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REVIEWS: CURRENT TOPICS

Regulatory role for the arginine–nitric oxide pathway in metabolism of energy substrates

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Abstract

Nitric oxide (NO) is synthesized from L-arginine by NO synthase in virtually all cell types. Emerging evidence shows that NO regulates the metabolism of glucose, fatty acids and amino acids in mammals. As an oxidant, pathological levels of NO inhibit nearly all enzyme-catalyzed reactions through protein oxidation. However, as a signaling molecule, physiological levels of NO stimulate glucose uptake as well as glucose and fatty acid oxidation in skeletal muscle, heart, liver and adipose tissue; inhibit the synthesis of glucose, glycogen, and fat in target tissues (e.g., liver and adipose); and enhance lipolysis in adipocytes. Thus, an inhibition of NO synthesis causes hyperlipidemia and fat accretion in rats, whereas dietary arginine supplementation reduces fat mass in diabetic fatty rats. The putative underlying mechanisms may involve multiple cyclic guanosine-3′,5′-monophosphate-dependent pathways. First, NO stimulates the phosphorylation of adenosine-3′,5′-monophosphate-activated protein kinase, resulting in (1) a decreased level of malonyl-CoA via inhibition of acetyl-CoA carboxylase and activation of malonyl-CoA decarboxylase and (2) a decreased expression of genes related to lipogenesis and gluconeogenesis (glycerol-3-phosphate acyltransferase, sterol regulatory element binding protein-1c and phosphoenolpyruvate carboxykinase). Second, NO increases the phosphorylation of hormone-sensitive lipase and perilipins, leading to the translocation of the lipase to the neutral lipid droplets and, hence, the stimulation of lipolysis. Third, NO activates expression of peroxisome proliferator-activated receptor-γ coactivator-1α, thereby enhancing mitochondrial biogenesis and oxidative phosphorylation. Fourth, NO increases blood flow to insulin-sensitive tissues, promoting substrate uptake and product removal via the circulation. Modulation of the arginine–NO pathway through dietary supplementation with L-arginine or L-citrulline may aid in the prevention and treatment of the metabolic syndrome in obese humans and companion animals, and in reducing unfavorable fat mass in animals of agricultural importance.

Keywords: Amino acids; Glucose; Lipid; Mitochondria; Nitric oxide; Oxidation

1. Introduction

Fat deposition in humans or animals depends on the balance between dietary caloric intake and whole-body energy expenditure. A chronic imbalance in energy metabolism (more energy input than energy output) due to complex genetic and/or environmental factors results in excess fat accretion or obesity in humans, which is currently a major public health problem worldwide [1,2]. Recent data from the 1999 to 2000 National Health and Nutrition Examination Survey show that 65% and 31% of the U.S. adult population are overweight (a body mass index >25 kg/m^2) and obese (a body mass index >30 kg/m^2), respectively [1]. The prevalence of overweight and obesity increased by 16% and 35%, respectively, compared with the survey conducted between 1988 and 1994. Children and adolescents have not been immune to this epidemic, as 15% of them in the United States are obese, representing a 36% increase within the past decade [1]. Most other countries are also experiencing the obesity crisis. For example, the prevalence of overweight in China doubled in women and

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almost tripled in men between 1989 and 1997 [2]. Worldwide, more than 300 million adults are obese and over 1 billion are overweight [1]. Obesity is a major risk factor for insulin resistance, type II diabetes, atherosclerosis, stroke, hypertension, impaired vascular function, sleep disorders and some types of cancer (including colon and breast cancers) [3]. Consequently, obesity claims an increasing number of lives and contributes to tremendous costs of health care worldwide. In the United States alone, about 300,000 people die of obesity-related diseases every year, the incidence of type II diabetes among children has increased 10-fold over the past decade and obesity accounts for 6–8% of all health care expenditures [1]. Unfortunately, clinicians have few tools to fight the obesity epidemic, because current antiobesity drugs are not highly effective and are fraught with side effects [3]. Because even a modest (5–7%) weight loss in obese subjects is associated with a significant improvement in risk factors for cardiovascular disease and diabetes [3], identifying new means to reduce body fat will be extremely beneficial for human health.

