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DIETARY REGULATION OF EXPRESSION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE

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■ **Abstract** The family of enzymes involved in lipogenesis is a model system for understanding how a cell adapts to dietary energy in the form of carbohydrate versus energy in the form of triacylglycerol. Glucose-6-phosphate dehydrogenase (G6PD) is unique in this group of enzymes in that it participates in multiple metabolic pathways: reductive biosynthesis, including lipogenesis; protection from oxidative stress; and cellular growth. G6PD activity is enhanced by dietary carbohydrates and is inhibited by dietary polyunsaturated fats. These changes in G6PD activity are a consequence of changes in the expression of the G6PD gene. Nutrients can regulate the expression of genes at both transcriptional and posttranscriptional steps. Most lipogenic enzymes undergo large changes in the rate of gene transcription in response to dietary changes; however, G6PD is regulated at a step subsequent to transcription. This step is involved in the rate of synthesis of the mature mRNA in the nucleus, specifically regulation of the efficiency of splicing of the nascent G6PD transcript. Understanding the mechanisms by which nutrients alter nuclear posttranscriptional events will help uncover new information on the breadth of mechanisms involved in gene regulation.

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INTRODUCTION

Glucose-6-phosphate dehydrogenase (G6PD) activity is needed in all cell types for the production of NADPH and for control of carbon flow through the pentose phosphate pathway. G6PD catalyzes the first reaction of this pathway, oxidizing glucose-6-phosphate to 6-phosphogluconolactone and in the process reducing $NADP^+$ to NADPH + H^+ (Figure 1). This reaction is the rate-determining step of the oxidative portion of the pentose phosphate pathway. Together with 6-phosphogluconate dehydrogenase, these enzymes provide NADPH for reductive biosynthetic reactions, such as fatty acid, cholesterol, and amino acid synthesis, and for maintenance of reduced glutathione concentrations. In addition, it regulates the rate of glucose conversion to ribose-5-phosphate, the precursor for nucleotide biosynthesis.

The G6PD gene spans 18 kb on the X chromosome (xq 28) and contains 13 exons. The G6PD promoter is embedded in a CpG island that is conserved from mice to humans (50, 85). The promoter of the G6PD gene contains a TATA-like

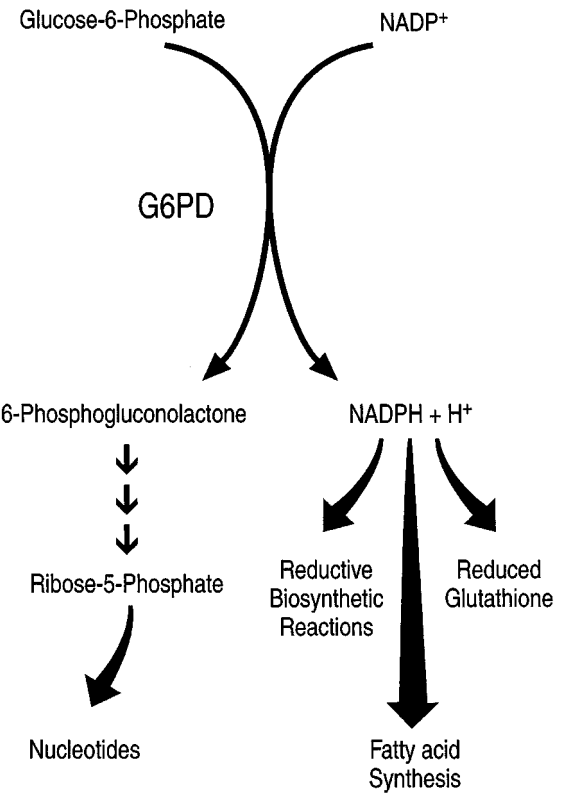

Figure 1 Role of glucose-6-phosphate dehydrogenase in cellular metabolism.

sequence, TTAAAT, and numerous stimulatory protein 1 (Sp1) elements, but no CAAT element (50, 65, 67, 85). S1 nuclease and primer extension analyses of mouse G6PD mRNA indicate that the transcriptional start site used in liver and adipose tissue, in which lipogenesis is regulated, is the same as in kidney, in which G6PD is expressed constituitively (37). These results indicate that the 5′-end of the mRNA is the same in all cell types. Three DNase I hypersensitive sites have been localized in the 5′-end of the G6PD gene. Hss-1 (−1000 bp) and Hss-2 (−400 bp) are present in all tissues, but Hss-3, which is located in intron 2 (+1500 bp), is liver specific (37). The translation start site is located in exon 2 and has been mapped in rats, mice, and humans (19, 36, 50, 79). The number of exons and introns and the size and sequence of the exons are conserved in higher eukaryotes. The sequence similarity between the human G6PD cDNA and that of mice or rats is 87%. The similarity between the mouse and rat cDNA sequences is even greater (93% identity). Most of the dissimilarity is in the 3′-untranslated region (3′-UTR). Exon 13 is at least 800 nucleotides long and contains the translation stop codon. The 3′-UTR is approximately 600 nucleotides long and contains a single poly(A) site (LP Stabile & LM Salati, unpublished data). The structure of the gene is unusual in that the second intron is 11 kb and accounts for almost half of the gene; the large size of this intron is also conserved between humans, rats, and mice.

