

ENERGY, METABOLISM AND MITOCHONDRIA: AN OVERVIEW.

INTRODUCTION.

SIGNALING TO AND WITHIN ORGANS AND CELL: THE BASICS THAT APPLY TO METABOLISM.

EATING: HOW MUCH AND WHEN TO STOP.

CONTROL OF NUTRIENT UPTAKE INTO TISSUES BY HORMONES.

INTERMEDIARY METABOLISM: THE BALANCE BETWEEN ENERGY PRODUCTION AND CELLULAR BIOGENESIS.

THE LIVER, GLUCONEOGENESIS AND GLYCOGEN SYNTHESIS.

KETOGENESIS.

CONTROL BASED ON ATP LEVELS: AMP KINASE. DECIDING BETWEEN THE USE OF GLUCOSE OR FATTY ACIDS FOR ENERGY (ATP) PRODUCTION: THE RANDLE CYCLE.

CONTROL BASED ON NAD⁺ LEVELS.

CONTROL BASED ON O-GL_cNACYLATION.

THE ATP PRODUCING STEPS: KREBS CYCLE AND OXIDATIVE PHOSPHORYLATION.

OXPHOS AND FREE RADICAL GENERATION

THE SPECIAL ROLE OF GLUTATHIONE.

INTRODUCTION.

We are what we eat, a maxim often used but what does this mean at the physiological and cell biological level? And, how does it pertain to people with mitochondrial disease for whom energy is at a premium? The process of using foodstuffs for energy, and to build up our bodies, which is by definition “metabolism”, begins with food intake in the oral cavity and involves every organ of the body as nutrients are released by digestion. Here I provide an overview starting with appetite and satiety, which involves interplay between the brain and gut directed by neuropeptides and hormones. Then I review how nutrients glucose, fatty acids and amino acids are distributed to cells in the body under the control of hormones including insulin and glucagon and more. The brain cannot use fatty acids for energy and I review how this is overcome by careful blood glucose modulation by the liver. Finally I cover the process known as intermediary metabolism, whereby the use of breakdown products of the food we ingest is parsed between energy generation and cell growth and repair. This involves the monitoring of ATP, NADH and acetyl CoA levels to ensure the optimum deployment of metabolic intermediates, and uses several strategies including different modifications of key enzymes in the various pathways.

SIGNALING TO AND WITHIN ORGANS AND CELL: THE BASICS THAT APPLY TO METABOLISM.

The human body is an incredible machine, or more accurately a set of machines called organs, that are orchestrated to work together. The “maestro” is the brain, which uses a series of strategies to ensure that the whole is finally tuned to meet the demands of life from birth to death.

The brain has direct channels through which to send messages to different organs and their constituent cells. Nerve impulses continually flow back and forth between brain and the multiple organs. This area is beyond the scope of the review, but see references. Adding to the information flow is release of a broad set of hormones, cytokines and neuropeptides into the blood stream. To communicate between the brain and tissues these peptides must be able to pass through the blood-brain barrier, which most described here can. With respect to metabolism there are a number of key messengers signaling when to eat, when to stop, the relative distribution of nutrients between tissues, and use of these nutrients for energy production or cell biogenesis.

Included are insulin, glucagon, ghrelin, leptin, thyroid hormone, cholecystikinin glucagon-like peptide-1 and neuropeptide Y. Tissues and cells recognize and bind these peptide-signaling molecules via cell surface receptors, which characteristically extend from outside the cell across the plasma membrane and into the interior of the cell cytosol where they initiate the appropriate cell responses. Not all tissues respond identically to insulin, glucagon or other hormones: it depends on the key functions of the tissue. Tissue specificity is ensured because many hormone receptors occur as variants or isoforms. The presence of receptors isoforms primes different cells to bind the circulating hormone at different concentrations and with different affinities. Variations in the C terminal part of the receptor allow for differences in intracellular signaling of hormonal responses by changing the interaction of the receptor with downstream activators of various cellular pathways.

Intracellular signaling in response to hormone binding can arise via generation of small molecules. For example, several cell receptors, including the insulin receptor, promote the synthesis of PI3K, which then activates, or in some cases inactivates, cellular metabolic pathways. Also, interaction of the receptor partner-protein can be post-translationally-modified by its interaction with the receptor, thereby propagating the signal down the cascade. The most common modification in this context is phosphorylation, but as will be seen, others are used in metabolism.

So far I have listed how signals external to the cell affect functioning. We now know that cells contain multiple “internal receptors” that monitor the levels of key metabolites in the cell, including ADP/ ATP, NAD⁺/NADH, free radicals, fatty acids etc. to induce various cascades of events to try and maintain cell homeostasis. Several are covered here including AMP activated protein Kinase (AMPK), which is widely used in metabolic control.

One final control mechanism becomes important in energy metabolism. This involves so-called product inhibition of individual enzymes in metabolic pathways. The inhibitory product can be the specific product of the enzyme in question, or can be a product of an enzyme further down the pathway. Also, product inhibition can involve blockage of the catalytic site or can be allosteric e.g. the product/inhibitor compound binds outside the catalytic site to inhibit a conformational rearrangement of the enzyme needed for functioning. A good example we will discuss is control of gluconeogenesis versus glycolysis as part of glucose degradation versus synthesis.

EATING: HOW MUCH AND WHEN TO STOP.

The process by which food materials are broken down to provide nutrients begins in the oral cavity and continues in the stomach,

pancreas, duodenum and intestines. What we eat, when we eat, and how we know when we have had enough i.e. are satiated, is determined by brain responses and by the set of hormones, neuropeptides, cytokines made by the brain and the digestive system and listed above. Food smell, taste and appearance, along with composition e.g. whether fats, carbohydrates etc. are all important factors in establishing the desire to eat. The main site of brain control of eating is the hypothalamus. A key neuropeptide involved in the brain activity is neuropeptide Y. Also involved are agouti-related peptide and a particular isoform of the enzyme AMP kinase present in the brain. Several signaling molecules synthesized in the stomach and intestine also participate, with each able to cross the blood brain barrier. One peptide synthesized in the stomach and pancreas, and called ghrelin, is involved along with agouti-related peptide in establishing appetite. Three other peptides counteract ghrelin and inhibit food intake and these are cholecystinin, glucagon like polypeptide 1 and peptide YY. The process of hunger and fullness is further controlled by the hormone leptin, which is secreted by fat cells and intestinal cells.

Ghrelin is a 28 amino acid peptide the blood level of which shows circadian fluctuation, spiking before eating, followed by rapid reduction after a meal. Thus ghrelin is a regulator of meal initiation, stimulating a cascade of events to prepare the body for an impending food intake. Ghrelin also contributes to the regulation of body weight by potently stimulating growth hormone secretion from the pituitary gland, increasing adiposity and reducing energy expenditure. This peptide has been shown to also affect reward processes, mood, memory and learning, and stress response.

Ghrelin is also important for the regulation of blood glucose homeostasis. It is expressed in the pancreas where it is thought to exert an inhibitory effect on secretion of insulin

Leptin, is a 167 amino acid hormone, secreted by adipose cells and to a much lower extent by the intestinal mucosa which functions to regulate glucose metabolism and adiposity. The levels of leptin fall to near zero during fasting, high impact exercising or other energy limitation, and increase during feeding. Thus low levels of leptin increase appetite while high levels restrict the desire to eat. Interestingly, low levels are seen to increase the liking for high fat foods relative to low fat foods. In this connection, lack of response to leptin has been suggested to lead to obesity, with obese individuals more likely to respond by over eating highly palatable food compare to lean individuals.

