Transcriptional switches in the control of macronutrient metabolism

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This review shows how some transcription factors respond to alterations in macronutrients. Carbohydrates induce enzymes for their metabolism and fatty acid synthesis. Fatty acids reduce carbohydrate processing, induce enzymes for their metabolism, and increase both gluconeogenesis and storage of fat. Fat stores help control carbohydrate uptake by other cells. The following main transcription factors are discussed: carbohydrate response element-binding protein; sterol regulatory element-binding protein-1c, cyclic AMP response element-binding protein, peroxisome proliferator-activated receptor-α, and peroxisome proliferator-activated receptor-γ.

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INTRODUCTION

The subject of how diet is involved in controlling metabolism is very complex, but it can be split into different types of mechanisms involving transcription factors and modification of enzyme activities, frequently by involving selective phosphorylation and dephosphorylation of specific enzymes. There are times when cells are predominantly metabolizing carbohydrate and others when fatty acids are the main source of energy. This review provides select examples of how transcription factors act as switches to control the relevant metabolic pathways, but it is not intended to be comprehensive. Figure 1 illustrates the main interactions that are discussed; it will not be referred to again in the text.

CARBOHYDRATES INDUCE ENZYMES FOR THEIR METABOLISM

When diets high in carbohydrate are consumed, enzymes in the glycolytic and fatty acid synthesis pathways are needed increasingly, and mechanisms are in place to produce more of these proteins that involve the activation of several genes via the transcription factor carbohydrate response element-binding protein (ChREBP). One of the key enzymes affected is pyruvate kinase. The mechanism proposed involves selective dephosphorylation of the transcription factor ChREBP, which is produced in the cytoplasm; it has the following two domains: one for its targeting to the nucleus and one for its binding to the carbohydrate response element on the DNA. In the inactive state, these domains are phosphorylated, preventing travel to the nucleus and blocking the subsequent binding to the DNA domain. Using a rat model, Kabashima et al. discovered the mechanism by which carbohydrate causes dephosphorylation of these domains. They found that the activity of the protein phosphatase, which is specific for the phosphate in the nuclear targeting domain, was increased by diet and specifically by carbohydrate and xylulose 5-phosphate, whilst other metabolites of glucose had no effect. The next step in the research was to fractionate the proteins and assay the activity of all the fractions with and without xylulose 5-phosphate; a phosphatase was found that was greatly stimulated by this...
compound. There appears to be a similar phosphatase enzyme in the nucleus that removes the phosphates on the DNA-binding domain.

CARBOHYDRATE INCREASES FATTY ACID SYNTHESIS

SREBPs were purified as nuclear factors that bind to specific DNA sequences (sterol regulatory elements or SREs) found in the control regions of the genes that encode enzymes needed in the lipid synthesis pathways. SREBPs are structurally composed of four domains with two membrane-spanning regions. The release of SREBP-2 from the membrane responds to cholesterol and is involved in control of cholesterol synthesis; this accounts for the naming of similar molecules, such as SREBP-1c. This is a transcription factor that regulates fatty acid synthesis through selective induction of hepatic glucokinase (GK) and an array of lipogenic genes. The release of SREBP-2 involves transport to the Golgi body where it is proteolytically released from the membrane; while this is true for SREBP-1c as well, it seems that control of this transcription factor does not involve the amount of cholesterol in the membrane. Much of its control appears to depend on regulating transcription of its own gene.

When there is excess carbohydrate, the liver may use it to synthesize fatty acids for export to the adipose tissue store, and it is understandable why insulin controls this. When hepatocytes were treated with insulin, there was an increase in the transcription of glucokinase. The next step in the research was to investigate the amount of SREBP-1c in the precursor form that was bound to the membrane and the nuclear form that was released from the membrane. Provision of insulin for 6 hours caused a great increase in both forms. When insulin was given as a pulse and incubated for only 30 min, there was not enough time for an increase in the membrane form to occur and when there was little in the membrane, not much was released from it. However, when the SREBP-1c had already been produced in the membrane, a large amount of it was released as the nuclear form from the membrane within 30 min. This shows that insulin has a speedy effect as well as the slow one via increasing transcription.