The amount of G6PD activity and mRNA abundance differs between tissues (27, 37) and most likely reflects tissue-specific differences in growth rate, reductive biosynthetic reactions, and oxidative stress. Moreover, G6PD expression is regulated by hormonal and nutritional factors in only a few tissues. G6PD expression is regulated in liver and adipose tissue, and its activity correlates with the rate of fatty acid biosynthesis. It is also induced in lactating mammary glands by dietary carbohydrate (31). The majority of the research on the nutritional regulation of G6PD expression has been in liver; thus, this review predominantly covers regulation of G6PD in liver tissue.

CELLULAR ROLES OF G6PD

G6PD: A Lipogenic Enzyme

The family of lipogenic enzymes includes fatty acid synthase, acetyl-coenzyme A (CoA) carboxylase, ATP-citrate lyase, malic enzyme, and G6PD; these enzymes catalyze reactions for the de novo synthesis of fatty acids. G6PD is considered a lipogenic enzyme because it provides a substrate, NADPH, for production of palmitate by fatty acid synthase. NADPH is also produced by the malic enzyme reaction using malate supplied by the ATP-citrate lyase and malate dehydrogenase reactions and reducing equivalents from cytosolic NADH. The relative contribution of these two pathways, as sources of NADPH for the fatty acid synthase reaction, varies with species. In rodents, and presumable other mammals, the contribution of each pathway is equivalent (18, 42, 68). In contrast, in avians, most of the NADPH is provided by the malic enzyme reaction, and flux through the oxidative reactions

of the pentose phosphate pathway is very low (29, 61). The redundancy of the NADPH supply protects the animal from mutations that would influence this supply. In this regard, mice homozygous for a null mutation of malic enzyme are viable and have normal lipid stores (48). In humans, G6PD deficiency, also called favaism, causes disease, with varying levels of severity, but the deleterious symptoms are a consequence of reduced glutathione depletion, not attenuated lipogenesis. Yet the importance of G6PD as a source of NADPH for fatty acid synthesis is highlighted by the decrease in lipogenic rate and in serum lipoprotein concentrations in individuals with G6PD deficiency (17, 57).

The activities of the lipogenic enzymes, including G6PD, change in parallel with lipogenic rates in cells. Their activities increase coordinately to achieve high rates of lipogenesis when substrate is in excess, such as after a high-carbohydrate meal, and their activities are inhibited when substrate is limiting, such as during starvation. The humoral factors signaling these changes effect the expression of all members of this enzyme family, yet the details of the intracellular mechanisms resulting in the changes in enzyme activity differ greatly between enzymes. The lipogenic enzymes are all regulated at a pretranslational step (34). In most cases, these genes undergo large changes in the rate of transcription. In contrast, G6PD is regulated posttranscriptionally. Acetyl-CoA carboxylase is unique in the lipogenic family in that it also undergoes both allosteric and covalent modifications, resulting in short-term changes in its activity in the absence of changes in the amount of enzyme protein.

G6PD and Oxidative Stress

Physiological conditions resulting in oxidative stress can also result in changes in G6PD activity. NADPH is required for detoxification of free radicals and peroxides. G6PD gene expression is essential for protection of the cell against even mild oxidative stress (6, 62). It provides the only means of generating NADPH to maintain reduced glutathione levels in mature erythrocytes, and in its absence, the erythrocytes are particularly vulnerable to hemolysis. Induction of G6PD expression due to oxidative stress has been observed in many cell types (71, 87). G6PD expression is increased along with other antioxidant enzymes when rat alveolar type II cells are exposed to oxidative stress (44). Incubation of rat hepatocytes with acetaldehyde or ethanol also increases G6PD activity and mRNA abundance (43, 76, 78). Moreover, maintenance of high levels of G6PD activity protects both neuronal cells and fibroblasts from hydrogen peroxide–induced cell death (81). In contrast, a high glucose concentration in the medium of endothelial cells results in a decrease in G6PD activity and cellular death (97). This adverse regulation of G6PD is thought to be involved in the pathogenesis of diabetes mellitus. The complete inactivation of the G6PD gene results in extreme sensitivity to oxidative stress in cells in culture (62). In humans, G6PD deficiency is not extreme; clinical manifestations occur primarily when an exogenous stress, such as an oxidative agent or a viral disease, is present. Of the more than 300 reported mutations in the G6PD gene, most are point mutations; there are no large deletions or insertions (88). Thus, a null mutant for G6PD is most certainly lethal to an animal.

