

Diet composition and insulin action in animal models

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Critical insights into the etiology of insulin resistance have been gained by the use of animal models where insulin action has been modulated by strictly controlled dietary interventions not possible in human studies. Overall, the literature has moved from a focus on macronutrient proportions to understanding the unique effects of individual subtypes of fats, carbohydrates and proteins. Substantial evidence has now accumulated for a major role of dietary fat subtypes in insulin action. Intake of saturated fats is strongly linked to development of obesity and insulin resistance, while that of polyunsaturated fats (PUFAs) is not. This is consistent with observations that saturated fats are poorly oxidized for energy and thus readily stored, are poorly mobilized by lipolytic stimuli, impair membrane function, and increase the expression of genes associated with adipocyte proliferation (making their own home). PUFAs have contrasting effects in each instance. It is therefore not surprising that increased PUFA intake in animal models is associated with improved insulin action and reduced adiposity. Less information is available for carbohydrate subtypes. Early work clearly demonstrated that diets high in simple sugars (in particular fructose) led to insulin resistance. However, again attention has rightly shifted to the very interesting issue of subtypes of complex carbohydrates. While no differences in insulin action have yet been shown, differences in substrate flux suggest there could be long-term beneficial effects on the fat balance of diets enhanced in slowly digested/resistant starches. A new area of major interest is in protein subtypes. Recent results have shown that rats fed high-fat diets where the protein component was from casein or soy were insulin-resistant, but when the protein source was from cod they were not. These are exciting times in our growing understanding of dietary factors and insulin action. While it has been clear for some time that ‘oils ain’t oils’, the same is now proving true for carbohydrates and proteins.

Dietary macronutrients: Energy balance: Insulin resistance

Background

Insulin resistance is a major player in the etiology of the metabolic syndrome cluster of diseases. Dietary factors undoubtedly influence insulin action, but it often appears that agreement stops with these two statements. Ambrose Bierce, in his wonderful tome *The Devil’s Dictionary*, defined a Trinitarian as ‘one who denies the divinity of a Unitarian’. Of course, a Unitarian was ‘one who denies the divinity of a Trinitarian’. In many ways the argument between the high carbohydrate versus fat-modified schools of dietary advice have fallen into Bierce’s dichotomous trap. Non-insulin-dependent or type 2 diabetes mellitus is the most linear disease descendent of insulin resistance. The prominent symptom, which provides the definition for the disease, is the inability to handle carbohydrate. The logical question is, why advocate a high-carbohydrate diet to individuals whose core problem is that macronutrient?

The answer, at least partially, concerns the macronutrients available to substitute for carbohydrate. There is limited ability to realistically manipulate the levels of protein, and therefore reducing carbohydrate comes at the cost of increasing fat. Since obesity is also part of the metabolic syndrome disease cluster, and insulin resistance is closely linked with adiposity, then the argument that ‘eating fat makes you fat’ comes into play. Fortunately, there is a way forward. Experimental animal investigations of insulin action, following manipulation not only of macronutrient proportions, but more particularly of macronutrient subtypes, is writing an exciting new story on diet and insulin action.

‘Oils ain’t oils’

The experimental animal literature on dietary fat subtypes is now quite large and coherent. There is evidence for both

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direct effects on insulin action and indirect effects via induction of obesity. The literature on both has recently been reviewed, and only a summary is needed here with the focus on mechanistic insights.