The role of leptin in humans has proved much more varied than at first thought. Recent work shows that leptin plays a major role in the regulation of the immune system. It does this by inducing increased levels of T cells and inhibiting transformation of naïve T cells into Th2 cells, which are anti-inflammatory. Leptin also increases macrophage and monocyte proliferation rates thereby increasing the levels of inflammatory cytokines e.g TNF1alpha, IL-1 etc.

The strongest inhibitor of appetite is the hormone cholecystinin (CCK) secreted by the intestine. Release of this peptide after food intake, mostly stimulated by fat and protein, leads to reduction of food intake by reaction with receptors in the alimentary canal that inhibit gastric motility, and binding to a brain receptor (CCKbrain). Another satiety hormone is glucagon-like peptide1, which is produced in the intestine. Its levels rise shortly after eating a meal (15min) and peak again later through direct contact with nutrients to reduce food intake. This hormone affects more than satiety as GLP1 signaling, and in particular, the early rise in levels amplifies glucose-induced insulin release so that oral glucose provokes a higher insulin response than that resulting from intravenous glucose. Interest in the mechanism of GLP1 has increased with

recent evidence that treatment with the hormone is effective in obesity treatment.

The signal of hunger provided by ghrelin and the neuropeptide agouti-related peptide together inhibit, while the signal of satiety provided by leptin and GLP1 activate, AMP kinase (see later for more details of this enzyme.) Activation of AMPK results in increased release of hormones from the pituitary and thyroid. These include CRH (corticotropin releasing hormone) and TRH (thyrotropin releasing hormone), which stimulate the pituitary to release ACTH (adrenocorticotropic hormone) and TSH (thyroid stimulating hormone), respectively. The actions of TSH on the thyroid gland result in an increased production and release of the thyroid hormones T4 and T3. The metabolic effects of T3 are diverse resulting in increased carbohydrate digestion and absorption in the gut, increased hepatic gluconeogenesis, and increased protein metabolism in skeletal muscle. As the level of T3 increases, it eventually feeds back and inhibits hypothalamic AMPK. The ACTH that is released stimulates the adrenal cortex to produce cortisol, which in turn stimulates lipid metabolism and hepatic gluconeogenesis.

SELECTED READING.

Brain-gut axis and its role in the control of food intake. Konturek, S.J. Konturek, J. Pawlik, T. Brzozowski T. *J Physiology and Pharmacology* 55. 137-54 (2004)

Leptin: molecular mechanisms, systemic pro-inflammatory effects, and clinical implications. Paz-Filho G. Mastrorardi , Bertoldi Franco C. Wang K.C , Wong M, Licinio J. *Arq Bras Endocrinol Metab.* 56 597-606 (2012)

Biological, physiological, and pharmacological aspects of ghrelin. Hosoda H, Kojima M, and Kangawa K *J Pharmacol Sci* 100, 398 – 410 (2006)

Fasting leptin is a metabolic determinant of food reward in overweight and obese individuals during exercise training. Hopkins M. Gibbons C and Finlayson G. *Int. J. Endocrinol.* 2014. 323728 (2014)

Stimulation of leptin secretion by insulin. Tsai M. Asakawa A and Inui A. *Indian J. of Endocrinology and Metabolism. Suppl3* 543-549 (2012)

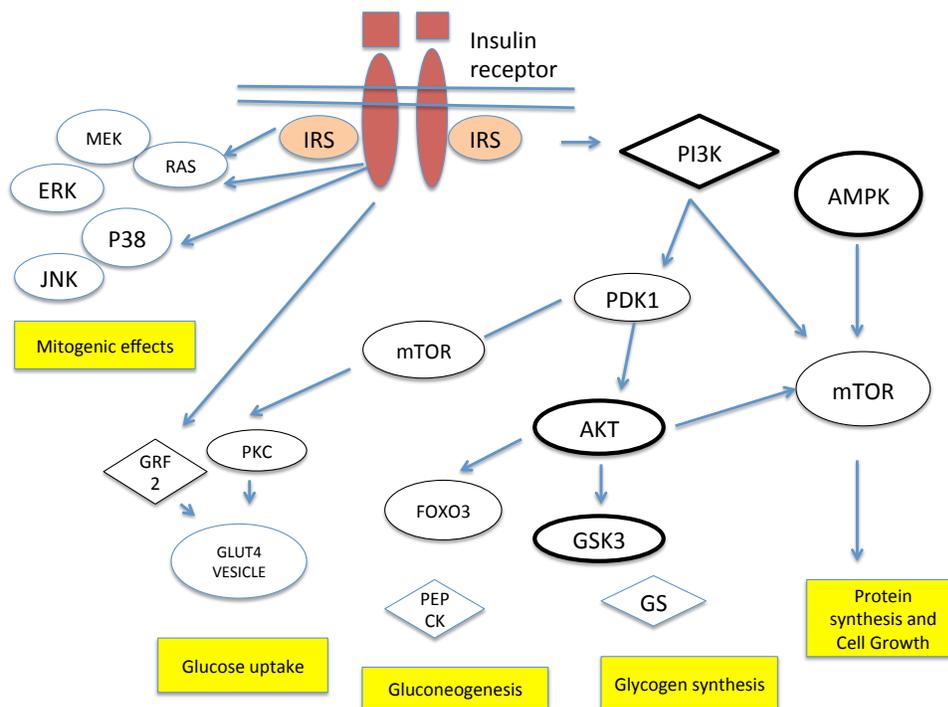
Ghrelin's second life: from appetite stimulator to glucose regulator. Verhuist P-J. World J. of Gastroenterology 18. 3183-95 (2011)

CONTROL OF NUTRIENT UPTAKE INTO TISSUES BY HORMONES.

As foodstuffs pass down the alimentary canal they are reacted on by sets of enzymes that break down carbohydrates to glucose, fats to fatty acids, and proteins to amino acids, which then circulate in the blood stream. Entry of these nutrients into cells in various tissues is under control of several hormones. For example the uptake of glucose and overall glucose homeostasis is regulated primarily by the opposing actions of insulin and glucagon, hormones that are secreted by pancreatic islets from beta-cells and alpha-cells, respectively. Insulin secretion is increased in response to elevated blood glucose to maintain normoglycemia by stimulating glucose transport in muscle and adipocytes and reducing glucose production by inhibiting gluconeogenesis in the liver. Glucagon secretion is stimulated during hypoglycemia, promoting hepatic glucose production and ultimately raising blood glucose levels.

Insulin in the blood stream binds to insulin receptor substrates (IRS1, IRS2, and IRS4) on the plasma membrane of cells, the short term effects of which are to activate the PI3K/AKT/mTor pathway which in concert with modulation of the ERK and MAP kinase pathways prepares the cells for glucose intake by inhibiting lipolysis, and gluconeogenesis while activating glycolysis and oxidative phosphorylation along with protein synthesis and glycogen synthesis. The end point is to drive glucose use for energy production, cell growth, and finally, glucose "energy" storage as glycogen in the liver.

Glucagon exerts a variety of biological actions via the activation of the glucagon receptor present in the islet β -cells and a wide array of tissues, including the liver, kidney, adipose tissue, brain, adrenal gland, duodenum, and heart. This hormone protects against hypoglycemia by stimulating net hepatic glucose production through promotion of glycogenolysis and gluconeogenesis and simultaneous inhibition of glycolysis. Glucagon has other functions in the cell including modulation of heart muscle contractility, ghrelin secretion, and gastrointestinal motility.



Key proteins involved in insulin signaling.

