]. These workers employed very high carbohydrate versus very high fat diets (60 % of calories from the major macronutrient) and assessed expenditure directly in a whole-room indirect calorimeter in order to accurately determine energy expenditure. They concluded that, "Diet composition did not affect total daily energy expenditure but did affect daily nutrient oxidation by rapidly shifting substrate oxidation to more closely reflect the composition of the diet". This contrasts with a study carried out under field conditions which came to the opposite conclusion; that a high percentage of calories in the diet from fat is associated with a relatively lower caloric intake to maintain body weight [89]. This latter study showed a quite remarkable apparent increase in metabolic inefficiency on a very low fat, high carbohydrate, diet (20 % of calories as fat) with weight and adipose tissue loss despite caloric intake almost 20 % more than that on a higher fat diet (37 % of calories from fat). It is not obvious how these results can be reconciled with those of Leibel and of Hill, except to say that there is a question mark over dietary intake data under non-hospital controlled conditions. Overall, the reasonable conclusion from both the animal and human work would seem to be that high fat diets can lead to obesity through increased metabolic efficiency although this may be particularly true only of diets with a more saturated fatty acid profile. Clearly this is an area deserving of considerable further investigation.

If we turn again to the epidemiological literature, there has been a wealth of reports in the last few years on the relationship between dietary fat intake and adiposity. Many of these studies have shown relationships between fat intake, as a percentage of calories, and a range of measures of increased adiposity [44, 46, 90–93]. However, a recent analysis of the NHANES I Epidemiologic Follow-up Study surprisingly shows very little correlation between baseline percentage of fat intake and subsequent weight change in the next 8 to 10 years [94]. Analysed in quartiles these are quite remarkable data. In a sample of 4567 women those in the lowest quartile of fat intake (25.6 ± 0.2 % of energy as fat) had a 10-year follow-up weight gain of 2.53 ± 0.3 kg while those in the highest quartile of fat intake (47.2 ± 0.2 % of energy as fat) had an almost identical weight gain of 2.47 ± 0.3 kg. While in this study, on a multivariate analysis and excluding all men with any morbidity, there was a just significant positive relationship between fat intake and weight change in men, the relationship was inverse in women. Either the initial estimates of fat consumption were entirely erroneous (the 24-h recall method is prone to within-person variation), there was major change in intake patterns over the 10 years, there was a direct correlation between fat intake and increased physical activity, or these data stand in marked contrast to the work noted above.

There is also some work in the epidemiological literature which speaks to the issue of fat subtypes with analysis of total fat intake into saturated, monounsaturated and polyunsaturated. Work by both Dreon et al. [90] and Romieu et al. [92] found that percentage of body fat was significantly related to the percentage of saturated fat and monounsaturated fat but not to polyunsaturated fat. Finally, increased adiposity itself may not be a single variable. Certainly central adiposity appears to be the major culprit in a range of metabolic complications [95]. Recent work from our laboratory has shown, in a cross-sectional study on Pima Indians that both muscle triglyceride

accumulation and waist/thigh ratio, as a measure of central adiposity, independently relate to insulin action (Pan and Storlien, unpublished observations). Further, when those variables are taken into account total adiposity no longer significantly relates to insulin action.

Dietary fats and insulin resistance independent of obesity?

If on balance the evidence from cross-sectional and epidemiological studies in humans suggests that an increased percentage of dietary fat will lead to increasing obesity, then can this relationship totally explain the effect on insulin resistance? From the study of Lovejoy and DiGirolamo [44], which directly assessed insulin action, the answer would appear to be yes. When S_i, their minimal model-derived index of insulin sensitivity, was tested after adjustment for BMI, then the percentage of fat in the diet was no longer a significant predictor. However, they had relatively few subjects and the result may represent a type II error. In contrast, on much larger samples, four of the studies analysed above have shown relationships between measures of insulin action and percentage of dietary fat which are independent of the relationship with adiposity. Firstly, Maron et al. [72] showed that intake of saturated fats was significantly related to both BMI and fasting insulin. Further, the relationship between saturated fat intake and fasting insulin remained after adjustment for BMI. In the report of Parker and colleagues [45] percentage of dietary saturated fat also remained a significant predictor of both fasting and postprandial insulin after adjustment for measures of adiposity while dietary unsaturated fat was not a predictor before, or after, adjustment. Similarly Feskens et al. [56] have shown that both fasting C-peptide levels and the area under the insulin curve following an oral glucose tolerance test were significantly related to both percentage of saturated and percentage of monounsaturated dietary fat independently of a number of variables including adiposity. Importantly, in this study the percentage of polyunsaturated fat was inversely related to the area under the insulin curve after correction for adiposity. Finally, Mayer et al. [46] showed that as well as a relationship, independent of adiposity, between fasting insulin and saturated and monounsaturated fat intake, there was also a significant, positive relationship with linoleic, n-6 polyunsaturated fat.

