Cell membrane composition, insulin sensitivity and adiposity

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Summary

The cell membrane is a phospholipid bilayer and alterations in its fatty acid components can influence a range of the cell's activities. To what extent such alterations are due to diet or to genetic influences on fatty acid incorporation is not yet understood. In animals and humans diet can influence the fatty acid composition of the membrane but the relative quantities of the different fatty acids also appear to be influenced by the effect of competing microsomal elongase and desaturase enzymes that produce the longer chain n-3 and n-6 fatty acids. There is excellent evidence that diet affects insulin action and adiposity in animals, where direct measures are easily available and the relative life span is short. In humans there is evidence that the type of fatty acid in the diet modifies the phospholipid membrane composition and there is further suggestive evidence that this in turn may influence insulin action. Recent data also lead to speculation that the fat composition of the diet may influence adiposity in humans by its effects on membrane composition as well through more accepted mechanisms.

Introduction

The cell membrane is a phospholipid bilayer in which are embedded the cell surface receptors that influence much of the cell's activities. The composition of the membrane is regulated by genetic and environmental influences. Changes in the composition of the membrane lipids may affect the function of the cell possibly by affecting the fluidity of that membrane and thus the physicochemical properties of the structures embedded in it. The presence of polyunsaturated fatty acids rather than saturated fatty acids in the plasma membrane lipid bilayer increases membrane fluidity, but it is also quite possible that changes in the lipid composition can affect function by altering the composition of the fatty acids released to be incorporated into the second messenger molecules, such as diacylglycerol, which mediate insulin receptor function (1).

For metabolic studies, the muscle cell is of particular interest as muscle is the major determinant of the insulin sensitivity of the body. Insulin resistance is an essential and early component of non-insulin-dependent diabetes mellitus and is also closely related to obesity, both increasingly common metabolic disorders. Therefore it is clearly important to study and understand the variations in muscle cell membrane phospholipid composition and their relation to insulin sensitivity and adiposity. The interaction between genetic influences and dietary intake in influencing the membrane composition needs to be appreciated to implement successful interventions for improving insulin action and adiposity.

Evidence that diet affects membrane composition, insulin action and/or obesity

In looking at the impact of diet on membrane composition, insulin action or adiposity in man, due to the difficulties of the measurements, researchers are usually compelled to use surrogate measures, eg, the occurrence of diabetes rather than insulin sensitivity itself and the body mass index rather than the actual body composition. There is also the problem, understood by all nutritionists, of not being able to ascertain accurately what people habitually consume in their diet, particularly over several years.

We have reviewed the subject of diet and insulin action in a historical perspective elsewhere (2,3); in summary there is a body of evidence suggesting a direct link between dietary fat, possibly of the saturated subtype, and measures of insulin resistance. Our own short-term study contrasting

extremes of a high and low fat diet in normal subjects for three weeks at a time contradicts this (4). In eight normal subjects, we found that increasing dietary fat from 20% to 50% of total energy for three weeks at a time in random crossover fashion, did alter circulating lipid levels as expected but did not have any impact on insulin action as measured by the euglycaemic hyperinsulinaemic clamp. The dietary saturated fat intake increased from 8% to 24% of energy and the total carbohydrate content fell from 55% to 31%, while weight was unchanged. This study can not answer the question of longer term effects. Alternatively, unsaturated fatty acids could be reducing metabolic efficiency by inducing peroxisomes.

In the San Luis Valley Diabetes Study of Hispanic and non-Hispanic white adults, higher total fat intake at the time of diagnosis of impaired glucose tolerance, predicted the conversion to NIDDM at three year follow-up, unaffected by adjusting for adiposity variables (5). While this is interpreted as circumstantial evidence for an effect of diet on insulin action, it is quite possible to interpret this as an effect on insulin secretion because failure of insulin secretion is now regarded as of greater importance in the transition from impaired glucose tolerance to diabetes than increasing insulin resistance. This is quite consistent with laboratory evidence of an inhibitory effect of longterm elevation of fatty acids on insulin secretion, particularly saturated fatty acids (6).