G6PD and Cellular Growth

Changes in G6PD activity occur in parallel with changes in the rate of cell growth. This may stem in part from the role of the pentose phosphate pathway in providing the ribose-5-phosphate needed for nucleotide biosynthesis, and in part by the role G6PD plays in regulating redox state of the cell. Similar to the regulation of G6PD activity by oxidative stress, the coincident changes in both cell growth and G6PD activity have been observed in multiple cell types. G6PD activity is elevated in proliferating nodules and tumors (27, 47). Growth stimulation by factors such as serum, epidermal growth factor (EGF), and platelet-derived growth factor increase both G6PD activity and DNA synthesis in fibroblast and epithelial cell lines (75, 82). Stimulation of hepatocyte growth by EGF also results in parallel changes in G6PD activity and DNA synthesis (59, 94). Within the same cells, incubation with EGF resulted in a decrease in malic enzyme expression, which suggests that EGF was enhancing G6PD activity to provide ribsose-5-phosphate and NADPH to support cellular growth and not lipogenesis. The mechanisms resulting in this increase in G6PD activity have not been fully examined. Although nutritional regulation of G6PD is not thought to involve allosterism or phosphorylation, G6PD regulation by growth factors may involve the release and thereby activation of G6PD enzyme from intracellular membranes (75). The details of this mechanism will most likely prove to be quite complex. Investigators examining G6PD regulation by hormonal and nutrient factors in cells in culture must bear in mind that some changes in G6PD mRNA or enzyme activity may be a consequence of changing cellular growth rates.

REGULATION OF G6PD BY NUTRITIONAL AND HORMONAL STATUS

The purpose of regulating G6PD activity by nutritional and hormonal factors is to provide the cell with the capacity to synthesize fatty acids. Thus, this regulation of G6PD is only observed in tissues with high lipogenic capacity, such as liver and adipose tissue. Identification of relevant humoral factors requires model systems using both intact animals and cells in culture. The latter has been particularly challenging because regulation of G6PD expression by nutrients and hormones is lost in most immortalized cell lines. Thus, primary hepatocytes in monolayer cultures have provided the primary tool for these analyses.

Nutritional Status

Starvation and Refeeding Paradigm Hepatic G6PD expression has been studied frequently as a model of adaptive regulation by diet. Long-term starvation decreases rat liver G6PD activity (40, 51). Refeeding with a fat-free, high-carbohydrate diet increases the activity of G6PD to an amount greater than that of the "normal" fed state (4, 66). This phenomenon has been termed enzyme overshoot (4). The molecular basis for overshoot is not well understood but appears to require

insulin, glucocorticoids, and a high-carbohydrate, low-fat diet. The starvation/refeeding paradigm remains a useful tool for studying metabolic regulation because these dietary changes are unambiguous and result in large changes in enzyme activity.

Type and Amount of Dietary Carbohydrate Consumption of a high-carbohydrate, fat-free diet causes the largest increase in G6PD activity of any diet. The greatest change in G6PD activity is observed when rats are fed diets containing glucose or fructose, compared with starch, and stimulation by fructose is greater than by glucose (20, 40). Glucose and fructose increase hepatic G6PD activity in a manner apparently independent of hormones. In this regard, hepatic G6PD activity can be increased in diabetic rats by feeding them fructose instead of glucose (23). Furthermore, increasing the concentration of glucose in the medium of primary hepatocytes in culture from 0 to 25 mM increases G6PD activity in the absence of hormones (43, 70); however, these effects are not uniformly observed by all researchers (46, 49). Induction by monosaccharides has been described for other enzymes, such as malic enzyme, S_{14} (spot 14, a putative lipogenic protein of unknown identity), and pyruvate kinase (reviewed in 26, 86). The factor causing the carbohydrate stimulation is thought to be an intermediate in the metabolism of glucose or fructose (26, and the references therein). Variations between laboratories with respect to the effect of glucose may reflect differences in the content of gluconeogenic amino acids in the culture medium resulting in sufficient accumulation of this metabolic intermediate even at low glucose concentrations. Alternatively, the effect of glucose on G6PD expression may be indirect and reflect changes in the growth rate or redox state of the cells.