Taken with the work of Feskens et al. [51] showing an apparent protective effect of fish intake (with its high levels of n-3 polyunsaturated fats) on glucose tolerance, the above studies would appear to be in close agreement with the experimental animal literature and offer the following synthesis. Increased dietary fat intake in general, and saturated fat specifically, will lead to increased adiposity. This increased adiposity, particularly central adiposity, will lead to impairment of insulin action. However, increased fat intake also appears to lead to insulin resistance independent of adiposity. Again saturated fat, in particular, would appear to be the major culprit. Limited evidence suggests that the n-6/n-3 ratio is likely to be important and that there is a protective effect of n-3 polyunsaturated fats. The independent effect of dietary fats on insulin action may be mediated via their effects on the fatty acid profile of the structural lipids in membranes, particularly in muscle as discussed above.

Incorporation of these highly unsaturated fatty acids into phospholipid would depend, at least to some extent, on their availability. As well as dietary intake, availability will be influenced by their resistance to destruction by oxidative metabolism. It is interesting that prolonged treatment with high doses of vitamin E has been shown to significantly improve insulin action [96] and vitamin C also appears to have beneficial effects [97]. It is tempting to speculate that the antioxidant effects of vitamins C and E, leading to increased incorporation of highly unsaturated fatty acids into muscle membranes, might be a mechanism underlying these observations.

Finally, it has now been shown in humans [98], as it has been in rodents [25], that muscle triglyceride stores are directly associated with insulin resistance independent of measures of total adiposity. It is not entirely clear what governs the level of storage lipid in muscle although in the rodent studies isocaloric feeding of diets differing only in their fatty acid profile results in very different muscle lipid levels. Further, elevated triglyceride stores are related, in humans, to increasing saturation of muscle membrane structural lipids (Pan and Storlien, unpublished observations). Inhibition of glucose utilisation by increased lipid availability has been demonstrated [12, 99]. As Standl and co-workers [100] have shown, increased muscle triglyceride stores will result in increased rates of lipolysis for a given stimulus. This latter observation is particularly germane as it has been shown that hyperinsulinaemia during a euglycaemic clamp increases sympathetic nervous activity [101]. Increased stress responsivity has been shown to be predictive of high weight gain on a high fat/high sucrose diet [102] and, in turn, fat-feeding itself results in an increased responsivity to stress [103]. Increased muscle intracellular lipid stores, increased sympathetic nervous activity to elevated insulin levels and increased responsivity to stress are mechanisms which together, or independently, could lead to increased lipolysis and therefore decreased glucose uptake in high fat fed rats. Again, it is not known if saturated fats are particularly deleterious.

Summary and conclusions

The mechanisms underlying the insulin resistance of high fat feeding have not been fully elucidated. However, from the available data the following might be the most parsimonious synthesis of the available literature.

A higher percentage of fat in the diet in humans appears, on balance, to increase adiposity over time. This may come about through a number of mechanisms including the efficiency of conversion to storage fat [77], the failure of fat intake to drive fat oxidation [79, 80], and/or the relative failure of fats to provide appropriate satiety/satiation [104, 105]. However, this issue is still controversial with the experimental animal literature suggesting that the fatty acid profile of the diet is of major importance [86]. Supporting this, the epidemiological and cross-sectional work in humans shows saturated fat intake to be the likely culprit but even the epidemiological literature is not conclusive [94]. It is completely unknown whether dietary fat types have characteristic effects on the specific accumulation of central adiposity.

Evidence exists for an effect of dietary fat on insulin action independent of changes in adiposity. Again, it is the percentage of saturated fat that appears important. This finding is congruent with the recent work showing that dietary fatty acid profile affects the fatty acid profile of skeletal muscle structural lipids with insulin resistance being associated with a higher proportion of saturated fats (and conversely a lower percentage of polyunsaturated, particularly n-3 fats). Finally, dietary fat may have its effects on insulin action via alterations in storage lipid in muscle. Here again the sparse literature suggests that the type of fat is critical [98] and this effect may come about via changes in membrane lipid profile.

So where do we go from here? Clearly much more work is needed especially on fat subtypes in humans. The particular effects of individual fats on total energy expenditure, and specifically on prandial lipid oxidation, need to be known. Short-term interventions aimed at changing the amount and type of fat in humans have yielded unimpressive and often contradictory results. Longer-term dietary trials in 'free-range' humans are needed. Better (and validated) techniques for obtaining dietary intake data on free-living humans are needed. The dietary databases used in large-scale cross-sectional and epidemiological work need to be upgraded to allow a separation of n-6 and n-3 polyunsaturated fats with their very different metabolic products (thromboxanes, prostacyclins and prostaglandins). Available evidence raises some concern about the effects of a high dietary n-6/n-3 ratio, but the data are very preliminary and more work is indicated before any strong statement could be made. The role of monounsaturated fats needs to be clarified. A cross-sectional or epidemiological study in an area where monounsaturated fat comes largely independent of saturated fat would be instructive.

In conclusion, to slightly misparaphrase Professor K. Jungermann "More is known about dietary fat and disease than is true". Certainly this is currently the case in relation to dietary fats and insulin action.

Note added in proof. In an excellent publication just to hand, Feskens and co-workers have reported on dietary factors determining diabetes and impaired glucose tolerance, analyzing a 20-year followup of the Finnish and Dutch cohorts of the Seven Countries Study. The conclusions they reach are very compatible with those reached in this review. High fat intake, particularly saturated fats, were associated with subsequent development of NIDDM and glucose intolerance while intake of vitamin C and fish (possibly due to the n-3 fats) were protective [106].

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