THE BODY'S RESPONSE TO DIETARY CARBOHYDRATE IN HEALTH AND DISEASE: NEW INSIGHTS

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Summary

The last decade has seen major advances in our understanding of the body's response to dietary carbohydrate, particularly after a period of starvation (the starved-to-fed transition). This paper briefly reviews these advances and poses the question of whether lipid as opposed to carbohydrate is the major oxidative fuel for the body during the initial phase of the starved-to-fed transition. Evidence is presented showing that the respiratory quotient increases progressively rather than abruptly in response to chow refeeding after prolonged starvation, only reaching a steady-state value approaching unity after four hours. These results are interpreted to imply that carbohydrate refeeding after starvation is associated with a progressive as opposed to abrupt transition from lipid-based to carbohydrate-based oxidative metabolism.

I. INTRODUCTION

The metabolic response to dietary carbohydrate is dependent on nutritional status, an important factor being the antecedent period of fasting. Fasting initiates a complex sequence of changes in the activities of key regulatory enzymes mediated via allosteric effectors and protein phosphorylation and consolidated by changes in gene expression. Carbohydrate refeeding after a period of prolonged starvation initiates an equally complex series of metabolic events that culminate in the restoration of the metabolic profile characteristic of the fed state only after a considerable period. It is this transitionary phase following carbohydrate refeeding and before restoration of the fed state (the starved-to-fed transition: Sugden et al. 1989) that is the focus of this paper. The body uses two primary fuels in energy generation. These are glucose and fatty acids. This paper explores the relationship between these two fuels in the context of the starved-to-fed transition and asks the question whether, contrary to established views, fatty acid as opposed to glucose constitutes the major oxidative fuel during the initial phase of the starved-to-fed transition.

II. HEPATIC RESPONSE TO CARBOHYDRATE REFEEDING AFTER STARVATION

In vivo, carbohydrate refeeding after starvation is widely recognised to promote the rapid repletion of hepatic glycogen stores. Many textbooks still describe hepatic glycogenesis as a relatively simple process, involving a direct pathway of glycogenesis (viz. glucose → glucose 6-phosphate → glucose 1-phosphate → UDPglucose → glycogen). There is now overwhelming evidence that this textbook view is fundamentally flawed (Shulman and Landau 1992). The term 'glucose paradox' has been invoked to describe the observation that, despite its ability to promote hepatic glycogenesis in vivo, glucose per se, at least at physiological concentrations, is a poor precursor for glycogen biogenesis in liver cells in vitro (Katz et al. 1986).

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The reasons underlying the glucose paradox are complex but one important factor is that lactate and related 3-carbon metabolites have the capacity to act as precursors for hepatic glycogen synthesis. Thus, a portion of the dietary glucose is believed to be first metabolised to lactate via glycolysis before the lactate carbon is converted into liver glycogen via the gluconeogenic pathway. This pathway of glycogen biogenesis is termed the indirect pathway (viz. glucose → lactate → glucose 6-phosphate → UDPglucose → glycogen).

The operation of the indirect pathway, which at first glance may seem contrary to common sense, is now supported by a wealth of evidence from a variety of sources. This has been extensively reviewed elsewhere (Shulman and Landau 1992). What remains a controversial issue concerns the relative contributions of the direct and indirect pathways to net hepatic glycogen synthesis in vivo. Although some reports suggest that the liver has a restricted capacity to phosphorylate glucose and that the indirect pathway accounts for up to 100% of the glycogen synthesised, it seems most likely that both pathways are operative in vivo. Indeed, in man and other animals, whose pattern of eating involves multiple mixed meals, the direct pathway is likely to be the major route of glycogenesis. By contrast, in those species whose hepatic metabolism is orientated towards gluconeogenesis, including many ruminants and obligate carnivores, the presumption must be that the indirect pathway is the primary route of glycogenesis.

