SKELETAL MUSCLE MEMBRANE AND STORAGE LIPIDS, MUSCLE FIBRE TYPE AND INSULIN RESISTANCE

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Summary

Skeletal muscle plays a major role in insulin-stimulated glucose disposal. This paper reviews the range of evidence in humans and experimental animals demonstrating close associations between insulin action and two major aspects of muscle morphology: the fatty acid composition of the major structural lipid (phospholipid) in muscle cell membranes and the relative proportions of the major muscle fibre types. Work in vitro and in vivo in both rats and humans has shown that incorporation of more unsaturated fatty acids into muscle membrane phospholipid is associated with improved insulin action. As the corollary, a higher proportion of saturated fats is linked to impairment of insulin action (insulin resistance). The studies in vitro suggest a causal relationship. Among the polyunsaturated fatty acids (PUFAs) there is some, but not conclusive, evidence that the omega-3 (n-3) PUFAs may play a particular role in improving insulin action, certainly a high n-6/n-3 ratio appears deleterious. In relation to fibre type the more highly oxidative, insulin sensitive Type 1 and Type 2a fibres have a higher percentage of unsaturated fatty acids, particularly n-3s, in their membrane phospholipid compared to the insulin resistant, glycolytic Type 2b fibres. However, these variables can be separated and may act in synergy to modulate insulin action. It remains to establish whether lifestyle (e.g., dietary fatty acid profile and physical activity), genetic predisposition or a combination are the prime determinants of muscle morphology (particularly membrane lipid profile) and hence insulin action.

I. INTRODUCTION

Skeletal muscle is the major site of insulin-stimulated glucose disposal. Insulin resistance, the relative failure of insulin action, and associated hyperinsulinemia are strongly linked to the development of a cluster of prevalent diseases including non-insulin-dependent diabetes mellitus (NIDDM), obesity, hyperlipidemias, hypertension and heart disease (Björntorp 1991; Reaven 1988; Reaven 1993). While skeletal muscle insulin resistance has clearly been demonstrated in both human and experimental animal models of obesity and diabetes, the mechanisms are still poorly understood. Work from our laboratory, and others, has focused on the interrelationships between the fatty acid composition of muscle membrane structural lipids, levels of muscle storage triglyceride, muscle fibre composition and insulin resistance. This short review is basically limited to studies in skeletal muscle and concentrates on the work from our laboratory.

II. MUSCLE PHOSPHOLIPID COMPOSITION AND INSULIN ACTION

A number of lines of evidence led to the current work. First, it was known from the pioneering work of Felber and colleagues (see Bringolf et al. 1972) that diets high in fat led to

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increased accumulation of storage triglyceride in skeletal muscle. According to the glucose-fatty acid cycle of Randle and co-workers (Randle et al. 1963; Randle et al. 1988) and inhibitory effects of lipids on glucose storage (Ebeling and Koivisto 1994), this lipid accumulation was a plausible factor in the impairment of muscle glucose metabolism. The second important set of observations came out when the euglycemic clamp for measurement of insulin action was adapted for use in rats (Burnol et al. 1983; Kraegen et al. 1983), and the technique was further extended to use of the labelled 2-deoxyglucose method for assessing insulin action in individual tissues (Jenkins et al. 1986; Kraegen et al. 1985). Using these techniques it was possible to demonstrate directly that high-fat feeding induced insulin resistance in individual skeletal muscles (Kraegen et al. 1986; Storlien et al. 1986). Further, by combining ^{3}H-labelled 2-deoxyglucose and ^{14}C-labelled glucose bolus injection during the euglycemic clamp it was possible to show that fat-feeding induced insulin resistance in both the storage and oxidation/glycolysis components of muscle glucose metabolism.

The third line of evidence came from work (Wong et al. 1984) showing the hypotriglyceridemic properties of omega-3 (ω-3 or n-3) fatty acids. The question became, if high-fat diets lead to muscle insulin resistance via the accumulation of storage lipid, would inclusion of lipid-lowering n-3 fatty acids in the high fat diet be beneficial? Indeed that was the observation. When n-3 fatty acids were substituted into a high fat diet insulin resistance in skeletal muscle was prevented (Storlien et al. 1987). Importantly, both the storage and oxidation/glycolysis components of glucose metabolism were ameliorated by inclusion of the n-3s. The observation that both components of glucose metabolism in muscle were impaired by some high-fat diets, and both ameliorated by n-3 fatty acids, was suggestive of either multiple metabolic defects or a single defect at an early, common point in the glucose metabolic pathway, perhaps at the membrane level.