Type and Amount of Dietary Fat In contrast to the stimulatory effect of carbohydrates, polyunsaturated fatty acids inhibit G6PD both in intact animals and in primary hepatocytes in culture (11, 12, 33, 41, 70, 73, 74). The inhibition of G6PD activity by polyunsaturated fat occurs both when rats are switched from a high-carbohydrate, fat-free diet to one containing polyunsaturated fat (11) and when starved rats are refed a high-carbohydrate diet supplemented with polyunsaturated fat (3). Several lines of evidence indicate that the inhibition of G6PD activity is due specifically to the polyunsaturated fat content of the diet. First, in the presence of a similar carbohydrate intake between rats with and without fat added to the diet, the addition of polyunsaturated fatty acids decreases the activity of G6PD (12). Addition to the diet of saturated fatty acids, such as palmitate (16:0) and stearate (18:0), and of monounsaturated fatty acids, such as oleate (18:1), do not inhibit G6PD activity (11, 12). Furthermore, this indicates that the inhibition of G6PD activity is not merely a consequence of a decrease in carbohydrate intake. Second, inhibition of G6PD activity is not a consequence of reversing essential fatty acid deficiency in the animals because adding additional polyunsaturated fat to a diet adequate in essential fatty acids further inhibits G6PD activity (11). Third, the inhibition by polyunsaturated fat is not a consequence of changing the

protein-to-calorie ratio of the animals' diet. In rats, 20% additional dietary energy from safflower oil inhibits the activity of G6PD significantly better than does 20% additional energy from beef tallow (10, 33). Finally, the decrease in G6PD enzyme activity is also observed in primary rat hepatocytes incubated with arachidonate (20:4 n-6) and eicosapentaenoate (20:5 n-3) (70, 74). The inhibition of G6PD gene expression in primary rat hepatocytes is likewise caused by polyunsaturated fatty acids and not saturated or monounsaturated fatty acids, which is similar to results observed in intact animals (74). G6PD expression is highly sensitive to the quantity of polyunsaturated fat in the diet. A significant decrease in enzyme activity in rats is observed with the addition (by weight to a glucose-based diet) of as little as 2.5% safflower oil (11); in mice, addition of 5% corn oil (by weight to a glucose-based diet) maximally inhibits G6PD activity (33). Curiously, addition of 10% corn oil (by weight) is needed to maximally inhibit G6PD activity in mice fed a fructose-based diet (33). The inhibitory effect of polyunsaturated fat is unique to G6PD regulation in the liver. Regulation of G6PD in adipose tissue is not affected by the presence of polyunsaturated fat in the diet (12). What remains elusive with respect to G6PD is the signal transduction pathway by which the cell responds to changes in the polyunsaturated fat content of the diet.

Hormonal Status

Role of Hormones in the Response to Nutritional Status The effects of starvation and refeeding suggest a role for insulin and glucagon in the regulation of G6PD (51). Rats treated with streptozotocin, which destroys the pancreatic β-cells, thereby stopping insulin production, fail to induce G6PD activity on refeeding (4). G6PD activity in both the liver and adipose tissue of diabetic rats is similar to the activity in starved animals, and treatment with insulin restores G6PD activity to normal levels, implicating insulin as an important signal of the refed state (5, 25, 28). Glucagon or cAMP have an opposing effect on G6PD activity. Injection of rats with glucagon prevents the induction of hepatic G6PD activity during refeeding and does so by decreasing the rate of enzyme synthesis (24). Because glucagon levels rise during starvation, this hormone is implicated as a primary signal of the starved state.

Hormones from the thyroid and adrenal glands also regulate G6PD activity. Thyroidectomy decreases G6PD expression, and treatment with thyroid hormone (T3) increases G6PD expression through a mechanism that does not involve an increase in G6PD mRNA (21). In contrast, the increase in G6PD activity during the transition from normal thyroid status to hyperthyroidism is accompanied by a parallel increase in enzyme synthesis and mRNA abundance (52). A high-sucrose diet further augments the increase in G6PD activity and mRNA abundance due to hyperthyroidism (52). Adrenalectomy attenuates the large increase in G6PD activity caused by refeeding rats, and the refeeding-induced increase is restored by glucocorticoid administration (4). These effects on activity are the result of changes in the rate of enzyme synthesis (80). Thus, the ability of a fat-free,

high-carbohydrate diet to increase the activity of G6PD probably requires a minimum thyroid and adrenal status.

G6PD regulation by hormonal factors is observed in primary rat hepatocytes in culture. The induction of G6PD activity and mRNA level in response to carbohydrate and insulin in hepatocyte cultures mimics the response to refeeding in intact animals. Insulin induces G6PD activity in primary rat hepatocytes in culture (46, 70, 74, 95). The insulin-induced increase in G6PD activity is accompanied by parallel changes in the rate of enzyme protein synthesis and G6PD mRNA abundance (49, 74). Furthermore, inhibitors that block signal transduction by insulin also block its stimulatory effect on G6PD expression (89). Because insulin will also increase the metabolism of glucose in hepatocytes, it is difficult at this point to determine whether insulin acts directly on the expression of G6PD or whether its effect is indirect and via enhanced glucose metabolism. Glucocorticoids also have been shown to be positive regulators of G6PD activity in rat hepatocytes. Glucocorticoids and insulin both stimulate G6PD mRNA accumulation and in an additive manner, but the molecular mechanism of this regulation has not been defined (77). Curiously, glucocorticoids block the inhibition of G6PD expression by linoleate (70).