There are a number of unresolved questions surrounding the operation of the indirect pathway. The first of these concerns the mechanism that allows hepatic glycogen synthesis to be activated in response to dietary carbohydrate without the concomitant suppression of gluconeogenic flux. In liver the direction of carbon flux is determined to a significant extent by cAMP-mediated protein phosphorylation. In starvation glucagon promotes the net phosphorylation of regulatory hepatic enzymes, including those involved in glycogen metabolism (glycogen synthase, glycogen phosphorylase) and glycolysis/gluconeogenesis (6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase, pyruvate kinase). Hitherto it has been presumed that carbohydrate refeeding after starvation promotes the synchronous dephosphorylation of these enzymes resulting in the coordinate activation of glycogen synthesis and suppression of gluconeogenesis. The concept of the indirect pathway challenges this view since it implies that the activation of glycogen synthesis must precede the suppression of gluconeogenic flux. Consistent with this presumed mechanism, there is a marked lag before hepatic fructose 2,6-bisphosphate concentrations begin to rise in response to carbohydrate refeeding, at least in response to glucose or chow refeeding. Research in this laboratory has explored the hypothesis that protein dephosphorylation is an asynchronous process. Studies with isolated hepatocytes prelabelled with $^{32}PO_4^{3-}$ confirm that the dephosphorylation of hepatic phosphoproteins is a complex asynchronous process during the recovery phase from acute glucagon challenge.

A second unresolved question posed by the concept of the indirect pathway of glycogenesis relates to the site at which dietary glucose is converted to lactate. Although a number of tissues may contribute to this process (viz. intestine, erythrocytes, skin), skeletal muscle is recognised to be the major site of insulin-stimulated glucose disposal in vivo. The implication is that skeletal muscle is the primary site of glucose-to-lactate conversion during the starved-to-fed transition.

III. THE STARVED-TO-FED TRANSITION IN SKELETAL MUSCLE

A major fate of dietary glucose during the starved-to-fed transition is the repletion of glycogen stores in skeletal muscles, the levels of which are progressively and severely depleted in response to starvation. Glycogen synthesis in skeletal muscle is predominantly, if not exclusively, initiated by the phosphorylation of glucose via hexokinase and involves the pathway glucose → glucose 6-phosphate → glucose 1-phosphate → UDPglucose → glycogen. A comparison of rates of muscle glycogen deposition during the initial phase of the starved-to-

fed transition with rates of glucose uptake and phosphorylation indicates that a surprisingly high proportion of the glucose that enters the muscle cell is channelled into glycogen synthesis, at least in certain skeletal muscles (Sugden et al. 1993). One question posed by this finding concerns the energetics of glycogenesis. Glycogenesis is a energy-consuming process, two moles ATP being consumed per glucosyl unit incorporated into glycogen. Glycolysis, which produces 2 moles ATP per mole glucose converted to lactate, is unlikely to have the capacity to support the high rates of glycogen synthesis seen in vivo in response to carbohydrate refeeding. This conclusion poses the question of whether a proportion of the glucose-derived pyruvate must be oxidised via pyruvate dehydrogenase (PDH) and the tricarboxylic acid (TCA) cycle to generate additional ATP. Studies by Sugden and colleagues (see Sugden et al. 1993) indicate that muscle PDH is refractory to rapid reactivation during the starved-to-fed transition, the complete reactivation (by dephosphorylation) of the enzyme not being achieved until at least 4-6 hours. The implication is that glucose oxidation via PDH may not generate sufficient ATP to support glycogen biogenesis in muscle, at least during the initial phase of the staved-to-fed transition. The delay in the reactivation of PDH, however, may provide a mechanism to divert glycolytically-derived lactate to the liver to act as a glycogenic precursor via the indirect pathway.