Cells take up glucose, fats and amino acids by a set of specific protein transporters whose presence, distribution and affinities are in large part controlled by hormones, and in particular, insulin and glucagon.

Cell uptake of glucose is via the GLUT or SLC2A transporter of which there are several isoforms. GLUTs are integral plasma membrane proteins that contain 12 membrane-spanning helices. Beside glucose these can transport hexoses. The different isoforms of glucose transporters each play a specific role in glucose uptake determined by substrate specificity, transport kinetics and regulated expression. The best-characterized GLUT transporters are GLUT1-4. Thus GLUT1 is responsible for the low level of basal glucose uptake into cells. This isoform is often highly unregulated in cancer cells. GLUT 2 is a bidirectional transporter found in liver and pancreatic beta cells and able to export from, as well as take in, glucose to the cell. Export of glucose is required for gluconeogenesis. This isoform has a very high K_m for glucose (17mM). Uptake of glucose through this transporter is not regulated by expression levels as most others are, but through phosphorylation reactions. GLUT 3 is expressed mostly in neurons and placenta. GLUT4 is found in adipose tissue and in skeletal and cardiac muscle. Levels are controlled by insulin through translocation from the cytosol to the plasma membrane.

Long chain fatty acids (FA) are common dietary nutrients, a major energy source for most cells, and precursors for synthesis of cellular lipids with structural or signaling functions. Most tissues, except for liver and adipose tissue, possess little capacity for de novo FA synthesis and depend on FA uptake for their needs, emphasizing the physiological importance of cellular FA uptake. Long chain FA uptake is facilitated by membrane transport proteins (FATP1–6), present at the plasma membrane or in intracellular organelles, which have fatty acyl-CoA ligase activity and appear to function in coupling FA uptake to the first reaction of FA utilization. Ancillary proteins such as FABPpm participate. This protein is identical to the mitochondrial aspartate aminotransferase, an enzyme that functions in maintaining the cytoplasmic/mitochondrial NADH/NAD ratios. It couple FA uptake

to cellular redox shuttles that are crucial for oxidative metabolism. Several G protein coupled receptors, specifically GPR40–43, have been recently shown to recognize short (GPR41 and GPR43), medium and long chain FA (GPR40) and mediate signaling regulatory effects of these nutrients. GPR40 is expressed in pancreatic islets signals and increases intracellular calcium and insulin secretion in response to FA. An additional receptor, GPR120, abundant in the intestine, modulates FA-induced glucagon-like peptide 1 secretion.

A key receptor in FA uptake is CD36. This multifunctional scavenger receptor is an 88kDa membrane protein originally referred to as FAT (Fatty Acid Translocase). CD36 is expressed in a variety of cells including and not limited to monocytes, platelets, macrophages, microvascular endothelial cells, adipocytes, muscle cells, enterocytes, and hepatocytes. It can function in a range of processes unrelated to FA uptake such as binding of native and oxidized lipids/lipoproteins for apoptosis and phagocytosis. Muscle CD36 plays an important role in the metabolic adaptation to changes in nutrient availability. Thus muscle contraction and activation of FoxO1, a transcription factor induced with fasting, increase sarcolemmal CD36, FA uptake and oxidation. Further, during fasting, CD36-mediated FA uptake up-regulates muscle PPAR δ and FoxO1, two transcription factors that reinforce reliance on FA utilization.

There is a strong link between glucose and fatty acid uptake. In muscle from diabetic humans, more than the normal level of CD36 is recruited to the plasma membrane, resulting in persistent enhancement of FA uptake, and possibly contributing to the impairment of insulin-sensitive glucose utilization.

Amino acid uptake from the gut takes place mostly into and across the kidney and intestinal epithelia. Different mechanisms are used for neutral and cationic amino acids. There are very many different transporters involved, some of which are Na⁺ dependent, while others use a pH gradient and or membrane

potential to drive the import into cells and organelles. Details of this broad subject are provided in the references.

SELECTED READING.

Insulin as a physiological modulator of glucagon secretion. Bansal P, Wang Q
American Journal of Physiology - Endocrinology and Metabolism 295,E751-E761
(2008)

Regulation of glucose metabolism from a liver-centric perspective. Han HS, Geon Kang G, and Koo SH. Exp Mol Med 48. 218 (2016).

Liver glucose metabolism in humans. Adeva-Andany MM, Pérez-Felpete N, Fernández-Fernández C, Donapetry-García C, Pazos-García. Biosci Rep. 29;36 (2016).

FoxO integration of insulin signaling with glucose and lipid metabolism. Lee S, Dong HH.

J Endocrinol. 2017 Feb 17

Glucose-responsive insulin release: Analysis of mechanisms, formulations, and evaluation criteria.

Yang J, Cao Z. J Control Release. 2017 Jan 31

Glycogen metabolism in humans. Adeva-Andany MM, González-Lucán M, Donapetry-García C, Fernández-Fernández C, Ameneiros-Rodríguez E. BBA Clin. 5:85-100. (2016)

PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure.

Cantó C, Auwerx J. Curr Opin Lipidol. 20(2):98-105. (2009)

Energy metabolism in the liver. Rui L. Compr Physiol. 4:177-97. (2014)

GLUT, SGLT, and SWEET: Structural and mechanistic investigations of the glucose transporters.

Deng D, Yan N. Protein Sci.25. 546-58 (2016).

Cellular Fatty acid uptake. Su X and Abumrad NA. Trends Endocrinol Metab 20 72-77 (2009)

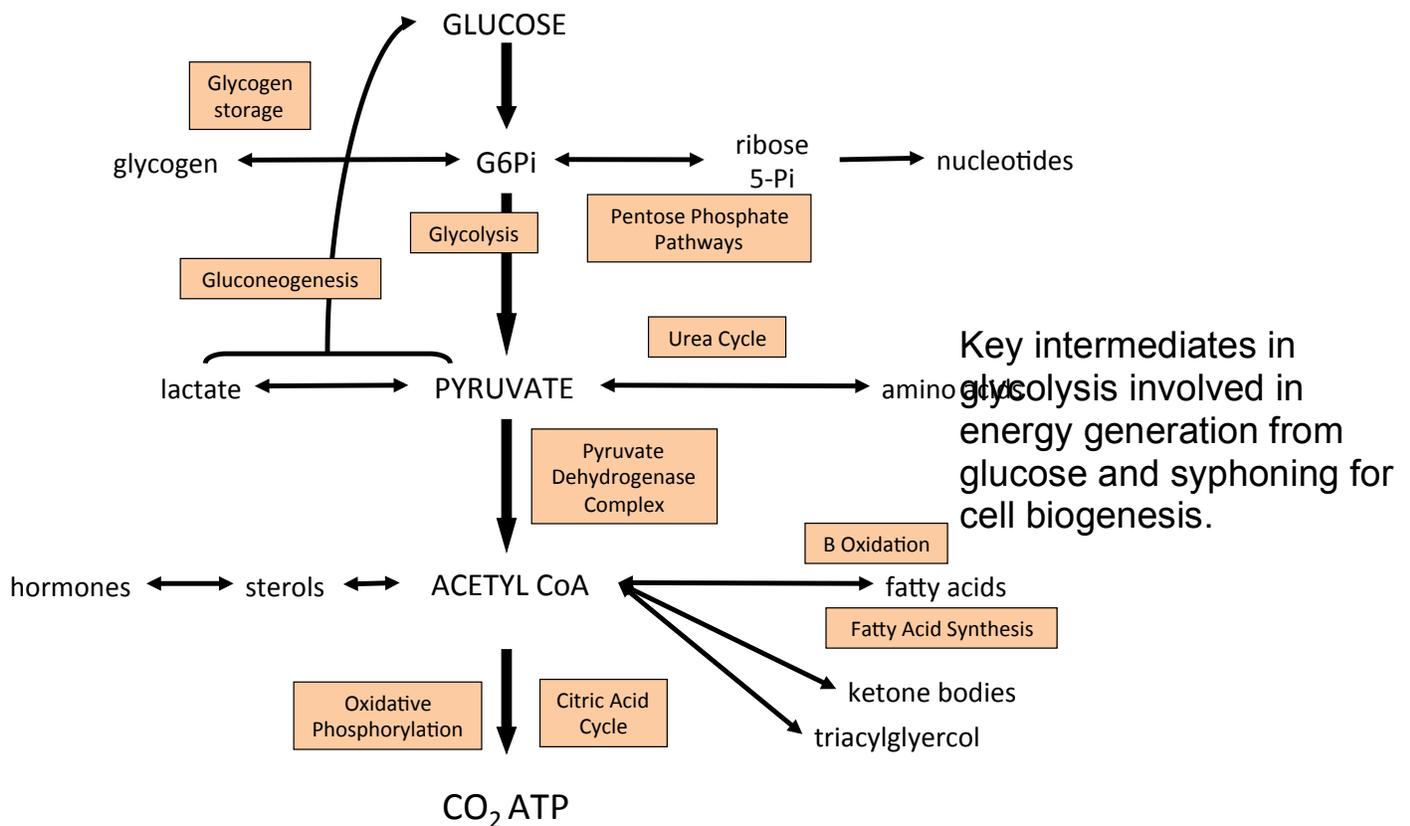
Dietary Lipids Inform the Gut and Brain about Meal Arrival via CD36-mediated transduction. Sundaresan S and Abumrad NA.. JNutr 2015;145:2195–200.