This interaction between glucocorticoid and fatty acid action may reflect an inhibition of fatty acid metabolism by glucocorticoids, thereby blocking the production of the active metabolite required to inhibit G6PD expression. In contrast, T3 and glucagon, both of which change G6PD activity in intact animals, have no effect on G6PD activity in cultured hepatocytes (58, 95). Therefore, in intact animals, the effect of T3 and glucagon may be indirect, possibly by altering circulating free fatty acid concentrations.

MECHANISMS REGULATING EXPRESSION OF G6PD

Translational/Posttranslational Mechanisms

The activity of any enzyme can be regulated at many steps. Modifications can occur that alter the catalytic efficiency of the enzyme and/or change the amount of enzyme present in the cell. The generally excepted dogma is that G6PD activity does not undergo allosteric or covalent modifications in response to nutritional modifications. Early reports have argued for an irreversible inactivation of G6PD enzyme. Palmitoyl-CoA, in vitro, has been shown to covalently bind to and inactivate G6PD and to reduce its apparent level when measured with antibodies (14–16). Furthermore, G6PD activity can be stimulated by growth factors and oxidant stress by a mechanism involving release of bound enzyme from intracellular membranes (75, 83). Most recently, Zhang et al (97) have observed phosphorylation of G6PD and a coincident decrease in G6PD activity in endothelial cells incubated in medium with high glucose concentrations. This phosphorylation is mediated via cAMP and protein kinase A. Changes in amount of G6PD by any of these mechanisms have not been observed during dietary manipulations. As is

discussed below, regulation of G6PD by nutritional status can largely be accounted for by changes in its synthesis, and thus, these short-term regulatory mechanisms have not been thought relevant to this type of metabolic control. Yet a change in the catalytic efficiency of G6PD has the potential to provide temporal regulation of cellular G6PD activity.

The effect of nutrients on the activity, the amount of enzyme protein, and the relative synthesis and degradation of G6PD have been widely studied. Using antibodies against G6PD protein and liver supernatants from rats fed a high-carbohydrate diet, the increase in G6PD enzyme activity was shown to parallel the increase in the amount of G6PD protein (63, 90). Likewise, starvation or the consumption of dietary lipids decreases the amount of G6PD protein (30, 63, 91). When examined in a variety of nutritional and hormonal conditions in both liver and adipose tissue, changes in G6PD activity can be accounted for by changes in the rate of enzyme synthesis (24, 25, 30, 52, 55, 63, 69, 84, 90, 92). For example, a 13-fold increase was observed in the relative rate of synthesis of G6PD and in G6PD enzyme activity in the livers of rats switched from a chow to a high-sucrose diet (52). Similarly, the rate of G6PD protein synthesis during dietary fat consumption is decreased 96%, coincident with a 91% inhibition of G6PD enzyme activity (91). Changes in the rate of degradation due to hormonal or nutritional modifications are more controversial. Consumption of a high-fat diet has been reported to decrease the half-life of the enzyme from 16 h to 6 h in rat liver (64). However, in other reports, dietary fat had no effect on the rate of enzyme disappearance (30). Multiple procedural differences could explain these conflicting results. Nonetheless, changes in enzyme synthesis can account for the changes in enzyme activity during nutritional manipulations, whereas any changes in the rate of enzyme protein turnover would serve to enhance the rapidity by which the cell can alter the amount of G6PD activity.

Pretranslational/Posttranscriptional Mechanisms

Changes observed in the rate of G6PD protein synthesis due to nutritional or hormonal factors are accompanied by similar changes in the amount of mature mRNA (38, 45, 52, 66, 73, 77). Such changes have been observed in intact animals in response to (*a*) changes in type of dietary carbohydrate (52, 53), (*b*) fasting and refeeding (45, 66), (*c*) changes in dietary polyunsaturated fat (73, 84), and (*d*) hyperthyroidism (52, 53); in rat hepatocytes in primary culture, such changes have been observed in response to insulin (49, 74, 77), glucocorticoid (49, 77), and polyunsaturated fatty acids (74). The rate of change in G6PD mRNA accumulation varies with diet. Refeeding mice or rats that had been previously starved results in a lag of 12 h or more before an increase in G6PD mRNA is detectable, and the maximal increase is observed 24 h into refeeding (66, 73). This lag is only observed with the starvation/refeeding paradigm. G6PD mRNA increases up to sevenfold during the diurnal cycle (22, 38). The increase occurs during the eating cycle (dark cycle) and is maximal 8 h into it. The increase in mRNA amount is a consequence

