REVIEW ARTICLE

Fuel selection and carbon flux during the starved-to-fed transition

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INTRODUCTION

Prolonged starvation is characterized by the mobilization of carbohydrate and triacylglycerol stores and the synthesis of glucose *de novo*, coupled with a restriction on glucose oxidation. Whereas re-feeding leads to the immediate cessation of glycogenolysis and lipolysis, there is increasing evidence that certain of the other metabolic adaptations to starvation may be refractory to rapid change. Consequently, re-feeding after prolonged starvation is associated with an asynchronous series of metabolic events, which lead to restoration of the metabolic profile of the fed state only after a considerable period. Such selective hysteresis results in a transient nutritional condition distinct from that observed in either the continuously-fed or the continuously-starved state, a metabolic pattern which is in the interests of fuel economy since it permits discrimination between transient or more sustained changes in fuel supply.

The aim of this review is to delineate those facets of mammalian metabolism which may be refractory to change during the starved-to-fed transition, and, where possible, to identify the molecular mechanisms involved. We have focused our attention on the responses of carbohydrate and lipid metabolism to the provision of carbohydrate after prolonged starvation in the rat.

GLUCOSE HOMEOSTASIS IN STARVATION

An early response to carbohydrate deprivation is the mobilization of hepatic glycogen which occurs as a result of decreased portal glycaemia and a decline in the circulating insulin/glucagon concentration ratio [1]. Increased lipolysis in adipose tissue generates glycerol, which acts as a gluconeogenic substrate, and non-esterified fatty acids (NEFA), which are oxidized. This leads to the suppression of glucose utilization and oxidation (the glucose/fatty acid cycle) [2], predominantly in working oxidative muscles which are characterized by high rates of glucose uptake/phosphorylation in the post-absorptive state [3,4]. As a result the requirement for gluconeogenesis for the maintenance of glycaemia is diminished (Fig. 1).

Inhibition of glucose uptake and phosphorylation may occur as a result of accumulation of hexose monophosphates secondary to a restriction of glycolytic flux at the level of 6-phosphofructo-1-kinase (PFK-1). This may be achieved via changes in the concentrations of citrate

(increased) and fructose 2,6-bisphosphate (Fru-2,6- P_2) (decreased) [5–7]. The oxidation of lipid-derived fuels also invokes a restriction on pyruvate oxidation via inhibition of the pyruvate dehydrogenase complex (PDH). This is mediated through end-product inhibition via increased mitochondrial acetyl-CoA/CoA, and possibly NADH/NAD⁺, concentration ratios (reviewed in [8]), and increased activity of PDH-kinase (which phosphorylates and thereby inactivates the complex) [9–13]. The decline in PDH activity permits cycling of carbon between glycolysis and gluconeogenesis (the Cori cycle; Fig. 1). Increased PDH-kinase activity may be accompanied in some tissues (notably adipose tissue, but also possibly in liver) by decreased PDH-phosphatase activity secondary to decreased blood insulin (see, e.g., [15]; reviewed in [14]).

In liver, a significant proportion of available NEFA is esterified rather than oxidized (Fig. 2). The disposition of long-chain acyl-CoA between mitochondrial oxidation or cytoplasmic triacylglycerol synthesis is influenced by the insulin/glucagon concentration ratio and the relative availability of carbohydrate and lipid (Fig. 2; reviewed in [16]). In starvation, a restriction on mitochondrial long-chain fatty acid oxidation imposed by a low activity of overt carnitine palmitoyltransferase (CPT 1) is attenuated, and an increased proportion of long-chain acyl-CoA is diverted towards β -oxidation. The energy demands of the liver are met by β -oxidation with only a limited contribution of glycolysis and pyruvate oxidation.

The oxidation of palmitate inhibits glucose phosphorylation, flux through PFK-1 and pyruvate oxidation in isolated hepatocytes [7,17]. The former effects occur concomitantly with a decline in Fru-2,6- P_2 concentration. Fatty acid oxidation also stimulates hepatic gluconeogenesis (reviewed in [18]). Thus, the oxidation of lipid fuels can be seen to exert a major role both in glucose conservation and in glucose production.

The role of hormones in the responses to starvation

Insulin and glucagon play a pivotal role in the response to starvation and re-feeding. Starvation is also accompanied by decreased circulating concentrations of tri-iodothyronine [19–21] which may be of major importance in restricting muscle protein degradation and reducing whole body energy expenditure (reviewed in [22]). Maintenance of a high circulating concentration of tri-iodothyronine by injection of exogenous tri-iodothyronine during the period of starvation reduces the

Abbreviations used: NEFA, non-esterified fatty acids; PFK-1, 6-phosphofructo-1-kinase; PEP, phosphoenolpyruvate; PFK-2, 6-phosphofructo-2-kinase; FBPAse-2, fructose 2,6-bisphosphatase; Fru-1,6- P_2 , fructose 1,6-bisphosphate; Fru-2,6- P_2 , fructose 2,6-bisphosphate; PDH, the pyruvate dehydrogenase complex; KAP, PDH-kinase activator protein; CPT 1, carnitine palmitoyltransferase 1 (overt carnitine palmitoyltransferase); PEPCK, PEP carboxykinase.

