Proceedings of the Nutrition Society (2004), **63**, 363–368 DOI:10.1079/PNS2004349

The 12th Conference of the International Research Group on the Biochemistry of Exercise was held at Maastricht University, Maastricht, The Netherlands on 13–16 July 2003

Symposium 6: Adipose tissue–liver–muscle interactions leading to insulin resistance

Metabolic flexibility

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Human physiology needs to be well adapted to cope with major discontinuities in both the supply of and demand for energy. This adaptability requires 'a clear capacity to utilize lipid and carbohydrate fuels and to transition between them' (Kelley *et al*. 2002*b*). Such capacities characterize the healthy state and can be termed 'metabolic flexibility'. However, increasing evidence points to metabolic inflexibility as a key dysfunction of the cluster of disease states encompassed by the term 'metabolic syndrome'. In obese and diabetic individuals this inflexibility is manifest in a range of metabolic pathways and tissues including: (1) failure of cephalic-phase insulin secretion (impaired early-phase prandial insulin secretion concomitant with failure to suppress hepatic glucose production and NEFA efflux from adipose tissue); (2) failure of skeletal muscle to appropriately move between use of lipid in the fasting state and use of carbohydrate in the insulin-stimulated prandial state; (3) impaired transition from fatty acid efflux to storage in response to a meal. Finally, it is increasingly clear that reduced capacity for fuel usage in, for example, skeletal muscle, as indicated by reduced mitochondrial size and density, is characteristic of the metabolic syndrome state and a fundamental component of metabolic inflexibility. Key questions that remain are how metabolic flexibility is lost in obese and diabetic individuals and by what means it may be regained.

Metabolic flexibility: Metabolic syndrome: Lipid and carbohydrate utilization

Man is a meal-feeder with a diet that emphasizes carbohydrates and lipids in a more or less balanced manner. The necessity to handle this discontinuous nutrient supply of both macronutrients focuses attention on the brain, pancreas, liver, skeletal muscle and adipose tissue as organs of importance in proficient handling of incoming nutrients, storing efficiently at times of surplus and providing energy at times of need. The complexity of metabolism and the constraints of the present brief review both require a limited focus on examples of metabolic flexibility that are illustrative rather than exhaustive.

Cephalic-phase insulin release

It may be Woody Allen's second favourite organ, but a good deal of metabolism is pretty sensible if put in the perspective of the brain's pre-eminence in the drive for a closely-regulated supply of glucose for energy. Efficient storage of incoming meal-associated nutrients, minimizing glucose levels after a meal (area under the curve), is a hallmark of healthy carbohydrate and lipid metabolism. This outcome involves coordinated regulation of the major organs of carbohydrate and lipid flux: pancreas; liver; skeletal muscle; adipose. The desired effect is rapid and appropriate insulin secretion from pancreatic β-cells, effective suppression of lipid mobilization and utilization, and activation of diverse metabolic pathways of tissue energy uptake and storage. An early metabolic event that initiates major elements of this coordinated pattern, and begins to occur even before the meal-related nutrient load has impacted on the gastrointestinal tract and bloodstream, is nervous system-driven or cephalic-phase insulin release.

Abbreviation: PGC, PPARγ co-activator.

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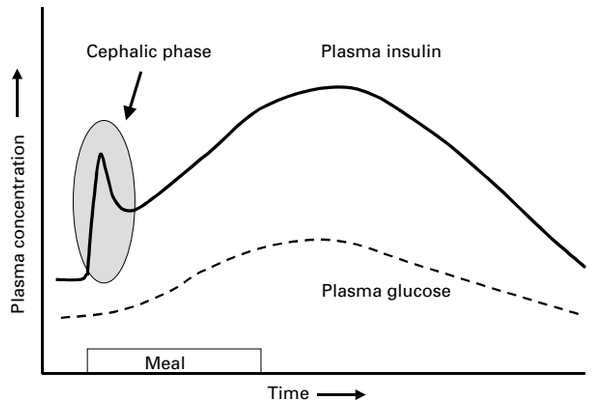

Fig. 1. Prandial insulin and glucose excursions in healthy individuals emphasizing the very early, nervous system driven, insulin spike (cephalic phase) so important for rapid and effective inhibition of hepatic glucose output and NEFA efflux, and glucose disposal.