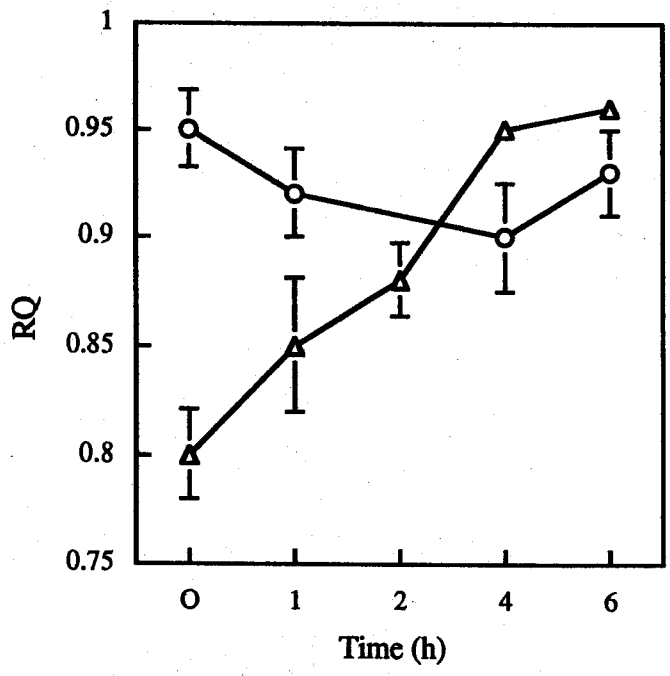

Figure 1. The response of respiratory quotient (RQ) to chow refeeding in 40 h-starved rats (s). For comparative purposes, changes in RQ values are shown for rats that were fed ad libitum on chow both before (Δ) and during the experimental period (o). Results are means ± S.E.M. for at least four rats.

The conclusion that glycolysis and glucose oxidation via PDH lack the capacity to meet the energetic requirements of glycogen synthesis in skeletal muscle raises the question of what other fuel(s) could support this process. Although muscle has the capacity to oxidise fatty acids, levels of plasma fatty acids (derived from triacylglycerol lipolysis in adipose tissue) decline rapidly in response to carbohydrate refeeding (Holness et al. 1988). This is commonly interpreted to imply that dietary carbohydrate consumption leads to the rapid suppression of fatty acid oxidation in muscle. This conclusion ignores the fact that skeletal muscle, like

adipose tissue, contains significant amounts of endogenous triglycerides located both within the cells and in the associated vasculature (Oscai et al. 1990). During muscular contraction, approximately 50% of the fat oxidised in muscle arises from intramuscular triglyceride stores (Oscai et al., 1990). Thus, endogenous triglycerides are major fuels in oxidative muscles, at least during exercise, and have the capacity to solely support muscle contraction in the absence of other exogenous energy substrates. The obvious question is whether endogenous fatty acid oxidation has a role during the starved-to-fed transition. To address this question, we examined changes in the respiratory quotient (RQ) of 40 h-starved rats in response to chow refeeding (Figure 1), the chow containing 55% digestible carbohydrate, 19% protein, 5% lipid and 21% non-digestible residue, by weight. In the starved state the RQ was 0.80±0.02 consistent with lipid and protein being the major oxidative fuels. The response of RQ to chow refeeding was progressive rather than abrupt, the value reaching a steady-state value of approximately 0.95 only after four hours. These results strongly suggest that carbohydrate refeeding after starvation is associated with a progressive as opposed to abrupt transition from lipid-based to carbohydrate-based oxidative metabolism. The fact that plasma fatty acid levels decline rapidly in response to carbohydrate refeeding implies that this lipid-based metabolism must involve endogenous stores in tissues, including those in skeletal muscle.

The data on RQ during the starved-to-fed transition need to be analysed in the context of the glucose/fatty acid cycle. The body uses two primary fuels in energy generation. These are glucose and lipid-derived fuels (fatty acids and ketone bodies). There is a long-established reciprocal relationship between the blood concentrations of glucose and lipid-derived fuels. This relationship (the glucose/fatty acid cycle: Randle et al. 1964) is commonly accepted as implying that lipid-derived fuels are preferentially oxidised in muscle and that their oxidation leads to the inhibition of glucose utilization and pyruvate oxidation. This inhibition may be mediated via changes in the concentrations of key regulatory metabolites, including acetyl-CoA, citrate, fructose 6-phosphate, fructose 1, 6-bisphosphate and glucose 6-phosphate, that result in the co-ordinate inhibition of the pyruvate dehydrogenase (PDH) complex, 6-phosphofructo 1-kinase (PFK-1), and hexokinase. The genesis of many of the metabolic abnormalities associated with non-insulin dependent diabetes and related disorders may be mediated via alterations in fatty acid oxidation in skeletal muscle.