SLC Transporters: Structure, Function, and Drug Discovery.

Colas C, Ung PM, Schlessinger A. Medchemcomm. 7:1069-1081.(2016)

INTERMEDIARY METABOLISM: THE BALANCE BETWEEN ENERGY PRODUCTION AND CELLULAR BIOGENESIS.

The utilization of glucose, fatty acids and amino acids once inside the cell is carefully orchestrated by a series of signaling pathways to ensure that a correct balance is made between the use of nutrients to generate energy as ATP, and their use for building new cell



components, and repairing damaged cell constituents. The overall process is termed intermediary metabolism. Glucose entering the cell is phosphorylated to glucose-6-phosphate by the enzyme hexokinase and this modification prevents the sugar from being returned across the plasma membrane. Glucose 6-phosphate is then modified to pyruvate in a series of enzyme reactions called glycolysis, (which is well covered in textbooks), or selected intermediates are syphoned off the glycolytic pathway to be used in biosynthetic reaction to make nucleotides, lipid derivatives or amino acids, depending on need. Further if glucose is plentiful and cell homeostasis is achieved, glucose- 6-phosphate can be converted to glycogen or creatine phosphate in order to store energy for later use. The key is that cellular signaling pathways constantly monitor the internal cellular milieu for ATP, ADP, AMP, NAD⁺ and NADP⁺ along with other needed metabolites. They then orchestrate which metabolic pathways should be on or off under the given environmental conditions e.g. nutrient levels and stress events. Importantly there is signaling between organs to maintain whole body homeostasis and this, again, mostly involves hormones. The energy needs of the various tissues of the body are different. Some like heart and kidney have a consistently large energy requirement. Thus the values for these two organs is close to 400 kcal/kg/day. Skeletal muscle has relatively low energy needs at rest (13kcal/kg/day), but at times of high aerobic exercise the need increases greatly. Glucose is the primary whole body energy source but this is not the preferred substrate for energy generation in heart and skeletal muscle. Instead these tissues use

fatty acids when possible. Brain, in contrast cannot use fatty acids and relies on glucose and ketone bodies (see later).

Abnormal concentrations of glucose in plasma result in deleterious effects, and levels must be kept relatively constant through periods of fasting or excess nutrient availability. As discussed already, the brain is unable to switch from glucose usage to use of fatty acid for energy. Therefore, maintaining brain functioning depends on keeping plasma glucose levels in a tight range. The liver takes the prime role in ensuring that there is sufficient energy for brain function. This organ is set up to both utilize glucose and synthesize glucose. It is the major storage site of glucose in the form of glycogen, and it can use various metabolic intermediate such as pyruvate and lactate to synthesize glucose, store this as glycogen, and release glucose in response to whole body need.

SELECTED READING

mTOR Signaling in Growth, Metabolism, and Disease. Saxton RA, Sabatini DM. Cell. 168. 960-976 (2017)

THE LIVER, GLUCONEOGENESIS AND GLYCOGEN SYNTHESIS.

Glucose synthesis in the liver is called gluconeogenesis. It involves the same set of reactions used to break down glucose to pyruvate: that is the reactions of glycolysis but it uses them in the reverse direction. Differential control of the forward reactions (glycolysis) and reverse reactions (gluconeogenesis) is assured by their being different enzymes in the glycolysis and gluconeogenesis pathways for interconversion of glucose-6-phosphate (G6P) to glucose, fructose 1,6-bisphosphate (F-1,6-P2) to fructose-6-phosphate (F6P) and of pyruvate to phosphoenolpyruvate .

Short-term regulation of the glycolysis/gluconeogenesis balance

involves allosteric regulators such as ATP, AMP, and fructose 2,6, biphosphate (F26BP). Long term the forward and reverse reactions are controlled at the transcription level. The two key transcription factors are SREBP-1c and ChREBP, which regulate not only the glycolytic enzyme genes but also the genes encoding enzymes for fatty acid biosynthesis and triacylglycerol synthesis. In addition to pyruvate, gluconeogenesis can use lactate as substrate, taking advantage of the ability of lactate dehydrogenase to work reversibly. Gluconeogenesis can also use amino acids after their conversion to generate pyruvate e.g. alanine, glycine, cysteine, threonine, serine and several other amino acids. Under fasting conditions insulin, glucagon and epinephrine together activate the PKA-driven kinase cascades that activate glycogen phosphorylase and thereby promotes glycogenolysis, enabling the liver to provide enough fuel for peripheral tissues such as the brain, red blood cells and muscles. These same hormones along with adrenal cortisol, also initiate the transcriptional activation of the key enzymes of gluconeogenesis. The major transcription factors involved include CREB, FoxO1 and members of nuclear receptors, along with transcriptional co-activators such as CRTC, PGC-1 α and PRMTs.