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Fig. 1. The glucose/fatty acid cycle and its relationship to the Cori cycle

The metabolic interactions between lipids and carbohydrate in starvation are indicated by $\longrightarrow$. The Cori cycle is shown thus $\longleftrightarrow$.

effects of starvation to diminish pyruvate oxidation [23]. The importance of hormonal changes in the establishment and maintenance of the metabolic characteristics of the starved state is demonstrated by the failure of an increase in fat-fuel availability alone to account quantitatively for the extent to which glucose utilization is restricted during starvation (see [5,24–26] for individual values).

CARBOHYDRATE RE-FEEDING AFTER STARVATION

Substrate supply and utilization during re-feeding

During the re-feeding transition, the disposal of dietary substrates may involve their further metabolism to fulfil energy requirements, and, in the case of dietary carbohydrate, storage as triacylglycerol or glycogen. Net storage of carbohydrate as lipid or glycogen necessitates inhibition of net triacylglycerol mobilization and glycogenolysis, together with stimulation of lipogenesis and glycogenesis. Re-feeding is accompanied by a precipitous decline in circulating levels of NEFA [27] and increases in blood glucose and lactate concentrations and the insulin/glucagon concentration ratio [5,28]. However, the time required for the replenishment of hepatic and muscle glycogen stores is quite considerable, up to 8–24 h in rats previously starved for 48 h [27,29,30].

Mechanisms must therefore operate to guarantee that a significant proportion of available carbohydrate is diverted towards glycogen synthesis, while, at the same time, tissue energy requirements are satisfied. The dual requirement for glycogen repletion and energy provision dictates the metabolic characteristics of the starved-to-fed transition.

The role of the liver in nutrient disposal

Although the liver may be the major site of disposal of some ingested amino acids on re-feeding, splanchnic uptake of the branched-chain amino acids is incomplete [31] and significant quantities are released to peripheral tissues. Dietary lipid (as chylomicrons) enters the body via the lymphatic system, thereby supplying peripheral tissues before reaching the liver. Furthermore, available evidence indicates that the primary sites of glucose utilization after re-feeding are extra-hepatic [32]. The role of the liver may thus be limited to the disposal of monosaccharides other than glucose. Nevertheless, evidence is accumulating which indicates that, although glucose metabolism is initiated predominantly at extra-hepatic sites, transfer of C_3 derivatives of glucose to the liver may be necessary to supplement the complete utilization of the carbon skeleton for oxidative or biosynthetic purposes (reviewed in [14]). An analogy may

Fig. 2. Relationship between lipid synthesis and oxidation in the liver

Interaction between metabolite and step in a metabolic pathway is indicated by $\cdots \rightarrow \ominus$ or $\cdots \rightarrow \oplus$. Sites of action of an increased insulin to glucagon ratio are also shown.

be drawn with the metabolism of branched-chain amino acids in the fed state, where the oxo-acid derivatives (and possibly their partial oxidation products) generated in muscle are mainly transferred to the liver for oxidation or utilization for glucose/lipid synthesis (reviewed in [33]).

THE RE-FEEDING TRANSITION IN LIVER

The transition from the fasted to the fed state involves a reversal of the metabolic response to starvation, including alterations in enzyme phosphorylation states and/or concentrations, as well as changes in the concentrations of key regulatory molecules. In this review, we do not attempt to undertake a comprehensive discussion of all of the factors which might be expected to influence the re-feeding response, particularly those responsible for effecting long-term adaptive changes in hepatic enzyme activities. These have been reviewed elsewhere (see, e.g., [34–38]). Instead, we have selected examples of where such long-term adaptive changes in concentrations in glycolytic, gluconeogenic and lipogenic enzymes have been documented to cause a significant change in metabolic flux during the starved-to-fed transition.