'Eating fat makes you fat' is catchy but not well supported as a global concept. Overall there is now an enticing literature which links intake of saturated fats with development of obesity, but contrasts a neutral, or even protective, effect of polyunsaturated fatty acids (PUFAs) (Pan *et al.* 1994; Storlien *et al.* 1998). Compared to saturated fats, PUFAs are more readily used for energy when initially ingested (Leyton *et al.* 1987). This was demonstrated by observing labelled CO₂ production after oral administration of identical amounts of differently labelled fatty acids. Dramatic differences were observed over the subsequent 24 h, with only a small proportion of the saturated fats being fully used for energy compared to unsaturated fats (Leyton *et al.* 1987). The implication is that the saturated fats are being stored in adipose tissue depots. Once stored there, saturated fats are also less readily mobilized by lipolytic stimuli (Mougios *et al.* 1995; Halliwell *et al.* 1996; Raclot *et al.* 1997). Increasing the degree of unsaturation at a given carbon chain length increases the relative mobilizability of stored fats. This has an interesting implication for physically active individuals, where selective mobilization of unsaturated fats (Mougios *et al.* 1995), combined with the greater propensity of these fats for utilization as energy, means that such individuals are in danger of 'saturating down' their body lipid stores. As we shall see, this has potentially negative consequences for a number of aspects of metabolism. Lipids are not just an efficiently stored energy reserve, they form the membranes of all cells and organelles. Here again, when saturated fats are incorporated into membranes they have the capacity to reduce metabolic rate and decrease receptor (e.g. beta adrenoceptor) binding (Matsuo & Suzuki, 1997) with the obvious implications for energy balance. Conversely, addition of *n*-6 PUFAs to the diet can increase beta adrenoceptor affinity (Nicolas *et al.* 1991).

In addition to their roles as energy and cell-structural elements, it is now clear that fatty acids act as potent gene regulators, notably in the current context on enzymes of endogenous lipid synthesis and adipocyte proliferation (Clarke *et al.* 1997). The beautiful pattern persists, with saturated fats up-regulating, and PUFAs down-regulating,

these enzymes. Saturated fats are then not only adept at finding their way into the body's stores and resistant to being winkled out, but proficient at making their own home – good friends in times of want but pernicious in the face of plenty. Finally, a more recent angle on fats and energy balance has been exposed (see Table 1). In mice fed diets high in saturated fats, neuronal activation was seen in the dorsal-lateral hypothalamus, the classic 'feeding centre', while activity in the ventromedial hypothalamic satiety centre was suppressed (Wang *et al.* 1999). This is in line with development of excess adiposity via a tilting of the autonomic nervous system balance to favour parasympathetic over sympathetic. This pattern was in contrast to PUFA feeding where only an increase in ventromedial hypothalamic neuronal activity was observed, and even less fat accumulated than in low fat-fed control mice (Wang *et al.* 1999). It is important to link this new information with previous work which has demonstrated an increase in stress responsivity (indexed by blood glucose and corticosterone response, and hypothalamic noradrenaline turnover) with high-fat feeding (Pascoe *et al.* 1991).

The pattern of low sympathetic nervous system activity/basal metabolic rate and increased stress responsivity is a feature of many animal models of the metabolic syndrome. It is perhaps not surprising that the antigluco-corticoid RU486 (Kusunoki *et al.* 1995), and the alpha-2 adrenoceptor agonist clonidine (Rocchini *et al.* 1999), have both been shown to ameliorate the insulin resistance of high-fat feeding.

To complete this compelling pattern of dietary fat subtype effects, there is now evidence that 'dietary fatty acid composition, independent of adipose tissue mass, is an important determinant of circulating leptin level', with PUFA-enriched diets leading to much higher leptin levels than those enriched in saturated fats (Cha & Jones, 1998). This suggests the possibility that there may even be an effect on the intake side of the energy-balance equation to help explain the anti-obesogenic effects of PUFAs.

As well as the indirect route via obesity, there is strong evidence that dietary fat subtypes are also able to modulate insulin action more directly (Storlien *et al.* 1996). Early *in vitro* studies provided evidence that changes in the composition of fatty acids within membrane phospholipids influenced insulin action, altering both insulin binding and

Table 1. Effects of 1 week of feeding diets high in carbohydrate, saturated fat or polyunsaturated fats on neuronal (c-fos) activation (high-carbohydrate diet set at 100%)

	Lateral hypothalamus*	Ventromedial hypothalamus†
Energy balance	Feeding	Satiety
ANS association	Parasympathetic	Sympathetic
Neuronal activity (%)		
high carbohydrate diet	100	100
high saturated fat diet	367	33
High PUFA diet	108	167

* The lateral hypothalamus has traditionally been viewed as a feeding centre and aligned with the parasympathetic arm of the autonomic nervous system (ANS). Activation of the lateral hypothalamus is thus associated with positive energy balance.