In the thirty year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study, the development of impaired glucose tolerance and NIDDM in men, assessed by glucose tolerance test, was directly related to total and saturated fat intake 20 yr before, independent of confounding factors like age, BMI and total energy intake (7). The relationships were independent of subsequent changes in fat intake. Similarly, a cross-sectional study examined diet and NIDDM incidence in genetic Mexicans living in two environments, Mexico City and San Antonio (8). There were marked dietary differences: the Mexico City group were leaner, exercised more and ate less fat and more carbohydrate and developed significantly less NIDDM than those in San Antonio. However, the significant relationship was abolished when the data were adjusted for the increased fatness of the latter group, suggesting the effect was mediated through the fatness rather than a fatty acid profile itself.

In elderly Danish men and women, high fish intake was related to a lower risk of developing impaired glucose tolerance at 4 yr (9). As recent diet (of at least several weeks) determines the composition of the fatty acids in cholesterol esters in humans, Vessby prospectively studied the relationship between the fatty acids in serum cholesterol esters and the subsequent development of NIDDM (10). He found that a high saturated, a low oleic and a high C 18:3 n-6, and C20:3 n-6 fatty acid content of the cholesterol esters in 50 yr old Finnish men predicted the development of NIDDM 10 yr later. This latter combination could imply a delta 5 desaturase enzyme defect similar to that described later in this article from the work of Borkman, although other interpretations are possible. Vessby has also reported a relationship in elderly Scandinavian men, between muscle membrane fatty acid composition and insulin action, finding a direct relationship between insulin resistance and the amount of saturated fatty acids in the muscle membranes (11). In this population there is a relatively high amount of n-3 fatty acids in the muscle membrane, which relates at least in part to the high dietary intake of n-3 fatty acids in Scandinavians.

Evidence for the effect of fatty acid composition of phospholipid membrane on insulin action and adiposity in animals

Professor Len Storlien, when working at the Garvan Institute, showed that rats fed a high fat diet developed insulin resistance, which was accompanied by changes in the muscle membrane. Muscle insulin resistance was accompanied by a change in muscle membrane phospholipid fatty acids towards less longchain unsaturated fatty acids, in particular of the n-3 group (12). In this animal model, insulin resistance develops with a high saturated fat diet, a mono-unsaturated fat diet or a high n-6 fat diet, and can be prevented by substitution of 11% of the dietary fat by longchain n-3 fatty acids and their incorporation into the muscle membrane. The n-3 fatty acids, predominantly long chain, can prevent insulin resistance in the high fat fed rat, while substitution

with the short chain n-3 fatty acid, linolenic acid, does not prevent it in the rat fed a high linoleic acid diet. Because both short and long chain n-3 fatty acids are successful in preventing insulin resistance in the high saturated fat diet, it is postulated that there is competition for the elongase and desaturase enzymes, when short chain n-3 fatty acids are administered with the high n-6 fat diet.

Paradoxically, in studies in normal or diabetic humans there is no improvement in glucose tolerance by fish oil supplementation and no positive impact on insulin sensitivity. This may relate to the fact that in humans, in contrast to rats, the ingested n-3 fatty acids are not incorporated into the phospoinositides which act as second messengers (1).

Evidence for the effect of fatty acid composition of phospholipid membrane on insulin action and adiposity in humans

A study, also from the Garvan Institute, in normal men and those having coronary artery surgery, made an exciting extension to the animal work (13). The normal men had euglycaemic hyperinsulinaemic clamps performed to assess insulin sensitivity and the coronary patients had fasting insulin levels measured as a surrogate estimate of insulin action. Both had measurements of the phospholipid fatty acid composition of the muscle membranes from biopsy samples.

In the coronary patients, there was a negative relationship between fasting insulin level (as a marker of insulin resistance) and long chain polyunsaturated fatty acids (particularly arachidonic), the unsaturation index and the ratio of C 20:4 n-6 to C 20:3 n-6 fatty acids. The main relationships for the normal subjects are shown in Table 1 (modified and simplified from the paper by Borkman et al (13) with the authors' permission).