Glycogenesis and glycolysis

Rapid responses of the liver to re-feeding normally include the suppression of net hepatic glucose output,

and stimulation of glycogen deposition [29,39–44]. A number of pathophysiological conditions, including hyperthyroidism [44], chronic fat feeding [45] and genetic obesity [46,47] are associated with modulation of the former response. It is unknown whether suppression of net glucose output is a consequence of changes in concentrations of regulatory metabolites, changes in the arterial-portal glucose gradient (see, e.g., [48,49]) or hormonal or neuronal changes associated with re-feeding. However, although hyperinsulinaemia inhibits hepatic glucose production in the fed and post-absorptive states [50,51], a role for an increased insulin concentration as the sole factor in suppression of glucose output after re-feeding can be excluded as it can be observed in rats treated with anti-insulin serum [29]. Suppression of glucose output is observed in the absence of increased glycogen synthesis in glycogen-storage-disease rats [52], and is therefore not a simple consequence of the preferential utilization of hexose monophosphate for glycogenesis [29].

The response of the liver to a glucose load normally involves the sequential inactivation of glycogen phosphorylase and activation of glycogen synthase ([53], reviewed in [54], see also [55]). Increases in blood (and therefore hepatic) glucose concentrations lead to an increased proportion of phosphorylase as the T-conformer, exposing a phosphorylated serine residue to the action of phosphorylase phosphatase. When the

Table 1. Changes in enzyme phosphorylation states after re-feeding

Measurements were made at 2 h after re-feeding glucose or chow to previously starved rats. The activities of active (dephosphorylated) glycogen phosphorylase (data from [27]) and active (dephosphorylated) PDH (data from [57]) are given as units/g of liver and munits/unit of citrate synthase respectively. Net dephosphorylation of L-pyruvate kinase and acetyl-CoA carboxylase may be inferred from changes in the activity ratios. For L-pyruvate kinase this refers to the activity measured at 0.15 mM-PEP relative to $V_{\max}$ (see [29] and [155]). Acetyl-CoA carboxylase activity refers to that measured at 0.5 mM-citrate relative to $V_{\max}$ (data from [156] and unpublished observations of M. R. Munday, M. J. Holness & M. C. Sugden). * $P < 0.05$, *** $P < 0.001$ compared with starved.

	Initial activity		Activity ratio (% of $V_{\max}$)	
	Glycogen phosphorylase <i>a</i>	PDH _a	Pyruvate kinase	Acetyl-CoA carboxylase
Starved	3.2 ± 0.2	9.7 ± 1.6	10.5 ± 0.6	22.5 ± 3.0
Re-fed	1.2 ± 0.1***	10.4 ± 1.8	20.7 ± 1.2***	33.1 ± 4.1*
Change (%) after re-feeding	-63	+7	+97	+47

concentration of phosphorylase *a* has fallen below a critical level (approximately 10% of total activity), glycogen synthase phosphatase becomes active, with concomitant conversion of glycogen synthase *b* to glycogen synthase *a*.

The threshold value of phosphorylase *a* for glycogen synthase activation is affected by the pre-existent glycogen concentration. In glycogen-storage-disease rats, where the glycogen concentration is high, the threshold value for phosphorylase *a* is decreased 5-fold [56]. As a consequence, glycogen synthase phosphatase remains inhibited, and glucose-induced activation of glycogen synthase occurs at a 10-fold lower rate than normal [56]. It should, however, be emphasized that in normal overnight-fasted rats re-fed chow *ad libitum*, neither activation of glycogen synthase [57] nor inactivation of glycogen phosphorylase [58] is obligatory for glycogen synthesis, which may be driven by an increase in substrate availability (a 'push' mechanism). Thus, for example, when fructose is provided as glycogenic substrate, activation of glycogen synthase occurs in parallel with activation of phosphorylase [54,58]. As a result the latency which normally precedes the activation of glycogen synthase is abolished (see, e.g., [59]).

Hormonal conditions affecting the glycogenic response to glucose administration in the intact animal include experimental diabetes (reviewed in [60]; see also, e.g., [61]), glucocorticoid-deficiency [62], and altered thyroid hormone status [29]. Abnormalities which may occur in diabetic or adrenalectomized starved rats include impaired inactivation of phosphorylase *a*, a phosphatase-resistant glycogen synthase *b* and/or decreased glycogen synthase phosphatase activity [54]. Thyroid hormones may also exert regulatory effects on hepatic glycogen synthesis via effects on glycogen synthase phosphatase [63] or phosphorylase phosphatase [64].

The extent to which glucose (via direct uptake and phosphorylation) and C₃ derivatives of glucose [via the gluconeogenic (indirect) pathway] are used as precursors for hepatic glycogen synthesis after re-feeding is controversial [65–70]. Nevertheless, the existence of the indirect pathway of glycogenesis [66,68] implies the co-existence of net gluconeogenic and glycogenic fluxes. This is difficult to reconcile with synchronous reversal of starvation-induced changes in activities of enzymes considered to be regulatory for glycogenesis, glycolysis

and gluconeogenesis, unless the liver is divided into functionally distinct glycolytic and gluconeogenic zones, as has indeed been suggested to be the case (reviewed in [72]).