† The ventromedial hypothalamus is considered a sympathetic nervous system-linked satiety centre, activation of which will induce negative energy balance.

Adapted from Wang *et al.* 1999.

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, there was some evidence that the highly unsaturated *n*-3 fatty acids were perhaps particularly beneficial (Sohal *et al.* 1992; Clandinin *et al.* 1993). These studies were followed by *in vivo* work combining the hyperinsulinaemic, euglycaemic clamp technique with bolus injection of labelled glucose and deoxyglucose to index insulin action in individual tissues after various feeding regimens (Storlien *et al.* 1991). Skeletal muscle was targeted as the single most important tissue of insulin-stimulated glucose uptake. These studies showed that feeding rats for short (3–4-week) periods on isocaloric diets, differing only in their fatty acid profile, was sufficient to provoke major insulin resistance in some, but not all, high fat-fed groups. Rats fed diets high in *n*-3 PUFAs, and with a low *n*-6:*n*-3 PUFA ratio, maintained normal insulin action. Diets high in saturated and monounsaturated fats led to profound insulin resistance in numerous tissues, as did diets with a higher level of PUFAs where those PUFAs were of the *n*-6 class (Storlien *et al.* 1991).

A variable linked early with whether or not a diet group developed skeletal muscle insulin resistance was the fatty acid composition of membrane structural lipids in skeletal muscle (Storlien *et al.* 1991). This work has been replicated a number of times in rodent models and in a number of human populations (Storlien *et al.* 1996). In addition, pharmaceutical insulin sensitizers such as bezafibrate (Matsui *et al.* 1997) and benfluorex (L. H. Storlien, unpublished results) also unsaturate lipids, which may be an important part of their mode of action. However, the mechanistic basis of altered membrane lipid composition directly influencing insulin action is not entirely established. There are a number of possibilities, including effects on translocation and intrinsic activity of the insulin-regulatable glucose transporter GLUT4 (Zierath *et al.* 1997; Hansen *et al.* 1998). These workers have shown substantial impairment of insulin-stimulated muscle glucose transport, along with reduced GLUT4 translocation to the membrane, following high-fat feeding in rats. However, no information is yet available regarding these metabolic elements and dietary fatty acid profiles which do not lead to insulin resistance. Specific diacylglycerol–protein kinase C interactions may be important. Activation of protein kinase C epsilon is associated with skeletal muscle insulin resistance in high fat-fed rats (Schmitz-Peiffer *et al.* 1997), and it is possible that interaction with specific fatty acid subtypes in diacylglycerols modulates this activation. Also, there are possible mechanisms via influences on nitric oxide production, eicosanoid balance and ion flux. However, no specific work has yet borne fruit in these directions.

One final aspect of fatty acid profile which must be considered is the potential effects at the level of the pancreas. It is now clear that insulin secretion is powerfully, and differentially, influenced by individual fatty acids (Stein *et al.* 1997), with saturated fats in particular leading to hypersecretion of insulin. Hyperinsulinaemia *per se* may lead to insulin resistance.

A second variable identified in early studies as important for insulin action was muscle storage triglyceride (TG)

levels, impaired insulin action being closely associated with high muscle TG levels (Storlien *et al.* 1991). This observation has now been replicated in human studies (Phillips *et al.* 1996; Pan *et al.* 1997). Some concern had been expressed about the location of the TG, but *in vivo* NMR studies have been used to show that the relevant TG was intracellular (Krssak *et al.* 1999). Using a more direct approach, we have recently measured insulin-stimulated glucose uptake in incubated soleus muscle using the 2-deoxyglucose method, and then measured TG in muscle fibres alone after stripping away all interstitial fat and connective tissue. Again, a highly significant inverse relationship was found between intramyocyte TG and insulin-stimulated glucose metabolism (T. C. Thomas & L. H. Storlien, unpublished results).