This seminal observation of the profound dependency of high-fat diet induced insulin resistance in vivo on the fatty acid profile of the diet was consistent with number of in vitro studies. These investigations have provided evidence that changes in the composition of fatty acids within membrane phospholipids strongly influence insulin action, altering both insulin binding and action. In general the more saturated the fatty acids in membrane phospholipid the more deleterious the effect (Grunfeld et al. 1981; Field et al. 1988). Further, the highly unsaturated n-3 fatty acids may be particularly beneficial (Sohal 1992; Clandinin et al. 1993). Our recent studies using L6 myocytes have extended these observations to a muscle-derived cell line (Pan and Storlien, unpublished results).

Follow-up work was aimed at extending both the in vivo and in vitro work in terms of elucidating mechanisms relating muscle membrane lipid profile and insulin action. An extensive study was carried out in rodents comparing a number of high-fat diets pair-fed and differing only in the fatty acid profile of the fat components (Storlien et al. 1991). The results demonstrated marked intergroup differences in insulin-stimulated glucose metabolism when assessed using the euglycemic clamp. Some high-fat fed groups progressed to major insulin resistance and others did not, with the effects particularly pronounced in skeletal muscle. As we had originally proposed, a variable closely associated with the muscle insulin resistance was accumulation of storage lipid, consistent with the accepted glucose-lipid interactions. Further, since the earlier results had suggested that the metabolic defect induced by some forms of high-fat feeding was at the membrane level, it was reasoned that changing the dietary fatty acid profile might also change the fatty acid profile of membrane structural lipid and that, in turn, may influence insulin action. Indeed, improved insulin action was tightly associated with an increased percentage of longer carbon chain, more highly unsaturated fatty acids in the muscle membrane phospholipid.

The association between membrane lipid profile and insulin action in experimental animals prompted studies in man. The first work assessed skeletal muscle phospholipid fatty acid composition in normoglycemic volunteers undergoing coronary bypass operations and from whom rectus abdominus muscle biopsies were obtained. The results were consistent with those observed in rodents. Fasting insulin level, used as an index of insulin resistance in this normoglycemic population, was inversely correlated with the percentage of long-chain polyunsaturated fatty acids (PUFAs) and with the Unsaturation Index (UI; the number of double bonds per fatty acyl moiety x 100) in the muscle membrane phospholipid. That is, the more unsaturated the membrane lipid, the more insulin sensitive the individual (Borkman et al. 1993).

A follow-up study in young, healthy subjects used the hyperinsulinemic / euglycemic clamp to assess insulin action directly and percutaneous biopsies of the vastus lateralis were obtained. The same relationships were found in this normal group between muscle membrane phospholipid profile and insulin action (Borkman et al. 1993).

Two other studies in man, using very different study populations have further explored these relationships. Both studies have used the euglycemic clamp to assess insulin action directly and analyzed vastus lateralis muscle obtained by percutaneous biopsy. Vessby and colleagues have studied an older, male Swedish population (Vessby et al. 1994). Relationships were again found between insulin action and muscle phospholipid fatty acid composition. However, differences between the Australian and Swedish results did occur. In the Swedish study the highest correlation occurred with saturated fatty acids (particularly palmitic). That is, the higher the proportion of saturated fatty acids in the muscle membrane phospholipid, the more insulin resistant the individual. In contrast to the Borkman et al. (1993) study, relationships with the highly unsaturated fatty acids were not significant.

The third study in this series of work was carried out in the diabetes- and obesity-prone Pima Indians (Pan et al. 1995). Again, results congruent with the two previous studies were found, the more unsaturated the phospholipids, the better insulin action. Here both individual saturated and polyunsaturated fatty acids were significantly linked to insulin action.

An interesting aspect of this latter study was that it confirmed and extended observations in the original study of strong relationships between insulin action and measures of activity of the Δ5 desaturase enzyme. It is clearly true that the Unsaturation Index 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 approximately 170 in our 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 determined by product/precursor ratios (for example the ratio of 20:4n-6 to 20:3n-6 gives an index of activity 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 (Borkman et al. 1993) and in Pima Indians (Panet al. 1995), have shown remarkably strong relationships between reduced Δ5 desaturase activity (20:4 n-6/20:3 n-6) and insulin resistance.