Table 1

The profile of fatty acids in the phospholipids fraction of the muscle membrane and simple correlations with insulin sensitivity

Fatty acid	% of total fatty acids	Correlation with insulin sensitivity
Saturated	33.3	0.03
C 16:0	16.6	-.27
C 18:0	16.5	0.46
Monounsaturated	10.3	- 0.59‡
C 16:1	0.7	- 0.46
C 18:1	9.6	- 0.59‡
n-6 polyunsaturated	51.5	0.32
C 18:2 n-6	34.1	- 0.02
C 20:3 n-6	1.5	- 0.30
C 20:4 n-6	14.6	0.76†
C 22: 4 n-6	0.8	- 0.23
C 22:5 n-6	0.5	- 0.16
n-3 polyunsaturated	4.9	0.19
C 20:5 n-3	0.8	- 0.28
C 22:5 n-3	1.6	- 0.03
C 22: 6 n-3	2.5	0.36
Derived indices		
C 20-22 polyunsaturated	20.8	0.76†
Unsaturation Index	170	0.62‡
Ratio of C 20:3 to C 18:2	0.045	- 0.28
Ratio of C 20:4 to C 20:3	10.0	0.84∬

‡ $p < 0.05$
† $p < 0.01$
∬ $p < 0.001$

Similarly to the coronary patients, in the normal men there was a positive relationship between the insulin sensitivity and arachidonic acid content, total C 20-22 fatty acids, the unsaturation index, and the ratio of C 20:4 n-6 to C 20:3 n-6 fatty acids. The inter-relationships between the fatty acids and their metabolism are shown in the Figure. The n-6 and n-3 polyunsaturated fatty acids must come from the diet. They are then modified by microsomal elongases and desaturases. The delta-5 desaturase enzyme affects the conversion of C 20:3 to C 20:4 and as the ratio of this product-precursor was directly related to the insulin sensitivity, it is possible that reduced activity of this enzyme is responsible for the lesser amounts of long chain fatty acids in the membrane.