The possibility has been suggested that the concentration of long-chain fatty acyl-CoA is a marker for skeletal muscle TG hydrolysis, and accounts for the inhibitory effect of muscle TG levels on insulin action (Oakes *et al.* 1997). The observation that addition of cholate to a high-fat diet improves insulin is interesting (Ikemoto *et al.* 1997), in particular given the data showing that, at least in the liver, changes in TG accumulation paralleled the cholate-induced decline in acyl-CoA synthetase mRNA.

This brings into focus our lack of knowledge about what actually controls the accumulation and distribution of TG in skeletal muscle. It is clear that it is not just the total intramyocyte TG that modulates insulin action, but its distribution is also critical. Muscle TG is substantially elevated in trained, compared to sedentary, individuals, but insulin action is improved. Female rats, given access to running wheels, will build up to an average of over 10 km/d. We have exploited this chronic exercise model to investigate, using electron microscopy, the effect of ‘training’ on both amount and distribution of intramyocyte TG. While the work is in its early stages, we can confirm that intramyocyte TG is greatly elevated, with lipid droplets very tightly coupled to mitochondria (P.L. Else *et al.*, unpublished results). What controls this increase in TG and its affinity for mitochondria and, in turn, why it is not deleterious to insulin action are questions of importance in our understanding of skeletal muscle insulin resistance.

Carbohydrate subtypes

Just as there are simple and complex carbohydrates, there are simple and complex arguments about the level and form of carbohydrate intake to optimize insulin action. From experimental animal work simple sugars, compared to complex carbohydrates, were found to have a negative influence on insulin action. These studies were conducted in rats and, as with the dietary fat subtype work described above, consisted of a series of pleasingly simple interventions of 3–4 weeks with caloric intake matched and only the carbohydrate type varied in the diet. Hyperinsulinaemic, euglycaemic clamps were performed with tracer administration to assess insulin action at the level of the liver and in individual skeletal muscles, and effects were found in both. Diets where starch was largely substituted by either sucrose or fructose were deleterious for insulin

action (Storlien *et al.* 1988; Thorburn *et al.* 1989). The hypertriglyceridaemic repercussion of elevated fructose intake was seen as a likely intervening variable. Certainly, changing the fat source to emphasize *n*-3 fatty acids ameliorates both the insulin resistance and hypertriglyceridaemia of high sucrose feeding (Lombardo *et al.* 1996). Interestingly, glucose itself had no deleterious effect on insulin action compared to starch. This suggests that the rate of carbohydrate absorption does not, in the short term, alter insulin action.

What is even less explored, and potentially of much greater importance to the broader picture, are the effects of various forms of complex carbohydrates. However, there is considerable controversy even in relation to an acceptable classification framework (Cummings *et al.* 1997) when we start impinging on the grey area of resistant starches, soluble fibre and large-bowel digestion (fatty acids again, of the short-chain type).

The glycaemic index is a concept which has been around for many years but still is shrouded in confusion and capable of inciting some acrimony (Coulston & Reaven 1997; Wolever 1997). There is still no evidence for direct beneficial effects of low glycaemic-index diets on insulin action either in animal or human studies. However, there are a number of very logical bases for suggesting that there may well be a beneficial effect of slowly digested carbohydrates in the long run.

Long-term feeding of diets high in carbohydrate, differing only in type of starch, has shown a differential influence on the insulin response necessary to maintain essentially normal glucose tolerance. Rats fed on a diet with amylopectin as the starch are markedly hyperinsulinaemic during an intravenous glucose tolerance test compared to rats fed a high-starch diet emphasizing amylose (Byrnes *et al.* 1995; Higgins *et al.* 1996). This relative hyperinsulinaemic response becomes more pronounced the longer the feeding period. Amylose is a straight-chain polysaccharide which, by virtue of that configuration, is slowly digested. The branched-chain configuration of amylopectin allows multiple access points for alpha-glucosidase attack and rapid absorption.