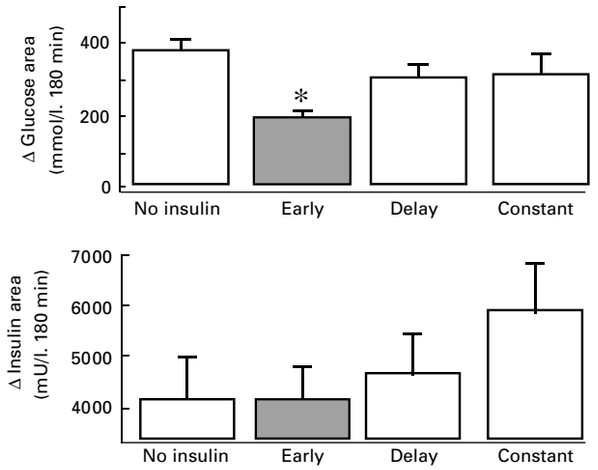

Fig. 2. (a) The effect of exogenous 'replacing' of the deficient early meal-related cephalic-phase insulin spike (early; ▩) on prandial glycaemic area under the 3 h curve. Values are means with their standard errors represented by vertical bars. Mean value was significantly different from that for the no insulin condition: * $P < 0{\cdot}005$. The delayed and constant conditions delivered the same amount of insulin delayed by 30 min or as a steady infusion over the 3 h following the start of the meal respectively. (b) The insulin area under the curve over the same period. (Adapted, with permission from the American Diabetes Association, from Bruce *et al.* 1988.)

As Fig. 1 shows, the pattern of prandial insulin secretion involves an initial 'blip' of insulin, the early or cephalic phase, followed by a more sustained release driven by rising blood glucose. There is good evidence from rodent studies that this initial insulin rise is nervous system driven. In these studies animals are conditioned to expect a meal. On the experimental day only the conditioning stimuli are presented but the meal does not appear. Despite the lack of any nutrient intake results clearly show a pronounced insulin rise, which can only be nervous system driven and closely linked to hypothalamic monoamine activation (Holmes *et al*. 1989).

In human subjects at a stage of the metabolic syndrome characterized by insulin resistance and glucose intolerance bordering on frank diabetes, but where there is still considerable β-cell capacity, a clear characteristic is the absence of this initial critical peak of insulin secretion (see Bruce *et al*. 1988). Associated with this factor are pronounced glucose levels after a meal (area under the curve) and failure to inhibit plasma NEFA levels. This condition is indicated by a marked blunting of the normal rapid decline in plasma NEFA in the early prandial period. 'Restoration' of this early insulin peak, by exogenous infusion of only 1·8 U insulin with the appropriate temporal profile, has a major effect of reducing glucose levels after a meal (area under the curve) by approximately 50 % of the excess, despite the major insulin resistance of the subjects and no net increase in total prandial insulin area under the curve (Fig. 2). Equally, this small insulin infusion is remarkably effective in restoring the appropriately steep curve of NEFA decline (Bruce *et al*. 1988). Similar insulin infusions either delayed by 30 min or as a continuous infusion over the prandial period are essentially ineffective in restoring either better glycaemic or lipaemic control.

The decline in plasma NEFA may be a particularly important index of 'flexibility' at the early prandial phase. The blood level will reflect a combination of inhibition of lipolysis and an increase in lipid uptake. The inhibition of lipolysis will be discussed later in relation to both skeletal muscle and adipose tissue. In relation to lipid uptake, there are data from Zucker fatty rats that very effectively show that the cephalic phase of insulin secretion is capable of eliciting a marked increase in lipoprotein lipase activity in adipose tissue and a decrease in skeletal muscle (Picard *et al*. 1999). This finding illustrates the coordinate metabolic patterning that is necessary to achieve optimal fuel handling.

Finally, it is worth noting here that the derangement of the cephalic phase is progressive. It is even likely that cephalic-phase insulin secretion is elevated early in simple obesity, when insulin levels are extremely high, with pancreatic β-cell capacity still sufficient for the maintenance of those levels. Insulin resistance at the organ level is, however, very likely to be already blunting insulin's physiological effectiveness, regardless of how it is driven. It is in the later phase, when the β-cell is metabolically *in extremis* and incapable of being driven further by nervous system input, that the full impact of this failure of metabolic flexibility becomes truly apparent. Whether it is too late by then to reverse back down this troublesome road by, for example, major weight loss, is not clear. Recent work by Polyzogopoulou and colleagues has shown that major weight loss following bariatric surgery is capable of restoring the early phase of insulin secretion by type 2 diabetics, suggesting this aspect of metabolic inflexibility can be ameliorated (Polyzogopoulou *et al*. 2003).