Some precedent for asynchronous de-phosphorylation of hepatic enzymes as one means to influence individual pathways selectively has been set by the sequential responses of glycogen phosphorylase and glycogen synthase to re-feeding. However, while selective modulation of one or more of the multiple protein phosphatases involved in the control of glycogenesis and gluconeogenesis is not excluded, an increase in glycaemia and in the insulin/glucagon concentration ratio would be expected to activate or de-inhibit most protein phosphatases known to be involved in the control of glycogenesis and glycolysis/gluconeogenesis, including types 1, 2A and 2C [74,75]. As reversal of many of the kinetic effects of phosphorylation on extra-mitochondrial enzymes is achieved within the first 2 h of re-feeding (Table 1), it would appear that control by substrates and allosteric regulators (such as long-chain acyl-CoA and citrate) or the relative persistence of stable adaptive changes in the concentrations of glycolytic and gluconeogenic enzymes (Fig. 3) is of primary importance for the control of hepatic carbon flux during the starved-to-fed transition. For example, while de-phosphorylation (activation) of L-pyruvate kinase occurs rapidly in response to re-feeding after prolonged starvation ([29,42]; Table 1), net flux from pyruvate to triose phosphate is favoured by a failure to reverse immediately starvation-induced increases in PEP-carboxykinase (PEPCK) activity (Fig. 3a; [76]). At the same time, while 6-phosphofructo-2-kinase (PFK-2) may be re-activated [28,42], the accumulation of Fru-2,6- P_2 (see, e.g., [66]) is limited by the preferential use of hexose monophosphate for glycogenesis, as opposed to Fru-2,6- P_2 synthesis [77], as the formation of glucose 6-phosphate via glucokinase may be limited (Fig. 3b; see also [65]). The time required for reversal of the stable, adaptive changes in protein concentrations are not a consequence of hysteresis at the level of genomic expression as effects of re-feeding on specific mRNA concentrations and enzyme synthesis rates are observed very rapidly (e.g. Figs. 3a and b; [78–81]). Instead, hysteresis is a consequence of the times taken to translate changes in rates of enzyme synthesis and degradation into changes in enzyme concentrations.

Fig. 3. Effects of re-feeding on mRNA activities (◆), rates of enzyme synthesis (●) and enzyme activities (▲) of hepatic PEP carboxykinase (a) and glucokinase (b)

Data for glucokinase are taken from [81] and for PEPCK from [76] and [166], from which further details of methodology may be obtained.

Oxidation

Hepatic PDH_a activity declines by 29–48% in response to starvation (see, e.g., [17,27,82–84]). In prolonged starvation, diminished hepatic PDH_a activity is associated with adaptive increases in PDH-kinase activity [85]. These are achieved by increases in the activity of a specific activator (PDH-kinase activator protein, KAP) [17,85].

While the time course of hepatic PDH inactivation bears a striking correlation with depletion of hepatic glycogen [1], substantial hepatic glycogen repletion is observed before PDH is re-activated on re-feeding after prolonged (24–48 h) starvation [27,29]. This latency contrasts with the rapid de-phosphorylation of glycogen phosphorylase, L-pyruvate kinase and acetyl-CoA carboxylase (Table 1), and may facilitate the preferential use

Fig. 4. Potential for carbohydrate and lipid oxidation in the liver after chow re-feeding

Time courses for hepatic PDH re-activation (▲), long-chain acyl-CoA depletion (●) and changes in the maximal activity of CPT 1 (◆) in response to re-feeding after starvation. Drawn from data in [27], [167] and [89] respectively, from which further details may be obtained.

of C₃ precursors for glycogen repletion [86]. Maintenance of an elevated circulating tri-iodothyronine concentration during starvation has been shown to accelerate PDH re-activation after re-feeding. This effect may involve increased activity of PDH-phosphatase [87].

The re-activation profile for hepatic PDH shows a striking correlation with depletion of long-chain acyl-CoA (Fig. 4), and although inhibition of CPT 1 by malonyl-CoA plays an important role to restrict mitochondrial fatty acid oxidation in livers of fed rats [88]; see Fig. 2), increases in maximal activity and decreases in sensitivity of CPT 1 to malonyl-CoA induced by starvation are not reversed during the first 6 h of re-feeding ([89]; see Fig. 4). During the initial phase of the re-feeding transition, it is therefore possible that mitochondrial fat oxidation is not suppressed and that there is an altered relationship between the lipogenic rate, the malonyl-CoA concentration and the rate of mitochondrial fat oxidation (see also [90–92]). For at least the initial 2 h of the re-feeding period, a proportion of available acetyl-CoA continues to be used for ketone body synthesis [93], implying hepatic energy requirements can continue to be met by the oxidation of non-carbohydrate substrates. It is tempting to conclude that continued oxidation of endogenous hepatic triacylglycerol or acyl-CoA immediately after re-feeding may permit the expression of a high PDH-kinase activity, stable increases in which are reversed only gradually (4 h in cultured hepatocytes) in response to insulin at concentrations found after re-feeding [83].