FATTY ACIDS REDUCE CARBOHYDRATE PROCESSING

Fatty acids in high amounts are activated to their CoA derivatives, leading to the production of AMP from ATP. Subsequently, the high levels of AMP are thought to activate AMP kinase (AMPK). This normally plays an important part in controlling the response to a lack of energy in the cell. In line with its proposed role in the management of cellular energy status, AMPK activation stimulates various pathways that generate ATP and switches off anaerobic pathways and other processes that consume ATP. It inhibits lipid biosynthesis by phosphorylation and inactivates...
vation of key metabolic enzymes such as acetyl CoA carboxylase (fatty acid synthesis) and glycerol phosphate acyl transferase (triacylglycerol synthesis). AMPK also inactivates the muscle isoform of glycogen synthase and hence prevents waste of energy by storing carbohydrate. Its capacity to phosphorylate the transcription factor ChREBP in the DNA-binding domain causes a reduction in transcription of proteins needed to process carbohydrate.

When carbohydrate is not available and fatty acids are the main source of energy, glucagon is secreted. Gluca
cagon is an example of a hormone that acts via G proteins that activate adenyl cyclase. The resulting cyclic AMP activates the enzyme protein kinase A (PKA), which phosphorylates ChREBP on an amino acid in the domain that targets the nucleus and in the domain that binds to DNA. This means that ChREBP does not enter the nucleus and this forms a second means of reducing carbohydrate processing.

**FATTY ACIDS INDUCE ENZYMES FOR THEIR METABOLISM**

There are several situations in which fatty acids are more available for metabolism than carbohydrates. This will happen some time after the carbohydrate in a meal has been taken up and, during starvation, when fatty acids are released from adipose tissue. Cells then need to have enzymes in place to oxidize fatty acids. A way in which this is controlled appears to involve the transcription factor peroxisome proliferator-activated receptor alpha (PPARα). This is an example of a transcription factor that needs to form a complex with retinoid X receptor (RXR), which requires 9-cis retinoic acid to activate it.

PPARα was discovered in 1990 as the receptor that activates DNA transcription in response to a diverse group of compounds called peroxisome proliferators. Certain pesticides, phthalates, and hyperlipidemic drugs belong to this category. PPARs are members of the nuclear hormone receptor superfamily, a group of nuclear proteins that mediate the effects of small lipophilic compounds, such as steroids, retinoids, bile acids, and fatty acids, on DNA transcription. They are grouped together based on a common structural motif consisting of a central DNA-binding domain containing two zinc-fingers, and a large C-terminal domain that binds the ligand.

The PPARα isotype is mostly expressed in organs with a high rate of fatty acid catabolism, such as brown adipose tissue, liver, kidney, and heart. It plays an important role in the regulation of intermediary metabolism, which has been studied extensively in liver. Ever since the first PPAR was cloned, an intensive search has been ongoing to identify their natural ligands. A unique feature of PPARs is that their ligand-binding pockets are relatively large, which allows the receptors to accommodate a range of different ligands. The class of drug called fibrates mimics the natural ligands for this transcription factor and are used to treat patients with dyslipidemia, especially patients with elevated triacylglycerols and/or reduced levels of high-density lipoprotein (HDL) cholesterol. Although a range of compounds has been shown to activate and bind to PPARs in vitro, including fatty acids, certain prostaglandins, and oxidized phospholipids, it is not fully understood which of these compounds serve as ligands in a living organism. Polysaturated fatty acids and some metabolites are considered the most potent ligands.