of diet rather than diurnal cues because presenting the diet at later times in the dark cycle delays the increase in G6PD mRNA accumulation (LP Stabile & LM Salati, unpublished data). Dietary polyunsaturated fat results in an 80% inhibition in G6PD activity accompanied by a parallel decrease in mRNA accumulation (73, 84). In mice, the decrease in G6PD mRNA abundance is observed within 4 h of polyunsaturated fat consumption and maximal inhibition occurs within 9 h (73). These effects are quite rapid considering the relatively slow rate of triacylglycerol absorption from the gastrointestinal tract. A similar time course has been observed in primary rat hepatocytes. In these experiments, incubation with arachidonic acid for 2 h resulted in a 14% decrease in the amount of G6PD mRNA relative to cells incubated with only insulin, and the maximum inhibition of 80% was observed by 8 h (73). The rapid effect of both dietary fat and fatty acids in culture suggests that the action of polyunsaturated fat on gene expression occurs via a protein(s) already present in the liver. In contrast, the lag in accumulation of G6PD mRNA during refeeding is consistent with a requirement for the synthesis of an intermediary protein involved in the induction. Despite these hypothesized differences in the intracellular details, these results are consistent with regulation at a pretranslational step.

Pretranslational regulation can be due to changes in transcriptional activity of the gene or to posttranscriptional regulation, such as mRNA stability, the processing of the pre-mRNA (including splicing and polyadenylation of the pre-mRNA), and nucleocytoplasmic transport. The transcriptional activity of the G6PD gene has been measured using nuclear run-on assays. The rate of G6PD transcription is not regulated by starvation, refeeding with a high-carbohydrate diet, or the inclusion of polyunsaturated fat in the diet (73). Similar results have been obtained for G6PD regulation by insulin, glucose, and arachidonic acid in rat hepatocytes in primary culture (74). Furthermore, the transcriptional activity of the G6PD gene occurs at a very low rate compared with constituitively expressed genes, such as β-actin and glyceraldehyde-3-phosphate dehydrogenase. The rate of G6PD transcription is as low as the transcriptional rate of the fatty acid synthase or stearoyl-CoA desaturase genes measured during starvation. Although the transcriptional activity of these genes increases 30-fold or more during refeeding, G6PD gene transcription remains at the same low level despite 27- to 30-fold increases in G6PD mRNA (73). In these experiments, multiple controls were used to make sure the measurements of transcriptional activity were valid (73). These included the use of probes to both the 5′ and 3′ ends of the gene, as well as single-stranded probes that would only hybridize to G6PD RNA, and not transcripts produced off the opposite strand. All probes were free of repetitive elements that could increase the background hybridization. Together, these results indicate that regulation of G6PD gene expression by nutritional and hormonal factors occurs at a posttranscriptional step.

Posttranscriptional regulation can occur at such steps as pre-mRNA processing in the nucleus or mRNA stability in the nucleus or in the cytoplasm. To investigate whether this posttranscriptional regulation of G6PD occurs in the nucleus or in

the cytoplasm, Hodge & Salati (38) compared the abundance of G6PD mRNA in these two cellular compartments. Refeeding starved mice resulted in an 18-fold increase in the cytoplasmic mRNA abundance and a 13-fold increase in pre-mRNA abundance in the nucleus. This suggests that regulation of G6PD in this dietary paradigm occurs primarily in the nucleus. Moreover, the changes in G6PD mRNA abundance in the cytoplasm of mice were parallel to those in the nucleus. Thus, nucleocytoplasmic transport of the mature mRNA does not appear to be regulated.

Regulation of G6PD gene expression by polyunsaturated fat is also by a nuclear posttranscriptional mechanism. Accumulation of G6PD pre-mRNA is inhibited 60–70% in the livers of mice fed high-fat diets compared with animals fed low-fat diets (38). Inhibition of G6PD in total RNA, which is more representative of cytoplasmic RNA, parallels the inhibition seen in the nucleus. Despite the major difference in the amount of nuclear mRNA with different diets, no significant differences in the half-life of either G6PD pre- or mature mRNA have been observed (38). Because dietary status causes little or no difference in the half-life of mRNA, all detected mRNA must be stabile, and changes in the amount of mRNA must involve rapid degradation in the nucleus.

Similar results have been obtained in primary rat hepatocytes in culture using arachidonate to inhibit the induction of G6PD by insulin and glucose (74). Incubation of rat hepatocyte monolayers with arachidonate causes a 50% inhibition of cytoplasmic G6PD mRNA abundance and a 60% inhibition of pre-mRNA abundance in the nucleus. Thus, primary rat hepatocytes mimic the regulation of G6PD observed in intact animals both qualitatively and quantitatively.

Because changes in the amount of G6PD mRNA occur in the nucleus and precede the changes in amount of G6PD mRNA in the cytoplasm, steps involved in processing of the nascent G6PD transcript may be sites for regulation. To isolate a pool of RNA that is enriched for mRNA in the processing pathway and to minimize the isolation of mature mRNA in the nucleus that is undergoing transport to the cytoplasm, we isolated RNA from the insoluble fraction of the nucleus. The insoluble fraction of the nucleus contains the proteins involved in transcription and processing of pre-mRNA as well as the pre-mRNA itself (7, 9, 54, 56, 93, 96). Thus, RNA in this fraction represents newly transcribed RNA that is undergoing processing. By using an RNase protection assay and probes that hybridized across an intron/exon boundary, the change in the amount of G6PD mRNA during splicing could be measured.