The glucose/fatty acid cycle has been proposed as a major determinant of the rate of glucose utilisation in skeletal muscles and, hence, of whole-body insulin sensitivity. Although there is now irrefutable evidence in support of the operation of the glucose/fatty acid cycle in cardiac muscle in certain physiological and pathophysiological states, there have been difficulties in establishing the operation of the cycle in isolated skeletal muscles. These difficulties have cast serious doubts on the operation of the cycle in vivo. Because skeletal muscle accounts for approximately 40% of total body mass, it is imperative that the glucose/fatty acid cycle operates in skeletal muscle if it is to influence whole-body glucose utilisation. There is overwhelming evidence of the operation of glucose/fatty acid cycle in rats in vivo during progressive starvation, during exercise, and after high fat-feeding and in man. The fact that skeletal muscle contains significant amounts of endogenous triacylglycerols (Oscai et al. 1990) raises the possibility that intramuscular lipolysis and in situ fatty acid oxidation may be a major factor determining glucose utilization in skeletal muscle. As a corollary, exogenous fatty acids may not be a prerequisite for the operation of the glucose/fatty acid cycle in skeletal muscle. Indeed, if the rate of endogenous fatty acid oxidation were to be high, glucose utilization may be refractory to further inhibition by exogenous fatty acids. In support of this hypothesis, recent research in this laboratory has established that 2-bromopalmitate (2-BP), a potent inhibitor in vitro and in vivo of fatty acid oxidation via the inhibition of carnitine palmitoyltransferase I, causes substantial changes in muscle carbohydrate metabolism consistent with its suppression of in situ fatty acid oxidation. Thus, 2-BP, in the absence of exogenous fatty acid, promotes glycogenolysis, inhibits net glycogen synthesis and stimulates in isolated skeletal muscles. These effects of 2-BP may explain why exogenous fatty acids, contrary to the predictions of the glucose/fatty acid cycle (Randle et al. 1964), do not consistently inhibit glucose utilisation and pyruvate oxidation in isolated skeletal muscles. Our research and a related study by Li et al. (1993) suggest that endogenous intramuscular triglycerides hold the

key to this controversy. These studies indicate that the rate of endogenous fatty acid oxidation is so high in isolated muscle preparations in the absence of 2-BP that glucose utilisation is maximally inhibited and is therefore refractory to further inhibition by exogenous fatty acids.

The progressive transition from lipid-based to carbohydrate-based oxidative metabolism following carbohydrate refeeding after prolonged starvation suggests that the glucose/fatty acid cycle hypothesis (Randle et al. 1964) needs to be refined to incorporate the situation that applies during the starved-to-fed transition. We propose that there are two elements to the glucose/fatty acid cycle, one extramuscular and the other intramuscular (Figure 2). The extramuscular glucose-fatty acid cycle is the cycle originally described by Randle et al. (1964) and focuses on the inhibition in starvation of glucose utilisation and oxidation in skeletal muscle by adipose tissue-derived fatty acids and ketone bodies. The intramuscular glucose/fatty acid cycle, by describes the situation during the starved-to-fed transition when lipolysis of endogenous contrast, triacylglyerol stores in muscle provides the fatty acids, the in situ oxidation of which acts to inhibit glucose oxidation and channel glucose into glycogen synthesis.