PPARα is probably important in regulating the adaptive metabolic response to fasting. Experiments with mice in which the PPARα gene has been deleted, have been very illuminating. Whereas these PPARα null mice are without overt symptoms when fed ad libitum, except for moderately elevated plasma triacylglycerol levels, they show a host of metabolic abnormalities upon starvation. This includes elevated plasma free fatty acid levels, hypoketone
mia, hypoglycemia, elevated plasma urea levels, hypothermia, a decreased metabolic rate, and fatty liver. PPARα stimulates the uptake, binding, activation, and subsequent oxidation of fatty acids. In addition, it stimulates the synthesis of ketone bodies and influences the synthesis of apolipoproteins such as apoAI and apoAII.

**FATTY ACIDS INCREASE GLUCONEOGENESIS**

Transcription factors like PPARs do not work alone; there are a number of other proteins that interact with them and initiate transcription. These proteins help make the process more specific in that some of them are required before certain transcription factors can have an effect and they may be needed for more than one transcription factor. It is known that some of the effects mediating the control of metabolism depend on alterations in these coactivators. An example is peroxisome proliferator-activated receptor-γ coactivator 1a (PGC-1α), which is involved in mediating effects of several transcription factors. In animal models, the expression of PGC-1 is correlated well with hepatic gluconeogenesis, which occurs when fatty acids rather than carbohydrates are the main energy source in the liver. Glucagon, acting via cAMP, and glucocorticoids are the major positive factors activating the genes of gluconeogenesis in liver. Treatment of hepatocytes with 8-bromo-cAMP, a cell-permeable analog of cAMP, induced mRNA expression of phospho-enol pyruvate carboxykinase (PEPCK) and glucose-6-phosphatase, key enzymes of gluconeogenesis. Primary hepatocytes were infected with adenoviruses encoding for the expression of either green fluorescent protein, a reporter
proteins, or PGC-1. PGC-1 expression stimulated the expression of mRNA for all three key genes of gluconeogenesis. Experiments performed at different doses of virus indicated that maximal induction of these genes occurred at approximately the same levels of PGC-1 protein that are present in fasted liver. Glucagon leads to release of cyclic AMP as described previously. This activates PKA, which phosphorylates cyclic AMP response element-binding protein, a transcription factor that enhances the production of mRNA for PGC-1. This, in turn, is needed to coactivate HNF-4 (hepatocyte nuclear factor), a hepatic transcription factor that enhances the production of mRNA for the synthesis of gluconeogenic enzymes.

**Fatty Acids Increase Storage of Fat**

When dietary fat is high, fatty acids and their metabolites bind to the transcription factor PPARγ in the liver and attempt to increase fatty acid oxidation, but they also bind to PPARα, which is expressed in adipocytes and plays an important role in the deposition of fat as storage. The process of adipogenesis can be modeled in a cell culture system, allowing detailed analysis of the sequence of molecular events that lead to adipocyte differentiation. The activation of PPARγ is both necessary and sufficient to induce an adipose phenotype, which is defined by lipid accumulation and expression of fat-specific marker genes. Genes that are under transcriptional control of PPARγ in adipose tissue include lipoprotein lipase, acyl-CoA synthetase, fatty acid translocase, and fatty acid transport protein, suggesting an important role is played by PPARγ in the uptake of fatty acids in adipocytes.

**Fat Stores Help Control Carbohydrate Uptake by Other Cells**

Yamauchi et al. developed a mouse model without adipose tissue by severely reducing PPARγ/RXR activity using an inhibitor. Four weeks of this treatment resulted in disappearance of visible white adipose tissue. This loss presumably results from decreased expression of molecules involved in triacylglycerol accumulation in white adipose tissue and whose expression is dependent on PPARγ/RXR activity. Substances produced by adipocytes are called adipokines; there is a wide range of these, but adiponectin will primarily be discussed here. Adiponectin appears to be produced only in adipocytes under the control of PPARγ and insulin. Continuous systemic infusion of a physiological dose of adiponectin ameliorated hyperglycemia and hyperinsulinemia, especially in the presence of added leptin, another adipokine that was missing in these animals. There are probably several factors whereby adipose tissue influences insulin sensitivity, of which adiponectin is just one prominent one. When people are starving, stores of fat may decrease to low levels, but when energy becomes available again, the lower insulin sensitivity of other cells may help divert it to the fat store. When there is a store of fat, adipocytes provide information about this so that other cells take up more of the energy.