The re-activation of PDH by de-phosphorylation may also be retarded by phosphorylation at sites in addition to the inactivating site (site 1; [97]). Thus, suppression of the activity of PDH-phosphatase may act synergistically with continued lipid oxidation to favour prolonged inactivation of the enzyme by phosphorylation of site 1.

Fig. 5. Relationship between acetyl-CoA carboxylase activities, malonyl-CoA concentrations and rates of lipogenesis after chow re-feeding

Initial (▲) and total (△) activities of acetyl-CoA carboxylase (unpublished results of M. R. Munday, M. J. Holness & M. C. Sugden) were assayed as described in [156]. Rates of lipogenesis (○) and malonyl-CoA concentrations (●) have been re-drawn from data in [27] and [99] respectively.

It is unknown whether covalent modification of other enzymes at multiple sites also influences their response to re-feeding, although, for example, multi-site phosphorylation has been demonstrated to occur for glycogen synthase (see, e.g., [94–96]), where the phosphorylation of site 5 enhances the ability of sites 3a–c to be phosphorylated.

A close temporal relationship exists between increases in utilization of citrate as a precursor for lipogenesis and re-activation of hepatic PDH ([27,29,40,44,87], reviewed in [14]). This suggests that restoration of PDH activity is necessary to permit the use of carbohydrate for hepatic fat synthesis during the starved-to-fed transition (reviewed in [14]). However, in livers of fed rats, parallel changes in rates of fatty acid synthesis and initial activities of PDH have not been observed [98]. Instead, a more close correlation is observed between the lipogenic rate and the initial activity of acetyl-CoA carboxylase [98]. The time courses of changes in initial and total activities of hepatic acetyl-CoA carboxylase in response to chow re-feeding after 48 h of starvation are shown in Fig. 5. An increase in the initial activity of acetyl-CoA carboxylase is observed within 2 h of re-feeding (see also Table 1), preceding increases in the total rate of lipogenesis (measured using $^3\text{H}_2\text{O}$). No change in acetyl-CoA carboxylase maximal activity is observed over this period. This relationship suggests that, while dephosphorylation of acetyl-CoA carboxylase is permissive for an increase in the rate of lipogenesis, substrate and co-factor availability may be of more importance in determining net lipogenic flux.

It is interesting to compare the time course of restoration of the rate of lipogenesis with changes in the

hepatic concentration of malonyl-CoA [99]. The malonyl-CoA concentration increases progressively over the first 8 h of re-feeding (Fig. 5) reaching a maximum after about 24 h (see [99]). The pattern of increases is most readily explained on the basis of an increased ratio of the activity of acetyl-CoA carboxylase to that of fatty acid synthase, achieved by selective, acute modulation of the activity of the former, but not the latter, enzyme.

THE RE-FEEDING TRANSITION IN MUSCLE

Evidence is available which suggests that muscle glycolysis and carbohydrate oxidation may not be immediately restored after re-feeding. As in liver, this metabolic pattern may be attributed to a failure to immediately reverse stable adaptive changes in enzyme activities and metabolite concentrations and to the continued preferential utilization of lipid fuels.

Glycogen synthesis and glycolysis

Skeletal muscle glycogen stores become progressively depleted during starvation, accounting for approx. 40 % of total body glycogen mobilization after prolonged starvation [100]. The repletion of skeletal muscle glycogen after re-feeding is therefore a quantitatively important aspect of carbohydrate disposal during the starved-to-fed transition. Glycogen synthesis in muscle occurs predominantly from glucose via hexokinase. In order to replete skeletal muscle glycogen it is therefore necessary to reverse the decreases in glucose uptake/phosphorylation which in working muscles constitute a major aspect of glucose conservation in starvation.

There is no evidence for any delay in restoration of high rates of skeletal muscle glucose uptake assessed on the basis of rates of glycogen deposition after re-feeding [30]. Moreover, rates of glycogen deposition in some skeletal muscles after re-feeding equal or may actually exceed the rates of glucose uptake/phosphorylation observed in the same muscles in the post-absorptive state [30]. The use of hexose monophosphate for glycogen synthesis after re-feeding is permitted by a change in the operational bias of a substrate cycle between glycogen and glucose 1-phosphate [101] to favour net glycogen deposition. Mechanisms may involve synchronous stimulation of glucose uptake and activation of protein phosphatase-1 by an increased insulin concentration ([102], reviewed in [103]). Rates of glycogen deposition after re-feeding are directly proportional to the extent to which glycogen depletion has occurred during starvation rather than being related to fibre type [30]. This suggests that the use of exogenous glucose for glycogen synthesis may be permitted via a diminished inhibitory effect of glycogen on glycogen synthase de-phosphorylation ([104,105], reviewed [54]), as occurs after the cessation of exercise [106,107].