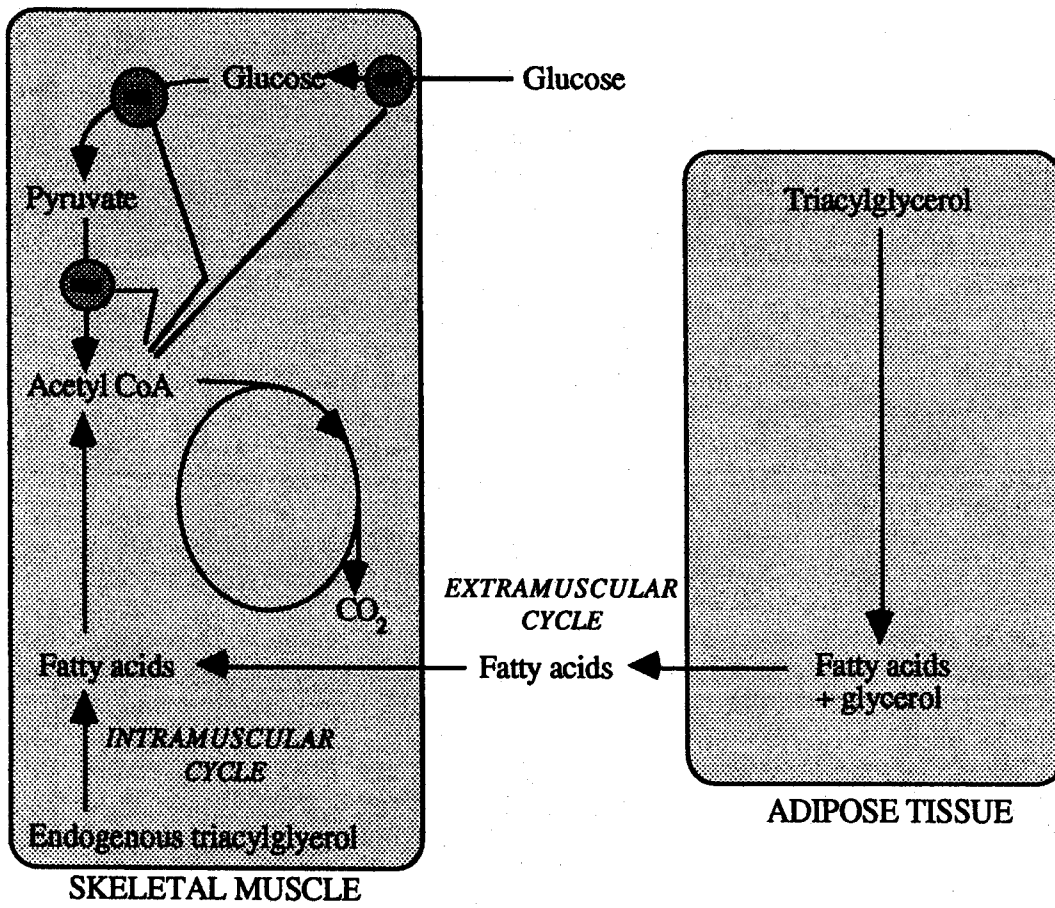

Figure 2. The extra- and intramuscular elements of the glucose/fatty acid cycle. The cycle denotes that acetyl CoA and other products of fatty acid oxidation suppress glucose utilisation via inhibitory effects on glucose uptake and phosphorylation, glycolysis and pyruvate oxidation.

IV. THE PATHWAY OF GLYCOGENESIS

No review of the starved-to-fed would be complete without mention of recent discoveries in relation to the pathway of glycogenesis. Many textbooks and reviews still describe glycogen synthesis from UDPglucose a one-step process in which a glycogen molecule is extended at its non-reducing chain ends via the combined actions of glycogen synthase and branching enzyme. This view now needs to be been revised. Recent research indicates that glycogenesis is a complex, multi-step pathway involving multiple synthases (Figure 3). Glycogen is now recognised to be synthesised on a protein backbone, the initial step in synthesis being the glucosylation of the 37 kDa glycogenin primer molecule via the creation of a novel glucosyl-1-*O*-tyrosyl linkage at tyrosine 194 (Smythe and Cohen 1991). Glycogenin functions not only as a protein backbone in this reaction but also acts autocatalytically to promote its own glucosylation. The glucosyltransferase catalyses the successive addition of approximately 8 glucosyl residues to the glycogenin backbone.

Recent research by Whelan and coworkers (see Lomako et al. 1993) has identified a stable polysaccharide intermediate, termed proglycogen, in the biosynthesis of glycogen. This 400 kDa intermediate contains glycogenin (10% by weight) and is insoluble in 10% trichloroacetic acid (TCA). The idea that glycogen exists in two forms, one TCA-soluble and the other TCA-insoluble, is not new but was proposed many years ago (see Stetten and Stetten 1960). Proglycogen is synthesised from the malto-octosyl derivative of glycogenin via the action of a specific glucosyltransferase, termed proglycogen synthase. The final step in the biogenesis of muscle glycogen is the conversion of proglycogen into macromolecular form of glycogen (Mr 10^7) (Lomako et al. 1993). This reaction is catalysed by the classical form of glycogen synthase which is regulated by multisite phosphorylation at 10 or more sites.