The insulin receptor (IR) responds to insulin by becoming an active tyrosine kinase. The insulin receptor substrate proteins (IRS) were initially identified as tyrosine-phosphorylated proteins in insulin-treated cells. The phosphorylated tyrosines become binding sites for downstream signaling molecules. A key signaling complex in insulin action is formed between IRS and the enzyme phosphoinositide 3 kinase (PI3-K), which phosphorylates phosphatidylinositol on the D3 position of the inositol ring. After hydrolysis from the membrane by phospholipase C, IP4 activates protein kinase B. This is then involved in the effects of insulin, such as translocation of GLUT4 (the glucose transporter) to the membrane and the release of SREBP-1c from the endoplasmic reticulum that was described earlier.

In order to study the role of adiponectin in more detail, Maeda et al. produced knockout (KO) mice for adiponectin. The KO mice showed some relatively mild metabolic disturbances in normal situations, but when they were challenged with a high-fat/high-sucrose (HF/HS) diet, the changes in metabolism were more pronounced. When fed a HF/HS diet that mimics the type of diet that humans consume compared to the normal mouse diet, the KO mice differed greatly from the wild-type (WT) mice in the development of insulin resistance. At 2 weeks post challenge, plasma glucose and insulin concentrations were significantly higher in KO mice than in WT mice. Mice that were fed a HF/HS diet for 2 weeks were subjected to glucose- and insulin-tolerance tests. Insulin stimulation of IRS-1-associated PI3-K on the HF/HS diet was lower in the muscle of KO mice than in WT mice. Using the KO animals, the effect of adenoviral overproduction of adiponectin was examined, which markedly decreased plasma glucose, insulin, and free fatty acid (FFA) levels in the KO mice fed a HF/HS diet.

At this stage it is too early to explain the effects of adiponectin completely, but there is evidence that it operates partially via mechanisms involving AMPK. After binding to the adiponectin receptor, AMPK activity increases rapidly and this may be part of the mechanism for improving insulin sensitivity. It may do this via more than one mechanism, and more research is needed to expand our understanding of this. AMPK may be linked to insulin sensitivity through IRS-1. The IRS-1 molecule contains many serine residues that can also be phosphorylated; this appears to be a very important way of influ-
enhancing how IRS-1 functions. Depending on which serine is phosphorylated, the cell may become more or less sensitive to insulin. There is some controversy about this at present due to conflicting studies, but it has been reported that AMPK phosphorylates a particular serine and this increases the activity of IRS-1 in the mouse.

The apparent beneficial effects of having some adipose tissue on insulin sensitivity in other cells do not continue when stores become excessive. Obesity is treated by the body as a stress situation leading to phosphorylation of IRS-1 that decreases insulin sensitivity.\(^{25}\) There is infiltration of adipose tissue, with macrophages secreting cytokines and, in particular, tumor necrosis factor alpha (TNFα). It is thought that most of the TNFα is produced by the macrophages in adipose tissue and that it has a paracrine action on neighboring cells, such as the adipocytes themselves.\(^{26,27}\) It has been shown that TNFα also leads to decreasing insulin sensitivity by reducing adiponectin secretion. The pathway proposed for the effect of stress on adiponectin production is that stress increases TNFα production, which reduces insulin sensitivity in adipocytes by phosphorylation of IRS-1 and that insulin is involved in regulating the production of adiponectin.\(^{28}\)

**CONCLUSION**

When either carbohydrates or fatty acids predominate in cellular metabolism, they signal via specific transcription factors to enhance the production of enzymes for the required pathways including oxidation, storage, and metabolic interconversions.

**REFERENCES**