SELECTED READING

Role of thyroid hormone in hepatic gene regulation, chromatin remodeling, and autophagy. Singh BK, Sinha RA, Ohba K, Yen PM. Mol Cell Endocrinol. S0303-7207(17)30105-3. (2017)

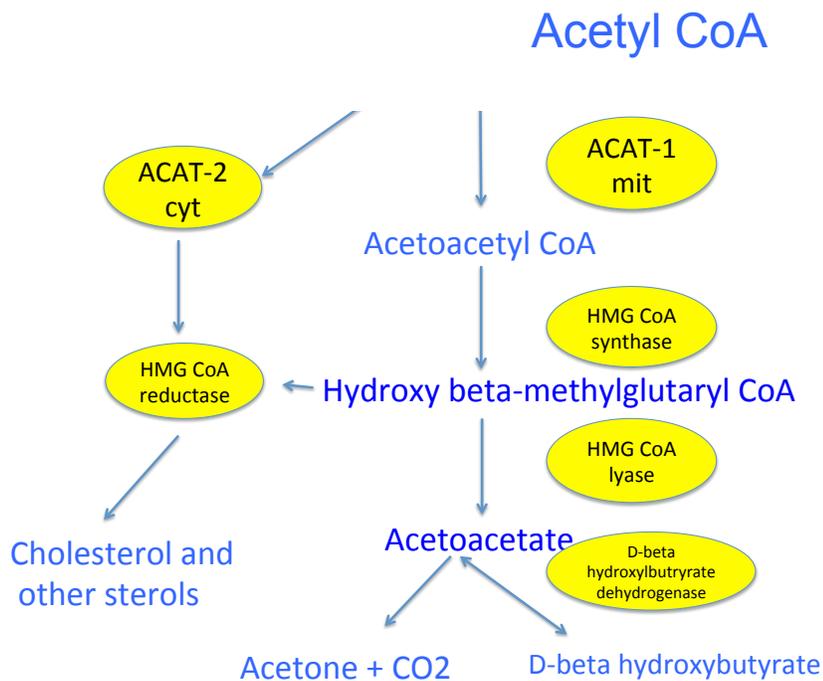
FoxO integration of insulin signaling with glucose and lipid metabolism. Lee S, Dong HH. J Endocrinol. 2017 Feb 17.

KETOGENESIS.

During high rates of fatty acid oxidation, primarily in the liver, large amounts of acetyl-CoA are generated that exceed the capacity of the TCA cycle. The excess is used in the synthesis of ketone bodies that provide additional energy reserves. The synthesis of

ketone bodies (ketogenesis) occurs in mitochondria, allowing this process to be intimately coupled to rate of hepatic fatty acid oxidation.

Conversely, the utilization of the ketones (ketolysis) occurs in the cytosol. The ketone bodies are acetoacetate, β -hydroxybutyrate, and acetone. Brain is able to use ketone bodies as a provider of energy, as are heart and skeletal muscle. The enzymes involved in ketogenesis are shown in the Figure below. β -hydroxy- β -methylglutaryl-CoA (HMG-CoA) is produced by mitochondrial HMG-CoA synthase (encoded by the HMGCS2 gene), an enzyme found in large amounts only in the liver. Humans express two HMG-CoA synthase genes, the mitochondrial HMGCS2 gene just described, and the HMGCS1 gene, which encodes a cytosolic enzyme that is involved in cholesterol biosynthesis. HMG-CoA in the mitochondria is converted to acetoacetate by the action of HMG-CoA lyase. This enzyme is also involved in catabolism of the amino acid leucine)). Acetoacetate can undergo spontaneous decarboxylation to acetone, or be enzymatically converted to β -hydroxybutyrate through the action of β -hydroxybutyrate dehydrogenase. The ketone bodies freely diffuse out of the mitochondria and hepatocytes and enter the circulation where they can be taken up by non-hepatic tissues such as the brain, heart, and skeletal muscle.



Ketone bodies are utilized by extra-hepatic tissues via a series of cytosolic reactions that are essentially a reversal of ketone body synthesis. Where tissues are utilizing ketones for energy production, $NAD^+/NADH$ ratios are relatively high, driving the β -hydroxybutyrate dehydrogenase-catalyzed reaction in the direction of acetoacetate synthesis. The second reaction of ketolysis involves the action of Succinyl-CoA:3-Oxoacid-CoA Transferase (SCOT) which is present at high levels in most tissues except the liver. The very low level of SCOT expression in the liver allows this organ to produce ketone bodies but not to utilize them. Among the regulators of ketogenesis is the release of free fatty acids from adipose tissue, which is effected by the activity of hormone-sensitive lipase (HSL). When glucose levels fall, pancreatic glucagon secretion increases resulting in phosphorylation of adipose tissue HSL, increasing hepatic ketogenesis due to increased free fatty acids delivery from adipose tissue. Conversely, insulin, released in the well-fed state, inhibits ketogenesis via the triggering of de-phosphorylation and inactivation of adipose tissue HSL.

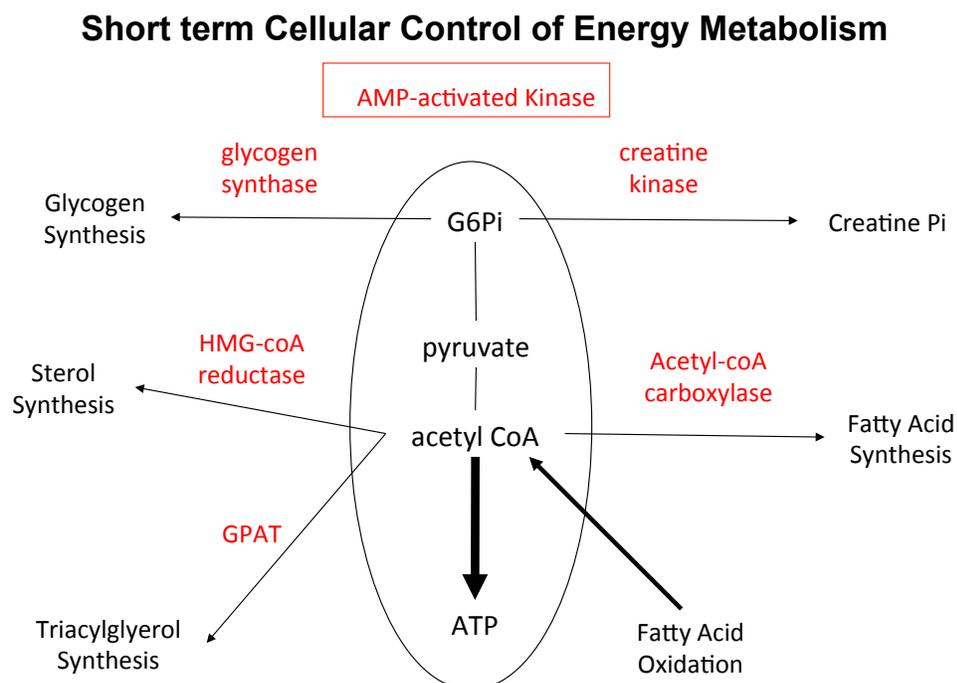
SELECTED READINGS

Multi-dimensional Roles of Ketone Bodies in Fuel Metabolism, Signaling, and Therapeutics. Puchalska P1, Crawford PA2 Cell Metab. 25:262-284 (2017)

Ketogenic diets, mitochondria, and neurological diseases. Gano LB, Patel M, Rho JM. J Lipid Res. 55:2211-28.(2014)

CONTROL BASED ON ATP LEVELS: AMP KINASE.

A key enzyme in initial regulation of glucose metabolism is AMP Kinase discussed briefly before. AMPK monitors the levels of ATP in the cell, and at low levels compared to the levels of AMP/ADP, this kinase blocks biosynthetic use of intermediates in glycolysis and conversion of glucose to glycogen by phosphorylation of glycogen synthase. The synthesis of sterols and triglycerides are both blocked by phosphorylation of HMG CoA reductase and GPAT respectively. This ensures that the vast majority of the glucose is converted to pyruvate, enters the Krebs cycle and produces NADH and succinate, which is then used by the oxidative phosphorylation machinery to make ATP.



Control by AMPK. The enzymes in red are inhibited by phosphorylation To favor ATP production when ATP levels are low.

AMP kinase also modifies the mTor complex, which is the master switch in control of cell fate by inhibiting autophagy, apoptosis. This complex also controls mitochondrial biosynthesis and levels of enzymes in a variety of pathways.