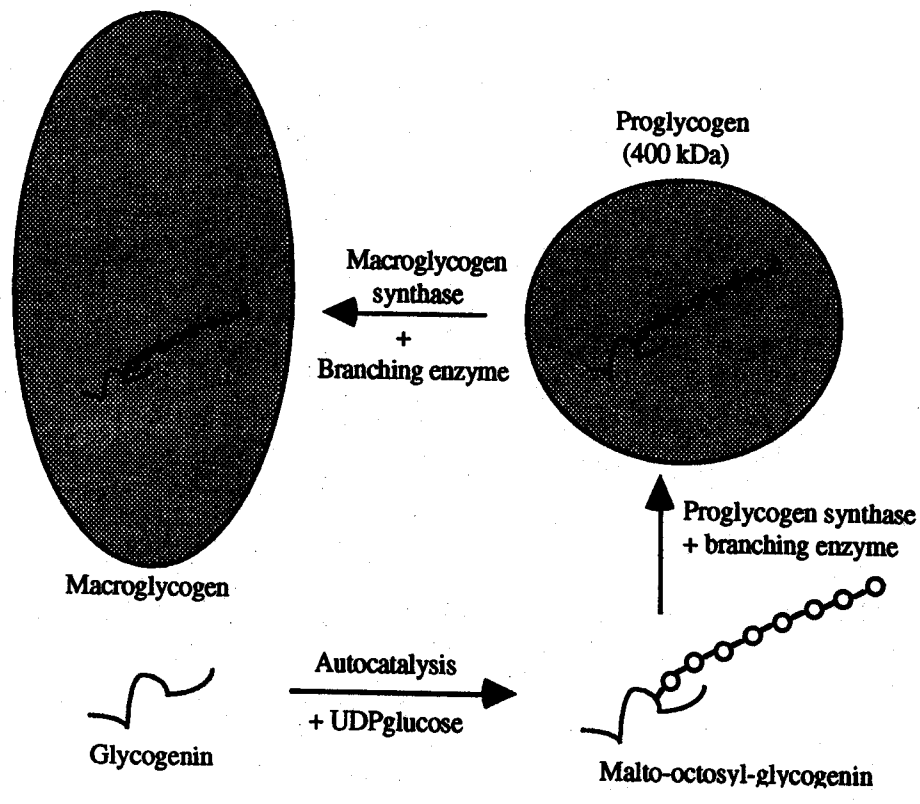

Figure 3. The newly-discovered glycogenin-proglycogen pathway of glycogen biogenesis.

What remains to be established are the physiological and pathophysiological implications of this newly-discovered pathway of glycogen biogenesis. Starvation is associated with the conversion of pro- to macroglycogen. An outstanding question is the extent to which modulation of the three component UDPglucose-dependent synthases of the pathway (viz. glycogenin, proglycogen synthase, macroglycogen synthase) contribute to the regulation of glycogen synthesis in vivo.

V. THE STARVED-TO-FED TRANSITION: MEDICAL PERSPECTIVES

This review has focussed on the advances that have occurred over the last decade in our understanding of the starved-to-fed transition. What are the implications of these advances in knowledge for our understanding of the genesis of metabolic diseases? Insulin resistance is a defect common to a broad spectrum of lifestyle-related metabolic disorders, including glucose intolerance, non-insulin-dependent diabetes mellitus (NIDDM), obesity, hypertension, dyslipidaemia, and atherosclerotic cardiovascular disease. It is now recognised that these insulin-resistant states are associated with a defect(s) in glycogen synthesis in skeletal muscle (DeFronzo et al. 1992; Felber et al. 1993). Results from this laboratory indicate that alcohol abuse may cause a similar type of defect (Xu et al. 1992). A new hypothesis proposes that the defect in net glycogen synthesis may be mediated via inhibitory effects of fatty acid oxidation on net glycogen synthesis in skeletal muscle (Felber et al. 1993), implying a major role for intramuscular triacylglyerol stores in the genesis of insulin resistance.

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