Skeletal muscle and metabolic inflexibility

There are two elements of skeletal muscle metabolism on which to focus in relation to metabolic flexibility. The first

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is fuel switching. The work from the Kelley laboratory (Kelley *et al*. 1999, 2002*a*,*b*; Kelley & Mandarino, 2000) has been informative in characterizing muscle fuel dynamics in normal healthy subjects and in obese and diabetic individuals. However, the present review will focus on one comprehensive set of studies (Kelley *et al*. 1999) of both lean and obese individuals under basal fasting and insulin-stimulated (hyperinsulinaemic euglycaemic clamp) conditions, with measurement of leg fuel balance. The leg represents a part of the body with a large muscle mass and comparatively little other metabolically-active tissue, and its total blood inflow and outflow can be sampled to allow balance studies to be performed. Equally, skeletal muscle is a major player in energy balance, it contributes >20% of the total energy expenditure, is the major tissue of insulin-stimulated glucose uptake and of glucose storage (≤4-fold the glycogen content of liver) and strongly influences metabolism via modulation of lipid flux (both circulating and stored). Thus, leg balance studies are particularly relevant to the characterization of whole-body metabolic flexibility. The main findings of this set of studies are that under basal fasting conditions glucose uptake and oxidation are normal or even increased in obese subjects compared with lean subjects. Fatty acid uptake is also normal, but fatty acid oxidation is lower and its storage elevated. In the meal or 're-fed' situation, achieved using a hyperinsulinaemic euglycaemic clamp to deliver higher insulin and sufficient glucose to maintain glucose levels, the changes in fuel utilization are strikingly different for lean and obese individuals. In the lean subjects glucose uptake increases 10-fold, with both oxidation and storage contributing strongly, while fatty acid uptake decreases equally dramatically, again with changes in oxidation and storage contributing. In the obese subject the changes in leg metabolism in response to clamp insulin elevation are severely blunted. Glucose uptake, oxidation and storage are much reduced. Of equal importance, while fatty acid uptake is comparable with that of the lean controls, oxidation is three times as high and there is evidence that fatty acids, instead of being stored, are still being mobilized for energy. This overall pattern was confirmed by leg RQ measurements (see Fig. 3). RQ in the lean subject shifts from approximately 0·82, indicating a substantial reliance on fat for fuel, to approximately 1·00, indicating that glucose is virtually the only energy source. In contrast, in the obese the RQ of 0·9 in the fasting state does not shift at all in response to the clamp (total metabolic inflexibility).

A very useful additional feature of the study reported by Kelley *et al*. (1999) is that the authors also attempted to answer the question of whether flexibility could be restored by substantial weight loss (very-low-energy diet-induced loss of approximately 15 kg over 12 weeks with a further 4 weeks of maintained weight stability). As Fig. 3 shows, the answer would appear to be a very qualified maybe! There is some evidence of a shift to carbohydrate use during the clamp, but substantial inflexibility remains. In this regard perhaps hope is more engendered by drug intervention in an animal model of the metabolic syndrome, the Zucker 'fatty' rat. This rat shows, compared with its lean siblings, an equally marked metabolic inflexibility.

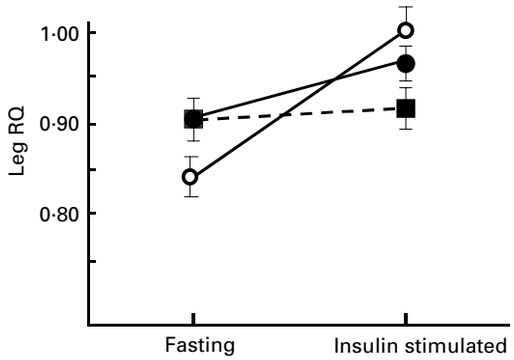

Fig. 3. Illustration of the metabolic response in the leg (largely indicative of skeletal muscle response) to elevation of insulin levels at euglycaemia in lean (○), obese (■) and obese maintained at plateau after substantial (approximately 15 kg) weight loss (●). Values are means with their standard errors represented by vertical bars.The results show the complete metabolic inflexibility of the obese individual compared with lean healthy individuals, which is only very partially restored by weight loss. (Redrawn, with permission from the American Physiological Society, from Kelley *et al.* 1999.)

Insulin-stimulated glucose uptake into skeletal muscle is badly impaired as is insulin suppression of plasma NEFA. Interestingly, treatment with a novel PPARα/γ agonist, tesaglitazar, has been reported to restore both factors, effectively almost normalizing the metabolic flexibility in these rodents (Oakes *et al*. 2002). Perhaps there is a way forward here.