When ATP levels are high, particularly in cells and tissues with intermittently high-energy fluctuations, the enzyme creatine kinase (CK) takes ATP as is it generated by glycolysis and oxidative phosphorylation and (reversibly) transfers the “high energy” of γ -phosphate to the guanidino group of Cr to generate PCr and ADP. In this way ATP can be stored in cells for use when levels are below needed for functioning. The creatine/phosphocreatine cycling is particularly important in muscle and measurement of the ratio is an important measure of the efficiency of energy utilization in patients with mitochondrial disease. The reaction is controlled by reversible phosphorylation of CR by AMP kinase. Creatine has other important roles that make its use as a supplement useful, including that it acts as a free radical scavenger.

DECIDING BETWEEN THE USE OF GLUCOSE OR FATTY ACIDS FOR ENERGY (ATP) PRODUCTION: THE RANDLE CYCLE.

A high glucagon/insulin ratio, characteristic of the fasted state, stimulates adipose tissue lipolysis and hepatic glucose production (gluconeogenesis) to preserve whole body glucose supply to those tissues that rely exclusively on this sugar. However this is not the only control mechanism as there is feedback within the cell that is independent of hormone action. The so-called glucose-fatty acid cycle developed by Randle and colleagues, adds nutrient-mediated fine-tuning on top of the more coarse hormonal control. Thus in heart and skeletal muscle the utilization of glucose or fatty acids inhibits the use of the other directly, and without hormonal mediation. The net result is that hormones that

control adipose tissue lipolysis affect circulating concentrations of fatty acids, and fatty acids, in turn, control fuel selection in muscle. In the fasted state, activation of lipolysis provides tissues with fatty acids, which become the preferred fuel for respiration. In the liver, β -oxidation of fatty acids fulfills both the local energy needs and fuels ketogenesis. Ketone bodies are preferentially oxidized in extra-hepatic tissues. By inhibiting glucose oxidation, fatty acids and ketone bodies provide the circulating glucose needed for the brain (which cannot use fatty acids directly) during starvation. Further, inhibition of glucose oxidation at the level of pyruvate dehydrogenase (PDH) preserves pyruvate and lactate, both of which are gluconeogenic i.e. glucose producing precursors. Additionally, heart and muscle produce lactate from anaerobic respiration, which inhibits the oxidation of both glucose and fatty acids, adding to the glucose pool for the brain.

The glucose fatty acid cycle is not restricted to the fasting state and is readily observed in the fed state after a high-fat meal or during exercise, when plasma concentrations of fatty acids or ketone bodies increase. These conditions are a signal for part of the glucose that is not oxidized, to be re-routed to glycogen. The impairment of glucose metabolism by fatty acids (or ketone body) at the individual cell level is mediated by a short-term inhibition of glucose transport and phosphorylation (i.e modification of hexokinase), of 6-phosphofructo-1-kinase (PFK-1), and of PDH. The extent of inhibition is graded and increases along the glycolytic pathway, being most severe at the level of PDH and less severe at the level of glucose uptake and PFK. The initial event, triggered by fatty acid oxidation, is an increase in the mitochondrial ratios of [acetyl-CoA]/[CoA] and [NADH]/[NAD⁺], both of which inhibit PDH activity which leads to an accumulation of cytosolic citrate. This in turn inhibits PFK-1, followed by an increase in glucose 6-phosphate, which eventually inhibits hexokinase.

PDH activity is controlled by reversible phosphorylation of three serine residues in the α -subunit of E1 of the PDH complex by pyruvate dehydrogenase kinase (PDK)]. Phosphorylation inactivates PDH, whereas pyruvate dehydrogenase phosphatases (PDPs) have the opposite effect. Four different isoforms of PDK have been identified with different tissue expression and phosphorylation site specificity. This allows for control of PDH activity under a range of cell conditions.

CONTROL BASED ON NAD⁺ LEVELS.

NADH is a critical coenzyme for catabolic reactions. The ratio of the electron acceptor NAD⁺ to NADH is kept high in mitochondria and in the cytosol to capture electrons from enzyme reactions in cellular breakdown of glucose, fats and amino acids. In turn, NADH in mitochondria donates the electrons it receives to complex I of the respiratory chain to initiate electron transfer as part of oxidative phosphorylation. The ratio of NAD⁺/NADH is around 8 in mitochondria, where NADH is reduced rapidly by transferring electrons to Complex I. Mitochondrial NAD levels can be maintained for at least 24 hours, and as long as 3 days, after strong depletion of the cytoplasmic pool, indicating that the mitochondrial and cytosolic pools of NADH are separate. The maintenance of a high mitochondrial NAD pool is critical for several aspects of cell metabolism. It is now known that the activity of many metabolic enzymes is regulated by acetylation/deacetylation reactions involving histone-like acetylases and a set of NAD⁺-dependent deacetylases, the sirtuins, 7 of which have been identified in humans. The reaction of acetyl CoA with lysine residues by the acetylases occurs in mitochondria and in the cytosol. A recent proteomic study identified acetylation of over 130 different mitochondrial proteins, most of which are metabolic enzymes that are acetylated at

anywhere from 1 to 8 different lysines. De-acetylation by sirtuins yields O-acetyl-ADP-ribose, the de-acetylated substrate, and NAM (nicotinamide).

Multiple lines of evidence indicate that the acetylation/de-acetylation reactions in the cell are metabolically linked and add a level of control of metabolism above that provided by hormones, phosphorylation/dephosphorylation reactions, or the Randle cycle discussed above. Three of the 7 sirtuins are localized to mitochondria (sirtuins 3-5), four are in the nucleus and or cytosol. All use acetyl CoA to modify the lysine residues. This, along with the dependence of the sirtuins on NAD⁺, strongly suggests that acetylation/de-acetylation is directly linked to the energy status of the cell. Also, the acetylation of mitochondrial proteins is dynamically regulated by changes in nutrient status. For example, calorie restriction, a high fat diet, as well as fasting and re-feeding can all cause dramatic shifts in global mitochondrial protein acetylation. Moreover, conditions that increase demand for mitochondrial energy production, such as fasting, exercise, and calorie restriction, induce SIRT3 expression while aging and high fat diet reduce it.

Acetylation by as yet undefined acetylases, and de-acetylation by the different sirtuins, modulates almost all aspects of cell metabolism. The obvious question is how the acetyl modification of large numbers enzymes in multiple pathways, which in mitochondria includes oxidative phosphorylation, Krebs cycle, urea cycle and more, affect metabolic control globally. The answer, as yet remains elusive

Another important way in which NAD⁺/NADH modulates metabolism occurs in the nucleus and involves poly(ADP-ribose) (PAR) polymerase-1 (PARP-1). PARP-1 functions at the center of cellular stress responses, where it processes diverse signals and, in response, directs cells to specific fates (e.g., DNA repair vs. cell death) based on the type and strength of the stress stimulus. Many of PARP-1's functions in stress response pathways are

mediated through its regulation of PAR synthesis. PAR is a negatively charged polymer that uses NAD⁺ as a donor of ADP-ribose units. Thus, PARP-1's functions are intimately tied to nuclear NAD⁺ metabolism and the broader metabolic profile of the cell. Recent studies in cell and animal models have highlighted the roles of PARP-1 and PAR in the response to a wide variety of extrinsic and intrinsic stress signals, including those initiated by oxidative, nitrosative, genotoxic, oncogenic, thermal, inflammatory, and metabolic stresses.

CONTROL BASED ON O-GLcNACYLATION.