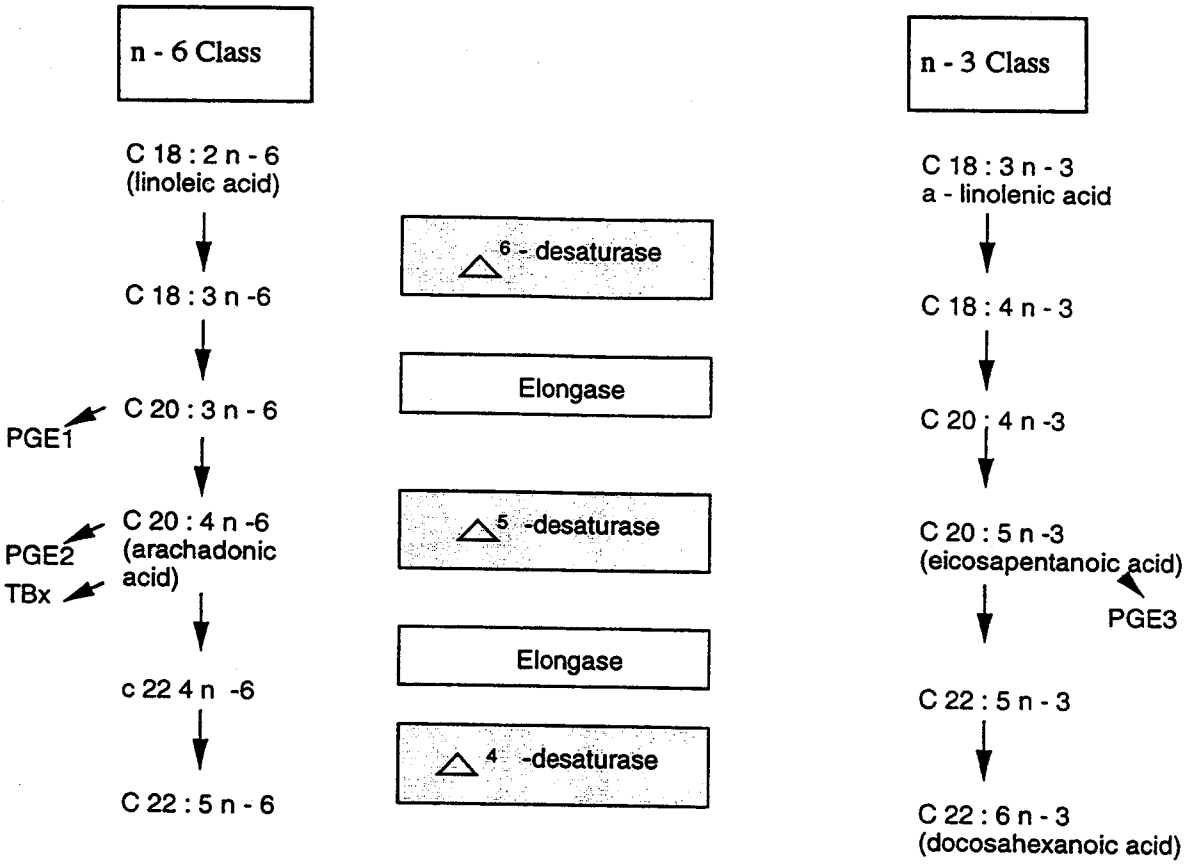

Figure 1. The major polyunsaturated fatty acid of the n - 6 and n - 3 series and the metabolic pathways by which they are elongated and desaturated. Humans cannot synthesise these fatty acids and must ingest them in the diet. The fatty acids of the two pathways compete for the same nzymes at each level of the pathway. The converastion to long chain n - 3 polyunsaturated fatty cids is slow in man; marine foods, however, are a good source, particularly, of eicosapentaenoic and docosahexaenoic acids. The seed oils are the major source of the C 18 fatty acids. PGEX prostaglandin; TBx thromboxane.

Evidence in rats and man for an effect of membrane fatty acid composition on adiposity

There is currently a growing body of evidence that a high fat intake plays a part in increasing adiposity in those susceptible to obesity. The post-obese have been shown to have a relative

impairment in fat oxidation (13) and fatty acids themselves exhibit differential rates of oxidation, with saturated fats being least and n-3 polyunsaturated fatty acids being most susceptible to oxidation (14).

However, there is as yet no conclusive evidence that there is also an effect on adiposity via membrane composition, although there is some interesting evidence from Professor Storlien's group. Rats were fed two isocaloric diets: the high saturated fat diet decreased insulin sensitivity and whole body metabolic rate. In contrast, the high n-3 fatty acid diet, maintained normal insulin sensitivity and a higher metabolic rate compared with rats fed saturated fat (15). There is as yet no evidence of the mechanism of this effect but it has been suggested that leakiness of sodium/potassium pumps in the cell membrane or mitochondrial proton pumps (15) is important.

In the Pima Indians, who have the world's highest incidence of NIDDM, there is evidence that leanness relates to increased muscle membrane unsaturation. Collaborative studies were done between Professor Storlien's group and the Phoenix, Arizona group studying the Pima Indians (16). Hyperinsulinaemic euglycaemic clamps were performed to study insulin resistance and muscle biopsies were taken in order to measure the muscle membrane phospholipid fatty acid content. Enzyme activities were assessed by product/precursor ratios. Reduced delta-5 desaturase activity showed strong and independent relationships with reduced insulin action (measured by clamp), and increased adiposity . The amounts of C 20-22 fatty acids and the unsaturation index correlated independently with insulin action. The elongase and the delta-9 desaturase enzymes (the latter inserts a double bond at carbon-9 of the fatty acid chain) were related to obesity alone. While not clearly explicable at present it is consistent with animal models.

The Pima Indians have a remarkably low n-3 polyunsaturated fatty acid content in their muscle membrane phospholipid and this may reflect a genetic effect as well as diet as the level is lower than that in Australians with a low marine product diet. There is some evidence that n-3 fatty acids are the most readily mobilised and oxidised fatty acids, particularly compared with saturated fatty acids.

Findings that membrane changes lead to both increased adiposity and insulin resistance are in some ways difficult to reconcile with the prospective evidence from Pimas that those with the greatest decrease in insulin action have the least gain in body fat over several years (17). However, at present more data is needed, particularly from intervention studies in humans. It is finally being understood that the type of fatty acid in the diet may be of equal or greater importance than the total amount of fat consumed.

Acknowledgements

This paper summarises the work done by several people at the Garvan Institute of Medical Research, Royal Prince Alfred Hospital and Wollongong University, Sydney in particular Dr M Borkman, Dr D Pan and Professor L Storlien.

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