Adipose tissue as a key player in the flexibility of lipid supply

Adipose tissue has a major role to play in the flexibility of metabolism. In this context Frayn (2002) has proposed 'that adipose tissue plays a crucial role in buffering the flux of fatty acids in the circulation in the postprandial period, just as the liver and, to a lesser extent, skeletal muscle buffer postprandial glucose fluxes'. This buffering role is illustrated by the data (from Frayn, 2002) presented in Fig. 4. In both the obesity and insulin-resistance states the function of the adipose tissue is disturbed (for review, see Frayn, 2002). These abnormalities include the relative failure of insulin to down regulate hormone-sensitive lipase and up regulate lipoprotein lipase (Coppack *et al*. 1992), and hence to suppress NEFA release and facilitate triacylglycerol clearance, and to increase adipose tissue blood flow prandially (Summers *et al*. 1996). This adipose tissue inflexibility is also seen in the blunting of the adrenergic control of the nervous system regulation of lipolysis in obese individuals (for a comprehensive review, see Dodt *et al*. 2003).

The abnormalities of the adipose tissue response to insulin may also extend to secretion of adipokines, particularly adiponectin. Adiponectin is now seen to be a major factor in the control of insulin sensitivity (Kern *et al*. 2003). Adiponectin levels are certainly low in obesity and diabetes, and most investigators may have been assumed

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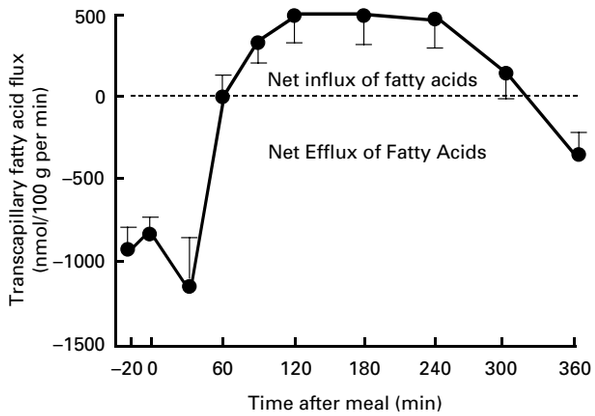

Fig. 4. The rapid and large shift in NEFA flux following initiation of a meal from efflux to storage in normal healthy individuals. Values are mean with their standard errors represented by vertical bars. (Redrawn, with permission from Springer-Verlag, from Frayn, 2002.)

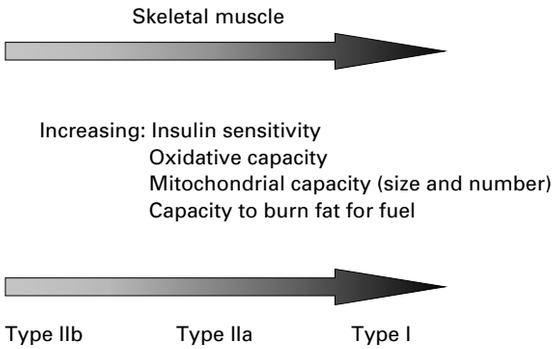

Fig. 5. The range of skeletal muscle fibre types with some of their relevant associated metabolic characteristics. A predominance of type IIb fibre type is associated with obesity and insulin resistance, both conditions of metabolic inflexibility.

that this factor reflects a chronic dysregulation. Interestingly, and contrary to what might be expected from the 'inflexible obese' profile, there is no change in circulating adiponectin levels in response to meals in lean individuals but there is a 4-fold rise in obese individuals (English *et al.* 2003). It would be interesting to know if this result relates to fully-processed and biologically-active adiponectin, what drives its release in response to meals in the obese and whether its release is accompanied by increased mRNA levels.

Capacity for fuel usage as a critical element of metabolic flexibility

As well as the ability to make the transition between carbohydrates and lipids for fuel, which has been the focus of the present paper thus far, the Kelley *et al.* (2002*b*) definition emphasizes the capacity to utilize these fuels. Given its sheer size as an organ, skeletal muscle is again critical in relation to the issue of capacity. Most major muscle groups in man are composed of a mixture of fibre types with very different metabolic characteristics (the most salient of which are summarized in Fig. 5). Given these metabolic characteristics, it is perhaps not surprising that a number of cross-sectional studies have shown relationships between muscle fibre type, and markers of metabolic capacity, and both obesity and insulin action (for references, see Kelley *et al.* 2002*a*). Consistently in these studies increased proportion of type I fibres and increased oxidative enzyme expression are associated with leanness and good insulin sensitivity, with the converse true for type IIb fibres. It is notable that the subject populations in most of these studies ranged from lean to obese, but were otherwise normal, and most sampled muscle was derived from the vastus lateralis, the large thigh muscle. What is striking is that there is a tremendous variation in the relative proportions of these muscle fibre types across individuals, which may reflect both genetic and environmental influences.