O-GlcNAcylation has emerged as an important regulatory mechanism underlying normal liver physiology and liver diseases. This post-translational modification uses UDP-GlcNAc as the substrate for the attachment of the acetylglucosamine (GlcNAc) moiety to the hydroxyl groups of serine and threonine residues of proteins. Since UDP-GlcNAc is synthesized via the hexosamine biosynthesis pathway (HBP), which involves nutrients such as glucose, free fatty acids, uridine, and glutamine, O-GlcNAcylation serves as a nutrient sensor, tuning various cellular processes in response to systemic metabolic status.

The cycling of O-GlcNAcylation involves O-GlcNAc transferase (OGT) which catalyzes the addition of O-GlcNAc to proteins, and O-GlcNAcase (OGA, which catalyzes its removal. This dynamic modification is prevalent on signaling proteins, transcription factors, metabolic enzymes, and histones, and has emerged as a key regulator of diverse cellular processes, including signal transduction, gene expression, and protein degradation. As discussed already, the response on eating to insulin is orchestrated by a set of phosphorylation/dephosphorylation reactions. Surprisingly, in parallel there are dynamic changes in O-GlcNAc modification of proteins that influence protein function by regulating protein–protein

interaction, protein stability, nuclear-cytoplasmic shuttling, and intrinsic protein activity.

In response to prolonged insulin stimulation, OGT translocates from the cytoplasm to the plasma membrane, leading to phosphorylation and activation of OGT by the insulin receptor. Active OGT is known to O-GlcNAcylate and deactivate key insulin signaling proteins, including IRS-1, PI3K, PDK1, and AKT, thereby facilitating insulin signal attenuation.

Insulin receptor substrate deactivation is an important mechanism for terminating insulin signaling. IRS-1 is a direct substrate of OGT.

Increased O-GlcNAcylation of IRS-1. PI3K and PDK1 are also direct substrates of OGT and are implicated in insulin signaling attenuation.

Decreased AKT activity is essential for insulin signal termination.

Increased O-GlcNAcylation of AKT decreases the activity of this kinase, thereby disturbing the AKT/PDK1 interaction. Decreased AKT activity also reduces glycogen synthesis by decreasing phosphorylation of GSK3 β . GSK3 β is also modified by O-GlcNAcylation. O-GlcNAcylation of glycogen synthase itself is responsive to high glucose or glucosamine treatment and reduces the activity of the enzyme.

SELECTED READINGS

Metabolic regulation by SIRT3: implications for tumorigenesis. Finley LW, Haigis MC. Trends Mol Med. 18:516-23. (2012).

*Nicotinamide phosphoribosyltransferase (Nampt): A link between NAD biology, metabolism, and diseases. Imai S. *Curr Pharm Des* 15 20-28 (2009).*

The dynamic regulation of NAD metabolism in mitochondria

*Stein LS and Imai S. *Trends Endocrinol Metab* 23 420-28 (2012).*

*Mitochondrial protein acetylation regulates metabolism. Anderson KA and Hirschey MD *Essays Biochem* 52 1042 (2012).*

*Sirtuins in metabolism, DNA repair and cancer Mei Z. Zhang X. Yi Y. Huang J, He J and Tao Y. *Journal of Experimental & Clinical Cancer Research* 35:182 (2016).*

The Randle cycle revisited: a new head for an old hat

*Hue L. Taegtmeyer H. *American Journal of Physiology - Endocrinology and**

Metabolism. 297. 3 (2009)

AMP-activated protein kinase: Ancient energy gauge provides clues to modern understanding of metabolism.

Kahn BB, Alquier T, Carling D, Hardie, DG. *Cell Metabolism* 1. 15-25 (2005).

O-GlcNAc: A Bittersweet Switch in Liver. Zhang K, Yin R, and Yang X
Front Endocrinol. 5. 22132 (2014)

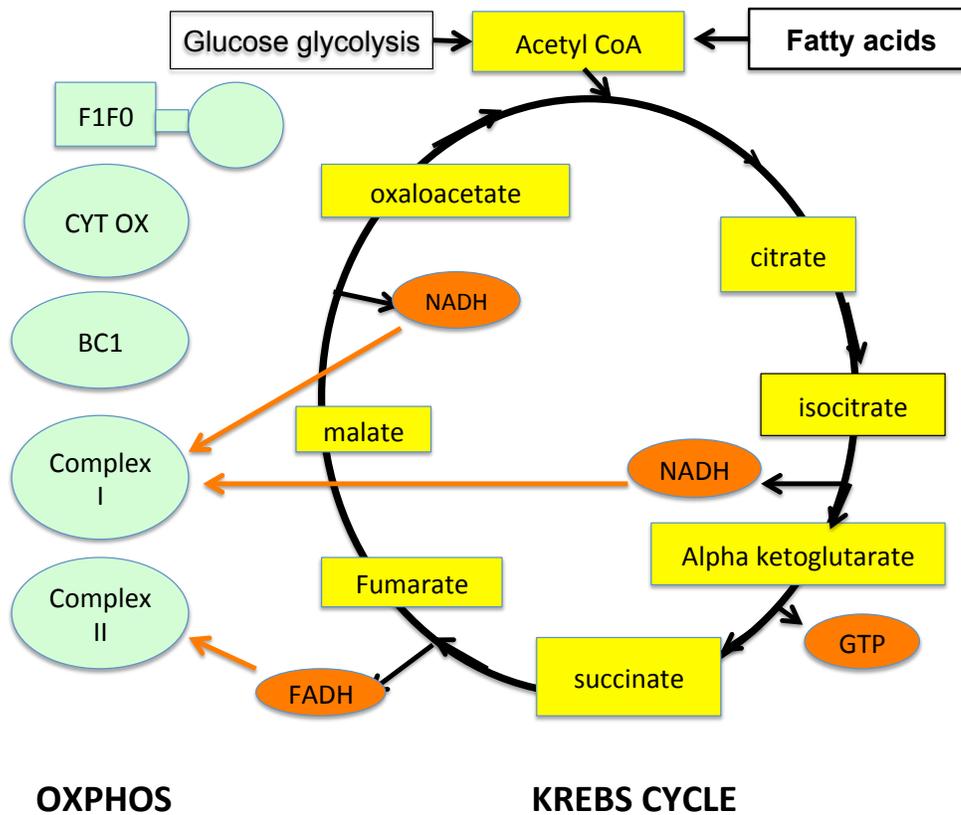
O-GlcNAcylation: a bridge between glucose and cell differentiation. Sun C, Shang J, Yao Y, Yin X, Liu M, Liu H, Zhou Y. *J Cell Mol Med.* 20. 769-81. (2016)

THE ATP PRODUCING STEPS: KREBS CYCLE AND OXIDATIVE PHOSPHORYLATION.

Two processes are involved in the final steps of generating ATP from glucose or from fatty acids and these are the Krebs cycle and oxidative phosphorylation respectively. As shown, glycolysis yields pyruvate, which is converted to acetyl CoA for entry into the Krebs cycle at citrate synthase. Fatty acids are degraded stepwise by the enzymes of the beta oxidation pathway to yield acetyl CoA which is reacted on by citrate synthase. The metabolism of glucose to produce ATP yields net 2 ATPs from the glycolysis steps and 36 ATPs from electron transfer coupled ATP. The overall reaction then is $C_6H_{12}O_6 + 6 O_2 \rightarrow 6 H_2O + 6 CO_2$. Contrast this with the full oxidation of the fatty acid palmitate where the overall reaction is $Palmitoyl-CoA + 23O_2 + 131Pi + 131ADP \rightarrow Palmitoyl-CoA + 131ATP + 16CO_2 + 146H_2O$. Clearly, oxidation of fatty acids can provide more energy per mole than glucose.