The relative contributions of glycogen synthesis and glycolysis/oxidation to total glucose utilization following re-feeding remains to be established. However, in isolated perfused hindquarters from starved rats, an 18-fold stimulation of glucose uptake by insulin was associated with enhanced net glycogen synthesis, but neither lactate output nor pyruvate oxidation was significantly increased [108]. Thus, the predominant fate of the additional glucose was sequestration as glycogen (Fig. 6). This pattern is also observed in preparations from fed rats, although in this instance there is also a modest additional

Fig. 6. Effects of insulin on glucose utilization in perfused hindlimbs from fed or 48 h-starved rats or in post-absorptive rats *in vivo*

Basal and insulin-stimulated rates of glucose uptake *in vitro* (perfused hindlimbs), and glucose utilization indices in selected skeletal muscles *in vivo* are indicated by open bars. The contribution of glycogen synthesis to total glucose utilization is shown by stippled bars. Drawn from data in [108] (*in vitro*) and [109] (*in vivo*), from which further details may be obtained.

increase in oxidation ([5,108]; Fig. 6). It might be argued that the perfused hindlimb is not an adequate model for the effects of insulin in the intact animal. However, James *et al.* [109] demonstrated that during euglycaemic hyperinsulinaemic clamp, increases in skeletal muscle glucose uptake observed in response to insulin can also be quantitatively accounted for in terms of glycogen deposition (Fig. 6). A similar pattern of glucose disposition may therefore be observed in the intact animal in response to re-feeding. The increase in skeletal muscle glucose uptake observed during re-feeding is not accompanied by parallel increases in lactate and alanine output (see, e.g., [110,111]) and it has been demonstrated that skeletal muscle PDH (like that of liver) may be refractory to re-activation after re-feeding [86,112]. This latency is restricted to muscles of the oxidative type (M. J. Holness & M. C. Sugden, unpublished work).

The metabolic fate of glucose in resting muscle differs markedly from that observed during muscle contraction. While the effects of muscle contraction on lactate release *in vitro* are not further increased by insulin [108], it remains possible that significant rates of glycolysis may be observed in working muscles during the starved-to-fed transition. In this connection, while measurements of arteriovenous concentration differences have been made across resting muscles, one study [113] has demonstrated that insulin and exercise act synergistically to enhance glucose disposal (predominantly by muscle) in man, and it is well established that exercise enhances muscle glucose metabolism (see, e.g., [114,115]).

The role of Fru-2,6- P_2 in the regulation of glycolysis in skeletal muscle, either in the resting state or during contraction, is less firmly established than it is in liver [116–119]. The regulatory characteristics of muscle PFK-2 are quite distinct from those of liver (reviewed in [117]). Nevertheless, as in liver, a possible restriction on glycolytic flux after re-feeding may be imposed at the level of PFK-1 by the failure to reverse rapidly starvation-induced decreases in concentrations of Fru-2,6- P_2 [5]. In rats starved for 48 h, skeletal muscle Fru-2,6- P_2

concentrations remain at less than approx. 65 % of the fed value throughout the most rapid phase of glycogen deposition, and are restored only after 8–24 h of feeding *ad libitum* [5].

Oxidation

Re-feeding after prolonged (40–48 h) starvation is associated with delayed restoration of the activities of cardiac [112] or skeletal muscle [86] PDH to those observed in fed rats. Consistent with these findings in the intact animal, an increase in insulin concentration does not immediately reverse the effects of starvation to diminish lactate oxidation in isolated perfused hindquarters from starved rats [108]. As in liver, the molecular mechanism by which re-activation of cardiac PDH is suppressed after re-feeding may involve stable, starvation-induced increases in PDH-kinase and KAP activities [112], and a similar mechanism may operate in skeletal muscle. These long-term changes become important for retarding PDH re-activation after re-feeding only if the duration of starvation exceeds 6 h [1].

As skeletal muscle glycogen synthesis occurs directly from glucose, there is no requirement for any delay in PDH re-activation to optimize the supply of precursors for muscle glycogen synthesis. It is more likely that the activity state of muscle PDH is closely coupled to the energy requirements for contraction. Thus, in the fed state, whereas insulin leads to a less than 2-fold increase in glucose oxidation in perfused hindlimbs from fed rats, muscle contraction leads to an 11-fold increase [108]. In contrast, in preparations from starved rats, while a 14-fold increase in oxidation is observed in response to exercise, the absolute rate of lactate oxidation is only 22 % of that observed in the fed state. Importantly, carbohydrate oxidation accounts for only 25 % of total oxygen uptake in the starved state, contrasting with 60 % in the fed state [108]. This finding implies the preferential oxidation of endogenous, non-carbohydrate substrates in starvation, of which triacylglycerol (see [120]) is likely to be of primary importance. A more detailed discussion of the possibility of continued lipid oxidation during the starved-to-fed transition is given elsewhere [5].