Together with enzyme measurements of oxidative capacity, recent work has investigated mitochondrial morphology in lean and obese individuals and individuals with type 2 diabetes. Obese individuals and obese individuals with type 2 diabetes have been shown to have markedly reduced mitochondrial size, even when corrected for fibre type distribution (Kelley *et al.* 2002*b*). This finding, together with the other data presented by Kelley *et al.* (2002*b*), strongly suggests impaired skeletal-muscle mitochondrial bioenergetic capacity in obese individuals. Furthermore, mitochondrial area has been shown to correlate well with insulin-stimulated glucose disposal. Interestingly, it is not only in obesity and diabetes that this reduced capacity has been observed. The same phenomenon of reduced mitochondrial oxidative and phosphorylation activity is associated with normal aging, and has recently been demonstrated in a non-invasive *in vivo* study using NMR spectroscopy (Petersen *et al.* 2003).

So, is there a way forward here to restoring tissue metabolic capacity? New work would suggest that the answer is quite possibly yes, and considerable excitement is being generated. PPARγ co-activator (PGC) 1α is a transcriptional co-activator that interacts with a number of nuclear receptors as well as with other transcription factors (nuclear respiratory factor 1, myocyte-enhancing factor 2). It is expressed in many tissues, but most highly in metabolically-active tissues such as skeletal muscle, brown fat, kidney, heart, brain and liver. PGC-1α expression is induced in skeletal muscle and brown adipose tissue; it can be induced in muscle by exercise (Goto *et al.* 2000) and in liver by fasting (Yoon *et al.* 2001). Retrovirus-mediated expression of PGC-1α in C_2C_{12} myotubes increases O_2 consumption and mitochondrial content, as well as the expression of uncoupling protein 2 and the transcription factor nuclear respiratory factor 1, which is important for mitochondrial biogenesis, and myocyte-enhancing factor 2, which is important for type I myofibre genesis. PGC-1α also serves as a target for calcineurin signalling, which has been implicated in type I myofibre expression (Wu *et al.* 1999, 2000). PGC-1α is mainly located in the type I muscle fibre, which, as described earlier, is rich in mitochondria, has an excellent capacity to utilize fatty acids as fuel and equally is very insulin responsive in terms of glucose

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metabolism. Very importantly, transgenic mice over-expressing PGC-1α under the control of muscle creatine kinase promoter, which directs expression to type II fibres, show conversion of type II to type I muscle fibres and increased endurance to electrical stimulation of those muscle groups (Lin *et al*. 2002). Equally, adenoviral-mediated overexpression of PGC-1α in cultured human white adipocytes induces the classic uncoupling protein 1, thereby giving these adipocytes a 'brown' heat-generating–energy-dissipating phenotype (Tiraby *et al*. 2003). Interestingly two recent papers have shown a coordinated down-regulation, in insulin-resistant and diabetic individuals, of a set of genes that are involved in oxidative phosphorylation and activated by PGC-1α (Mootha *et al*. 2003; Patti *et al*. 2003). In addition, a PGC-1 isoform, PGC-1β, has also been shown to be important in mitochondrial biogenesis and cellular fuel utilization capacity (Meirhaeghe *et al*. 2003). It is tempting to speculate that activation of PGC might be a way of restoring also capacity of cells to utilize lipid and carbohydrate fuels, and hence provide the basis for improving the flexibility to switch effectively between these fuel sources. However, PGC-1 is only one example, and work needs to be done to demonstrate that elevating its activity can actually improve metabolic flexibility. Also, there are undoubtedly other metabolic targets that will provide alternative modes for restoration of metabolic flexibility.

Summary

The intention here has been to give some flavour of the overall concept of metabolic flexibility, by looking at some of the elements involved and discussing what it is and what it does. Currently, the questions of why is it lost and can it be regained are more important. Lipid overload, insulin resistance, leptin resistance and β-cells near failure are prime suspects in the crime. However, there is still much to learn about how they fit together and how the way back from this state of metabolic inertia can be found.

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