In starved mice, the amounts of G6PD RNA at all stages of processing were very low (1). When the animals were refed, the amount of nascent G6PD transcripts (those containing at least the intron represented in the probe) increased in the nucleus but remained low. The amount of more processed forms of the RNA (those that had the intron spliced) increased in the nucleus and was consistently greater than the amount of its precursor. These results suggest that the abundance of G6PD RNA is regulated during the processing of the nascent transcript, steps that could involve either splicing or polyadenylation of the pre-mRNA. To differentiate between these two processing reactions, we first measured the amounts

of unspliced, partially spliced, and fully spliced RNA using probes that detected two consecutive exons and two intervening sequences. The amount of unspliced RNA (that still contain both introns) was present in very low amounts in RNA samples from both starved and refed mice. Enhanced accumulation of G6PD partially spliced RNA (RNA from which one intron had been spliced) was observed on refeeding and the accumulation was greatest for the fully spliced RNA (1). The accumulation of the partially and fully spliced RNA was greater in refed animals than in starved animals. Thus, refeeding enhanced the accumulation of splicing intermediates for G6PD, which suggests that the efficiency of splicing has been enhanced.

Next, we measured the amount of G6PD RNA that was nonpolyadenylated versus polyadenylated using a probe that hybridized across the cleavage site in the nascent RNA. The amount of nonpolyadenylated RNA was very low in livers of both starved and refed mice but did increase slightly during refeeding. Refeeding resulted in a four-fold increase in the rate of accumulation of G6PD polyadenylated RNA, to a rate similar to that for partially spliced G6PD RNA. The rate of increase in the amount of fully spliced RNA was greater still (eight-fold greater than nonpolyadenylated RNA). The observation that even nonpolyadenylated RNA was increasing in amount, albeit only slightly, suggests that it is the process of splicing that caused G6PD RNA to accumulate in the nucleus. Clearly, polyadenylation is necessary for the production of a stable mRNA but insufficient to cause the enhanced accumulation of fully processed RNA during refeeding. This enhancement is the result of an increase in the efficiency of splicing of the nascent transcript.

Regulation of G6PD expression by polyunsaturated fat appears to use the same nuclear mechanism. Consumption of a diet high in polyunsaturated fat resulted in a decrease in the rate at which more spliced forms of G6PD RNA accumulated in the nucleus (38). Although dietary polyunsaturated fat inhibits G6PD expression, consumption of a diet high in polyunsaturated fat does not prevent the normal diurnal variation in the expression of G6PD; it merely attenuates the magnitude of the increase. Most likely, the carbohydrate component of the diet stimulates the increase in the amount of G6PD mRNA during each eating (dark) cycle. During the first 2 h of the eating cycle, the amount of G6PD mRNA in the cytoplasm is less in mice fed high-fat diets (6% safflower oil) compared with mice fed low-fat diets (1% safflower oil). The diurnal increase in the amount of G6PD mRNA is not detected until 4 h into the eating period (38). Within the insoluble fraction of the nucleus, at 0 and 2 h into the eating cycle, the amount of G6PD unspliced RNA was the same between diets, consistent with the lack of transcriptional regulation of this gene. However, the amount of processed G6PD mRNA was 50% less in mice fed diets high in polyunsaturated fat (1). Thus, regulation of G6PD expression due to dietary polyunsaturated fat also involves changes in the efficiency of pre-mRNA processing.

Is this mechanism unique to G6PD? Posttranscriptional regulation in the nucleus has also been described for regulation of the S_{14} gene by dietary carbohydrate. Consumption of a diet high in carbohydrate increases the transcription of this

gene and the amount of the splicing intermediate for its single intron pre-mRNA (8, 32, 39). We extended these findings by examining amounts of unspliced and spliced S_{14} RNA during refeeding. The amount of S_{14} unspliced RNA increased during refeeding. This increase is greater than that seen for G6PD unspliced RNA and is consistent with transcriptional regulation of the S_{14} gene. Refeeding also resulted in a greater amount of the spliced S_{14} RNA compared with the unspliced RNA (1). The enhanced rate of accumulation of spliced RNA for the S_{14} gene suggests that nutritional regulation of splicing is not unique to G6PD. Regulated processing of the RNA for all the lipogenic genes would enhance the rate at which the cell could change the expression of these enzymes. Thus, we hypothesize that this mechanism is common to most members of the lipogenic enzyme family.