THE RE-FEEDING TRANSITION IN ADIPOSE TISSUE

The metabolism of white adipose tissue is important for glucose homeostasis in starvation only because triacylglycerol mobilization leads to increased availability of NEFA; the rate of glucose utilization in white adipose tissue accounts for < 5 % of total body glucose turnover [121], and is unaffected by starvation [3]. However, an increment in blood insulin to a value approximating that observed during carbohydrate feeding leads to a 2–3-fold increase in glucose utilization [24,122]. Consequently, it appears that a diminished supply of carbohydrate does not necessarily restrict glucose uptake, but instead that increased availability of carbohydrate stimulates glucose uptake and utilization (via an increase in insulin level).

The extent to which white adipose tissue triacylglycerol is mobilized is determined by the relative rates of fatty acid esterification and lipolysis. In starvation the triacylglycerol/fatty acid substrate cycle [123] is biased towards net release of fatty acids, whereas after re-feeding a rapid and marked decline in NEFA concentration indicates cessation of net lipolysis. As

increased rates of triacylglycerol/fatty acid substrate cycling are observed [124,125], it is unknown whether the predominant effect of re-feeding is to increase esterification or to decrease lipolysis [124,125]. However, it can be concluded that rates of glucose uptake/phosphorylation exceed rates of re-esterification, because, in contrast to the situation in liver, glycerol 3-phosphate (the co-substrate for esterification) is derived exclusively from glucose. High rates of triacylglycerol/fatty acid cycling further imply that glycerol continues to be released from adipose tissue, as it cannot be metabolized *in situ*. The continued release of glycerol from adipose tissue may contribute to the provision of precursors for hepatic glycogenesis via the indirect pathway.

Glucose can be utilized by white adipose tissue, not only as a precursor for the glycerol moiety of triacylglycerol but also as a precursor for lipogenesis. Marked diurnal rhythms in rates of white adipose tissue fatty acid synthesis and esterification have been observed in rodents provided with food *ad libitum* [126]. Peak rates of lipogenesis are observed during periods of food ingestion and absorption, which normally occur during the dark period of a light-dark cycle [126]. Nevertheless, marked 2–3-fold increases in lipogenesis can be elicited by the administration of carbohydrate to 'fed' rats during the light period [98,127]. Under these conditions, glucose is the major lipogenic substrate utilized (reviewed in [128]) and the increases in lipogenesis are mediated by insulin [98,127,128]. Increased rates of lipogenesis are paralleled by activation of pyruvate kinase, PDH and acetyl-CoA carboxylase (reviewed in [128]). In contrast, available evidence suggests that lipogenesis in white adipose tissue may show a delayed response to carbohydrate feeding after prolonged starvation [129,130]. The biochemical basis for this hysteresis has yet to be elucidated. However, in contrast to the situation in liver, a role for retarded PDH re-activation can be excluded as the addition of insulin results in a rapid 2–3-fold increase in PDH in fat pads from starved rats [131]. Triacylglycerol/fatty acid cycling in the absence of an increase in fatty acid synthesis *de novo* implies either that exogenous fatty acids are esterified, or that no net synthesis of triacylglycerol occurs.

It is of interest that in white adipose tissue an acute elevation of fatty acid concentration does not oppose the effects of insulin to stimulate either glucose uptake/phosphorylation (infusion of fatty acids during hyperinsulinaemic euglycaemic clamp [24]) or lipogenesis (administration of an intragastric load of medium-chain-length triacylglycerol [127]). Indeed, in isolated adipocytes, NEFA have been demonstrated to stimulate glucose transporter activity [132]. Increased glucose uptake may permit an elevated rate of esterification of endogenous fatty acid through provision of glycerol-3-phosphate (see above, and [24]).

In post-absorptive rats, brown fat carbohydrate metabolism responds to an elevation in the circulating insulin concentration in a manner qualitatively similar to that of white adipose tissue, with increased glucose uptake/phosphorylation, activation of PDH and increased fat synthesis [24,127,133–137]. However, even in the basal state, rates of glucose utilization are more than 10-fold higher than those of white adipose tissue [24]. Moreover, after increasing insulin concentrations to values similar to those observed after re-feeding, rates

are approx. 60-fold greater than in white fat. By analogy, rates of brown fat lipogenesis in fed rats are 3–4-fold those observed in white fat, while after additional feeding with glucose they are 11-fold higher [127].