The other aspect particularly of the Δ5 desaturase enzyme is that it is common to both the n-6 and n-3 fatty acid families; as such there is competition between n-6s and n-3s fatty acids as substrates for desaturation. Certainly it is true that n-3 fatty acids are elongated and desaturated to a greater extent (to 22 carbon 5 and 6 double bond fatty acids) than is true for the n-6 pathway where aracidonic acid (20:4) is the normal endpoint. This may be significant at a time in history when we have been undergoing the first major experiment in human history of a high level of dietary intake of n-6 fatty acids. This has been an outcome, driven by the cardiovascular literature, of the move to increase polyunsaturated fat intake and reduce saturated fat intake. The polyunsaturated seed oils available over the last 30 years have been largely n-6 in makeup. We have analyzed both our Australian and the Pima Indian data in terms of the relationship between the n-6/n-3 ratio, insulin action (as indexed by fasting insulin in these normoglycemic subjects) and obesity. Figure 1 shows the results. Clearly the higher the n-6/n-3 ratio, the worse the insulin action and the higher the relative weight level. Interestingly Pima Indians have a much lower level (<40%) of n-3 fatty acids than the Australian, largely Caucasian, sample. In turn, the Swedish subjects of Vessby had an n-3 percentage in skeletal muscle phospholipid much higher again than even the Australian group and over three times that of the Pimas. These observations presumably reflect at least in part dietary intake of n-3 fatty acids. However, there is very likely to be also a genetic predisposition to incorporation, elongation and desaturation of specific fatty acids. In dietary analysis of a limited number of subjects in the Borkman study, relative amounts of, for example n-3 fatty acids, did not relate at all to apparent dietary intake. It will be interesting to determine if the low levels of n-3 fatty acids in the Pima Indian muscle membranes relate at all to low dietary intake or reflects a genetic predisposition. It is interesting in this regard that an intestinal Fatty Acid Binding Protein (FABP) loci on chromosome 4q has been significantly linked

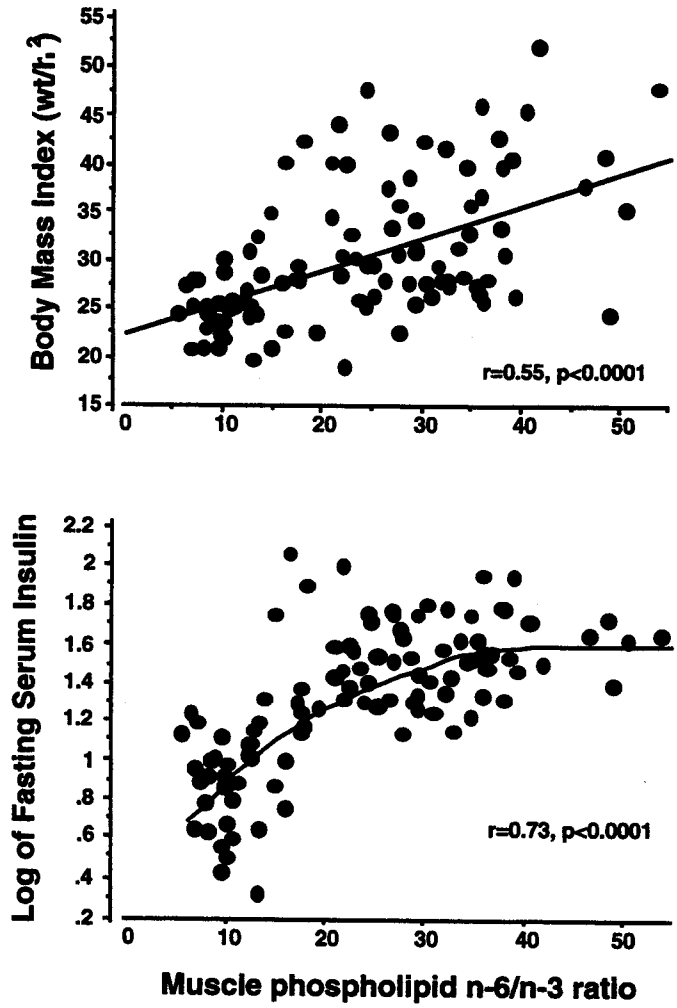

Figure 1. The relationship between the n-6/n-3 ratio and relative adiposity (Body Mass Index - top panel) and the n-6/n-3 ratio and insulin resistance (indexed by a high fasting serum insulin - bottom panel) The ratio of n-6 to n-3 fatty acids was measured in the phospholipid of muscle obtained by percutaneous biopsy of the vastus lateralis. Subjects were Australians, largely Caucasian, and Pima Indians (Storlien, Pan and Lillioja, unpublished observations).

with insulin resistance in this population (Prochazka et al. 1993). It is therefore possible that the 'thrifty gene' (Neel 1962; Ravussin and Bogardus 1990) may include the control and binding and uptake of specific fatty acids and their insertion into membranes.

The relationships between muscle phospholipid fatty acid profile and insulin action have been established in adult rats and humans; however, the relative contributions of lifestyle and genetic predisposition are still unclear. In an effort to shed light on this issue we have begun studies in human infants. Muscle biopsies are being obtained during surgery on infants who are not acutely unwell and who are near normal height and weight (operations range from talipes correction to minor gastric and cardiac repair). The initial results emphasize the major effect of diet. Breast-fed infants have a muscle membrane lipid profile much like insulin sensitive adults. Formula-fed infants, in contrast, present a profile characteristic of insulin resistance (Baur et al. 1994). Insufficient evidence has yet been collected to assess the genetic predisposition to a certain lipid profile independent of the powerful dietary effect.