CONCLUSIONS

During the expression of a gene, steps subsequent to transcription are essential for production of the mature mRNA. In this regard, mutations that effect splicing or polyadenylation result in degradation of the RNA in the nucleus (2, 13). A model for regulation of G6PD expression is shown in Figure 2. Clearly, the reactions are listed in this order for illustration purposes only. Because splicing and polyadenylation occur cotranscriptionally (reviewed in 35, 60), splicing (k_2 and k_4) most likely occurs coincident with polyadenylation (k_3).

In the case of G6PD, the rate of gene transcription is constant (k_1) across all dietary states. Consumption of a high-carbohydrate diet results in an increase in the accumulation of partially spliced and mature forms of G6PD mRNA. The slowest rates of accumulation of G6PD RNA were observed for the amount of partially spliced, nonpolyadenylated RNA (k_2). The increase in the rate of accumulation of polyadenylated but partially spliced RNA (k_3) is insufficient to account for the rate of accumulation of mature mRNA in the nucleus. It is during the process of splicing of G6PD pre-mRNA that the greatest increases in rate occur (k_2 and k_4), and it is the rate of accumulation of the mature RNA that is most enhanced (k_4).

In contrast, during starvation or consumption of a diet high in polyunsaturated fat, the rate of splicing (k_2 and k_4) is decreased and the inefficiently spliced RNA undergoes degradation in the nucleus (k_5, k_6, and k_7). Once fully processed, G6PD mRNA is stable in the nucleus, and no change in its rate of degradation (k_8) is detectable across dietary manipulations (38). What remains to be determined is whether starvation or dietary fat directly interacts with the machinery involved in degradation (k_5, k_6, or k_7) or whether their action is to interfere with the mechanisms resulting in the enhanced rate of splicing (k_2 and k_4) due to a high-carbohydrate diet. Our data to date favor the latter. In this regard, arachidonic acid decreases the induction of G6PD expression caused by incubation of hepatocytes with insulin and glucose, but arachidonic acid does not block the increase in G6PD expression caused by incubation in a high-glucose medium without insulin (74).

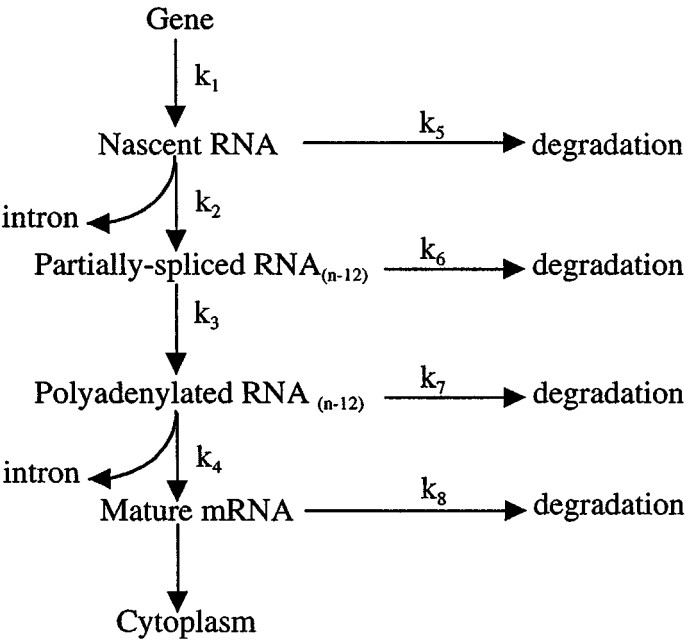

Figure 2 Model for the posttranscriptional regulation of glucose-6-phosphate dehydrogenase (G6PD) expression. The rate terms are as follows: k_1, the rate of transcription of the gene; k_2 and k_4, rates of splicing of the 12 introns; k_3, rate of polyadenylation; k_5 and k_7, rate of degradation of RNA that is not correctly or efficiently spliced; k_6, rate of degradation of RNA that has not been polyadenylated; and k_8, rate of degradation of mature mRNA in the nucleus.

Furthermore, the kinetics of mRNA change during dietary fat consumption are consistent with an interaction between the signal transduction pathway for dietary fat and existing protein(s).

How then can nutrients alter the rate of RNA splicing? Existing examples for regulated splicing have concentrated on alternatively spliced messages. In these cases, splicing enhancers in the RNA molecule appear to mediate this regulation (cf 72). Thus, a potential scenario is the presence of a *cis*-acting element in the G6PD RNA that is a splicing enhancer, and the activity of the protein that binds to this element can be regulated by nutrients. G6PD provides an excellent model to study the regulation of gene expression by dietary factors at a posttranscriptional level. The absence of transcriptional regulation makes interpretation of changes in the pre-RNA pool easier, and its regulation can be studied both in intact animals and in primary rat hepatocytes in culture. Understanding the mechanisms by which nutrients alter nuclear posttranscriptional events will help uncover new information on the breadth of mechanisms involved in gene regulation.

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