Brown fat PDH_a is rapidly increased in response to glucose administration after starvation, and, in contrast to the situation in white adipose tissue, parallel increases in lipogenesis are evident [137]. There is thus no evidence for a retarded response of this tissue to nutritional stimuli. The increases in lipogenesis observed after re-feeding are insulin-mediated [127,137] and are secondary to increased utilization of glucose as lipogenic substrate [137]. Under these circumstances glucose carbon is utilized for fatty acid synthesis via PDH and citrate [137]. The site at which insulin acts to increase the use of glucose as lipogenic precursor occurs proximal to citrate cleavage in the lipogenic pathway [137].

Incorporation into lipid may not be the sole fate of dietary glucose in brown fat after re-feeding. PDH is present at about five times the quantity of acetyl-CoA carboxylase (see [128]), and maximal activities of the enzymes of the citric acid cycle are high in brown fat ([138,139]). Therefore, unless for some reason citric acid cycle flux is inhibited, increased oxidation of glucose to CO₂ via the citric acid cycle is likely to occur, and thus may impose an irreversible drain on available carbohydrate.

CONCLUDING REMARKS

In this review we have alluded to the major mechanisms by which changes in metabolic fluxes may be achieved during the starved-to-fed transition. We have provided evidence that latency in the response to carbohydrate provision is introduced predominantly by the times taken to reverse any chronic adaptive change in enzyme concentration and/or possibly to exhaust any endogenous triacylglycerol which has accumulated as a consequence of starvation-induced increases in fatty acid supply. We have thus introduced the concept that the glucose/fatty acid cycle may operate not only in starvation, where it serves to permit maintenance of glycaemia via cycling of carbon via glycolysis and gluconeogenesis, but also during the re-feeding transition, where it may spare carbohydrate carbon for glycogen synthesis.

An analogy may be drawn between the accumulation of triacylglycerol which occurs in muscle in starvation with that which occurs in association with fat-feeding or genetic obesity (see, e.g., [140,141]; reviewed in [142,143]). The constraint on glucose metabolism imposed by lipids during the re-feeding transition may thus have relevance to other situations associated with increased lipid supply, including dietary obesity and pathological conditions of hormone deficiency or excess [157–165].

While we have described the responses to re-feeding carbohydrate, either as sole substrate or as the predominant component of a mixed diet, we have not commented on the response to the provision of proteins or amino acids. Decreased myofibrillar proteolysis after re-feeding requires dietary protein or amino acids [144] and, just as the re-activation of PDH in certain tissues may be refractory to the stimulus of glucose or chow re-feeding after prolonged carbohydrate deprivation, re-activation of hepatic branched chain 2-oxo acid dehydrogenase complex is not immediate when protein intake is resumed after protein-starvation [145]. In this

case, metabolic hysteresis may function to direct absorbed branched-chain amino acids towards protein synthesis [145]. In addition, while describing the regulatory significance of glucose itself, we have ignored the possible importance of amino acids as regulators. For example, as well as acting as a major respiratory fuel for the cells of the immune system [146], and serving to transport carbon and nitrogen between tissues (see, e.g., [147,148]), glutamine may be considered as an important anabolic modulator, stimulating hepatic glycogenesis [149] and lipogenesis [150], inhibiting lipolysis and ketogenesis [151], and possibly regulating skeletal muscle protein turnover ([152], reviewed in [153]).

We have described in detail the responses to re-feeding after prolonged starvation. It might be argued that this topic is of limited physiological significance in man, where nutrients are generally available after relatively short periods of food deprivation. We have ourselves demonstrated that the pattern of restricted carbohydrate oxidation after re-feeding in the rat is manifested only after extended (6–24 h) periods of starvation [1]. We would argue, however, that this nutritional manipulation may have more widespread applicability than is generally appreciated. For example, many of the features of prolonged starvation (lipid availability, low insulin, high glucagon) are also observed during suckling, with reversal upon weaning onto a high carbohydrate diet. Furthermore, the necessity for fuel mobilization and glucose conservation following nutritional deprivation may be exaggerated and accelerated in situations associated with increased substrate requirements, including pregnancy and lactation, where many of the characteristics of more prolonged starvation may be observed after relatively short periods of nutrient withdrawal (see e.g. [154]). Analogous pathological conditions where an increased metabolic demand is imposed include trauma, immunological challenge and cancer. Further investigation into the biochemical basis for the hysteresis mechanisms invoked during starvation will, we hope, provide further insight into the relationship between short-term and longer-term mechanisms for the control of metabolism, together with the molecular basis for a co-ordinated response.

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