At the molecular level, high-amylose starches have been shown to increase GLUT4, and decrease fatty acid synthase gene expression in rat epididymal tissue (Kabir *et al.* 1998*b*). In addition, maximal insulin-stimulated ¹⁴C-glucose oxidation was increased, whereas ¹⁴C-glucose incorporation into lipids was decreased (Kabir *et al.* 1998*a*), and glycogen synthesis in muscle was increased (Denyer *et al.* 1998), in amylose-fed compared to amylopectin-fed rats. Taken together, these results and those noted above are suggestive of potential effects of amylose-enriched starches on insulin secretion and/or on substrate repartitioning.

Finally, there are major developments in our understanding of the physiochemistry of starch and the increasingly blurred boundaries between resistant starches and fibre. There are few real data here in relation to insulin resistance, an important emerging field of research.

Protein subtypes

Compared to fat and carbohydrate subtypes, proteins have

been under-studied. Some years ago we noted that rats fed a synthetic diet were much more insulin-sensitive than those fed on laboratory chow (Storlien & Jenkins, 1996). This was puzzling as the synthetic diet had been prepared so as to closely match the chow macronutrient distribution and fatty acid profile. Since then, exciting new work has demonstrated that changing the protein source in synthetic diets can markedly dictate development, or not, of insulin resistance in medium- to high-fat-fed rats (Iritani *et al.* 1997). Follow-up work has shown that high-fat diets prepared with cod protein, as compared to soy or casein protein, do not lead to insulin resistance (Lavigne *et al.* 1999). Further mechanistic investigations showed that the cod protein improves GLUT4 translocation to skeletal muscle T-tubules, but not to the plasma membrane (Tremblay *et al.* 1999). The T-tubule GLUT4 protein correlates with insulin-stimulated glucose transport, and is most interesting in terms of the possibility that a specific protein might be critical in skeletal muscle insulin-stimulated glucose transport. The effect might be due to a specific protein which has a gene-specific effect at the intestinal level, a protein which escapes full digestion, a molecule which is co-extracted with protein, or indeed a particular amino acid pattern unique to cod. In this regard the observation that L-glutamine supplementation of a high-fat diet has beneficial effects on glycaemia and insulinaemia in mice may be relevant (Opara *et al.* 1996). The exploration of these possibilities offers interesting new lines of research.

Summary

Animal models have proven useful in studies of the influence of dietary variables and insulin action. This manuscript has focused on the macronutrients, and the overriding message is that we must look beyond the broad categories of fats, carbohydrates and proteins. A great deal of work has been done on fatty acid subtypes, and a harmonious pattern linking saturated fat intake, both indirectly and directly, to insulin resistance is evident. In contrast, PUFAs are, if anything, protective. Less is known about carbohydrate subtypes. While clear data have been obtained regarding the insulin resistance-inducing effects of sucrose, and in particular its fructose moiety, little other direct evidence is available on carbohydrate subtype–insulin action interactions. Finally, new work on protein sources and the profound differences in insulin action they induce is providing interesting new avenues to explore. What is now clear, and points in a particularly exciting direction, is that the macronutrients are all potent gene regulators – and subtype mix will undoubtedly be found important in the precise patterning of that gene regulation.

Research on experimental animals is important, for the most part, only in its capacity to inform directions for human work. This has happened in the field of diet and insulin action, and there is potential for new and exciting contributions. Our work has focused on macronutrient subtypes, *inter alia* from the recognition that persuading ‘free range’ individuals to introduce and sustain changes in the macronutrient proportions of their diet has proved remarkably difficult, at least in our hands. However, changing individual macronutrient subtype profiles is substantially

easier with the resultant increased capacity for a significant impact on the prevention and therapy of insulin resistance.

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