A key issue for patients with mitochondrial disease is how efficient ATP production is. Based on a capture of 7.3 kcal per mole of ATP, and a yield of 38 ATP per mole of glucose, oxidative phosphorylation yields around 280Kcal out of the maximal of 686kcal per mole possible, for an efficiency of around 40%. Energy not captured in

ATP is lost as heat. Note that the best car engine has an efficiency of less than 20%.



Optimal conditions means that the reactants including oxygen are present in equimolar amounts, which is never the case, and that the reaction occurs at 25 degrees C instead of the biologically relevant 37degrees C. Taking account of these factors, efficiency is probably higher i.e close to 60%.

Other issues combine to reduce the overall efficiency of conversion of glucose to ATP in vivo. This includes the presence of uncoupler proteins, and use of the membrane potential and pH gradient for ion transport functions instead of ATP synthesis. Also defects in the respiratory chain and ATP synthesis, as in mitochondrial diseases, further reduce efficiency and have other unwanted

side effects discussed below.

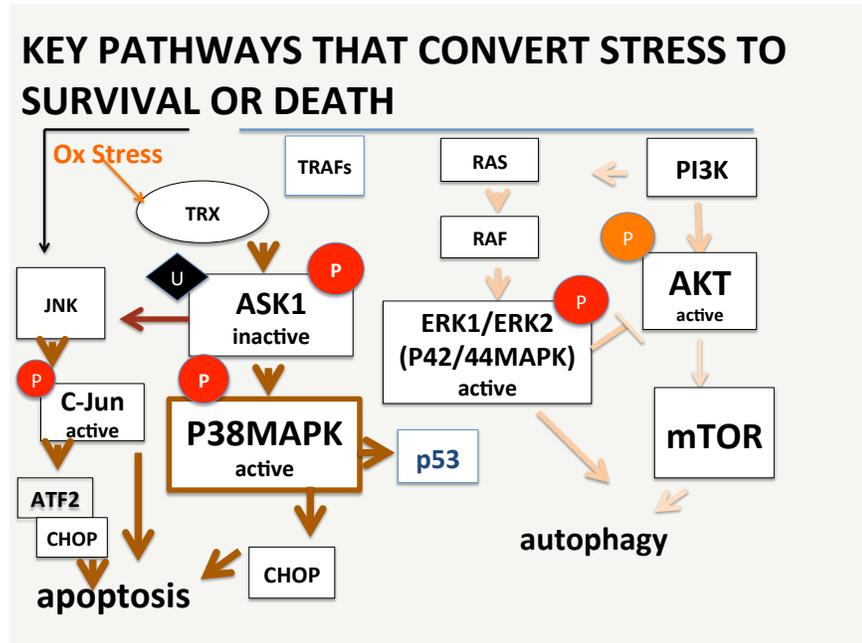
OXPHOS AND FREE RADICAL GENERATION.

One important consequence of slower rates of electron transfer, either because of component protein modifications or lack of sufficient O₂, is the generation of free radicals. At low levels, free radicals have important signaling functions: at higher levels these are toxic.

Under normal conditions of OXPHOS, approximately 1 to 3% of all electrons leak from the system and produce superoxide (O₂⁻), which is rapidly converted to the less toxic H₂O₂ by superoxide dismutases in the cell. Other ROS can be produced within mitochondria or in the cytosol that are of physiological significance including hydroxyl radical (•OH). Complexes I and III are thought to be the major contributors to ROS generation but complex II and cytochrome c oxidase can be involved under some conditions. Hydrogen peroxide is also produced by xanthine oxidase, amino acid oxidase, and NAD(P)H oxidase and in peroxisomes by consumption of molecular oxygen in metabolic reactions. In addition to ROS, cells also make toxic NOS from nitric oxide. NO is generated in biological tissues by specific nitric oxide synthases (NOSs), which metabolize arginine to citrulline with the formation of NO via a five electron oxidative reaction. NO acts as an important oxidative biological signaling molecule in a large variety of diverse physiological processes, including neurotransmission, blood pressure regulation, cell defence mechanisms, smooth muscle relaxation and immune regulation. It has a half-life of only a few seconds in an aqueous environment, but greater stability when oxygen concentrations are low (half life >15 s). Since it is soluble in both aqueous and lipid media, NO readily diffuses through the cytoplasm and plasma membranes. It reacts rapidly with superoxide anion to form the highly reactive product peroxynitrite (ONOO⁻). Peroxynitrite reacts with proteins to form nitrotyrosine (3-NT).

To combat toxic build up of free radicals, cells have considerable

antioxidant defences to detoxify ROS and NOS, which are activated through transcriptional regulation. These include superoxide dismutases in mitochondria (SOD2) and in mitochondria (SOD1), catalase, heme oxygenase-1 thioredoxins (TRXs), peroxiredoxins (PRXs), and glutaredoxins.



Common to these antioxidants is the requirement of NADPH as a reducing equivalent. NADPH maintains catalase in the active form and is used as a cofactor by TRX and GSH reductase. Intracellular NADPH, in turn, is generated by the reduction of NADP⁺ by glucose-6-phosphate dehydrogenase, the first and rate-limiting enzyme of the pentose phosphate pathway, during the conversion of glucose-6-phosphate to 6-phosphogluconolactone. By generating NADPH, glucose-6-phosphate dehydrogenase is a critical determinant of cytosolic glutathione (GSH) buffering capacity (GSH/GSSG) and, therefore, can be considered an essential, regulatory antioxidant enzyme.

THE SPECIAL ROLE OF GLUTATHIONE.

Reduced GSH is a powerful antioxidant in the cell armory. It can donate its electron to H₂O₂ to reduce it into H₂O and O₂. GSH is a linear tri-peptide of L-glutamine, L-cysteine, and glycine.

As electrons are lost, this molecule becomes oxidized, leading to dimerization by a disulfide bridge to form glutathione disulfide or oxidized glutathione (GSSG). This linkage is reversible upon re-reduction. GSH is under tight homeostatic control.

A dynamic balance is maintained between the synthesis, its recycling from GSSG/oxidized glutathione, and its utilization.

GSH synthesis involves two closely linked, enzymatically-controlled reactions that utilize ATP. First, cysteine and glutamate are combined by gamma-glutamyl cysteinyl synthetase. Second, GSH synthetase combines gamma-glutamylcysteine with glycine to generate GSH. As GSH levels rise, they self-limit further GSH synthesis; otherwise, cysteine availability is usually rate-limiting. Fasting, protein-energy malnutrition, or other dietary amino acid deficiencies limit GSH synthesis.

GSH recycling is catalyzed by glutathione disulfide reductase, which uses reducing equivalents from NADPH to reconvert GSSG to 2GSH.

SELECTED READING

The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Kurutas EB. Nutrition J. 15. 71-80 (2016).

Oxidative stress in health and disease: The therapeutic potential of Nrf2 activation. Hybertson B, Gao B, Bose K., McCord JM. Molecular Aspects of Medicine 32. 234-46 (2011).

*Hypoxia-Induced **Oxidative Stress** Modulation with Physical Activity.* Debevec T, Millet GP, Pialoux V. Front Physiol. 8:84. (2017)

Modulation of cellular thiol status affects FoxO activity and life span. Urban N, Tsitsipatis D, Gille A, Hamann I, Hou X, Klotz LO2 Free Radic Biol Med. 75 Suppl 1:S53 (2014)