III. RELATIONSHIP BETWEEN MEMBRANE LIPIDS AND STORAGE LIPIDS

As noted earlier, the inhibitory effects of lipids on glucose metabolism have clearly been described (Ebeling and Koivisto 1994; Randle et al. 1963; Randle et al. 1988). A significant feature of this glucose/lipid interaction appears to be the failure of insulin to suppress lipolysis of intramuscular storage triglyceride (Groop et al. 1992). We originally came at the issue of the relationship between dietary fat and insulin action via this issue. As noted earlier a study with a large range of dietary fat profiles showed a good, inverse relationship between intramuscular triglyceride levels and insulin action in that muscle (Storlien et al. 1991). In humans (Pan et al. 1994b) we have now been able to show that intramuscular triglyceride levels are also closely related to whole-body insulin action independent of total adiposity. In this context it is interesting that we have recently demonstrated relationships between muscle membrane lipid composition and muscle storage lipid: the more saturated the membrane lipids, the greater the accumulation of storage lipid (Pan et al. 1994a). This result is consistent with the observations of Leyton et al. (1987), showing that more saturated fats are poorly oxidized for energy in comparison to PUFAs and hence more likely to be stored (and incorporated into membranes). In addition it is significant that for a given carbon backbone number, the n-3 fatty acids were markedly more likely to be quickly oxidised for energy than the equivalent n-6 fatty acid which is more likely to be stored (see, for example, the 18 carbon n-6 and n-3 fatty acids).

IV. MUSCLE FIBRE TYPE, MEMBRANE LIPIDS AND INSULIN ACTION

Finally, there are a number of aspects of muscle morphology that undoubtedly influence insulin action. One receiving current attention is the relative proportions of Type 1, Type 2a and Type 2b fibres. It has been known for some time that different fibre types exhibit enormously different sensitivity in relation to insulin's ability to promote glucose uptake (James et al. 1985). Type 1 (slow-twitch oxidative) and Type 2a (fast-twitch oxidative/glycolytic) fibers are much more insulin sensitive than Type 2b (fast-twitch glycolytic) fibers (James et al. 1985). It has also been shown in man that insulin resistance is associated with a relative increase in percentage of Type 2b fibers (Kriketos et al. 1994b; Lillioja et al. 1987) and a relative decrease in Type 1 fibres (Hickey et al. 1995) and indices of oxidative capacity (Kriketos et al. 1994a; 1994b).

In order to determine the relationship between muscle fiber type and membrane lipid composition, we have recently accumulated data in our laboratory on rats fed the same diet where the phospholipid fatty composition was determined on hindlimb muscles that were predominantly Type 1 (Soleus), Type 2a (red quadriceps) or Type 2b (white quadriceps). It was clear that the percentage of PUFAs in membrane phospholipid was significantly higher in muscles which were predominantly of the Type 1 or Type 2a fiber type compared to muscles predominantly of the Type 2b fiber type (Kriketos et al. 1995). Interestingly, voluntary wheel-running was able to markedly increase the percentage of Type 2a fibers while lowering the percentage of Type 2b fibers without significantly changing phospholipid fatty acid profile. Data such as these provide a correlational link between muscle fatty acid composition and fiber type which may be important in insulin action. However these data support earlier suggestions (Ivy et al. 1986; Kraegen et al. 1989) that factors such as diet and exercise might work synergistically via variables such as membrane lipids and fiber type to influence insulin action.

V. CONCLUSIONS

Skeletal muscle plays a major role in insulin-stimulated glucose disposal. There is now a range of evidence in humans and experimental animals demonstrating strong relationships between the fatty acid composition of structural membrane lipids and insulin action. The in vivo work is

correlative but the in vitro studies suggest a causal relationship exists. There are a number of ways that a more saturated membrane lipid profile might come about. It may reflect dietary fatty acid profile; it may relate to a genetic alteration in the binding and subsequent incorporation of certain fatty acids (eg, a defect in certain FABPs); it may reflect a genetic defect in one or more of the enzymes of fatty acid elongation and desaturation which are necessary to turn the major dietary fatty acids into the longer-chain, more highly unsaturated fatty acids in membrane linked to good insulin action. Equally, there are a number of ways in which a more saturated membrane lipid profile might impair insulin action: these include altered insulin receptor binding and affinity as suggested by earlier work; altered ability to translocate and insert glucose transporters; altered intrinsic activity of those glucose transporters, hydrolysis of the phospholipids in membranes will yield diacylglycerols with differing fatty acid groups and these might interact, in their second messenger role, quite specifically with a particular phosphokinase C variant for example. Work is currently under way in both in vivo and in vitro model systems to attempt to extend the evidence for the causality of the relationship between membrane lipid profile and insulin action and also to understand the mechanisms involved.

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