Nitric oxide (NO), a signaling molecule that regulates nutrient metabolism, is a free radical produced from L-arginine by various isoforms of NO synthase (NOS). This synthetic reaction occurs in virtually all mammalian cells and tissues, including adipocytes, brain, endothelial cells, heart, hepatocytes, macrophages and skeletal muscle [4–8]. As a small hydrophilic gas molecule, NO readily diffuses into cells and acts on the heme of target molecules. Through multiple cyclic guanosine-3′,5′-monophosphate (cGMP)-dependent pathways, NO plays a crucial role in regulating vascular tone, neurotransmission, host immunity and whole-body homeostasis [9,10]. Excitingly, we recently found that dietary supplementation with L-arginine reduced fat mass (Fig. 1) and enhanced expression of key genes responsible for glucose and fatty acid oxidation (Fig. 2) in the Zucker diabetic fatty (ZDF) rat, an animal model of type 2 diabetes secondary to a defect in the leptin receptor [11]. Physiological levels of NO stimulate glucose uptake and oxidation as well as fatty acid oxidation in insulin-sensitive tissues (muscle, heart, liver and adipose tissue); inhibit the

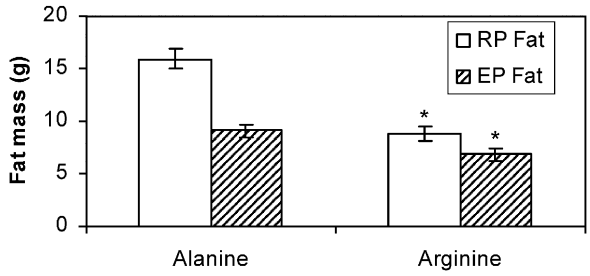

Fig. 1. Dietary L-arginine supplementation reduced fat mass in Zucker-diabetic fatty rats. Zucker diabetic fatty rats (9 wk old) received drinking water containing either 1.51% L-arginine–HCl or 2.55% L-alanine (isonitrogenous control) for 10 weeks. *P<.01 vs. the alanine control group. RP, retroperitoneal; EP, epididymal. Data are adapted from Fu et al. [11].

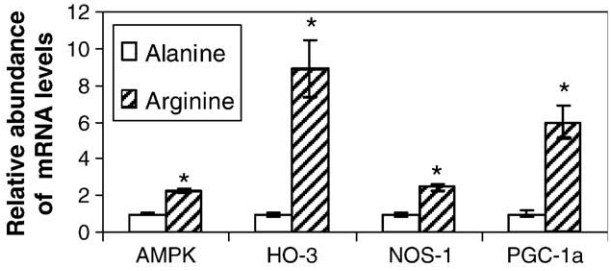

Fig. 2. Dietary arginine supplementation enhanced expression of key genes for mitochondrial substrate oxidation. Abbreviations used: HO-3, heme oxygenase-3; NOS-1, NO synthase-1. Values of mRNA in the isonitrogenous control ZDF rats are 0.13±0.01, 2.25±0.26, 0.053±0.006 and 0.045±0.01 (means±S.E.M., n=6), respectively, for AMPK, HO-3, NOS-1 and PGC-1α. *P<.01 vs. the alanine control group. Data are adapted from Fu et al. [11].

synthesis of glucose, glycogen and fat in target tissues (e.g., liver and adipose); and enhance lipolysis in adipocytes (Fig. 3). The underlying mechanisms may involve multiple cGMP-dependent pathways: (1) phosphorylation of adenosine-3′,5′-monophosphate-activated protein kinase (AMPK), (2) phosphorylation of hormone-sensitive lipase (HSL) and perilipins, (3) increased expression of peroxisome proliferator-activated receptor-γ (PPAR-γ) coactivator 1α and (4) increased blood flows to tissues. Modulation of the arginine–NO pathway may provide a potentially novel means to reduce fat mass in obese subjects. The major objective of this article is to critically review the role for NO in the regulation of the interorgan metabolism of energy substrates (fatty acids, glucose and amino acids) as well as its nutritional and pathological implications.

2. Nitric oxide synthesis in animal cells

2.1. Nitric oxide synthases

The biosynthesis of NO requires L-arginine (a basic amino acid at physiological pH) and oxygen as substrates, as well as tetrahydrobiopterin (BH4), NADPH, Ca^{2+}, calmodulin, FMN and FAD [5]. Nitric oxide synthase can be present in plasma membrane, cytoplasm, nucleus, rough endoplasmic reticulum and mitochondria of cells (Table 1). There are three isoforms of the NOS: nNOS (type 1 NOS), which was first discovered in neuronal tissues; iNOS (type 2 NOS), which was originally found to be inducible under certain conditions in macrophages and hepatocytes; and eNOS (type 3 NOS), which was first identified in endothelial cells. These NOS isoforms are encoded by three different genes, and their nucleotide sequences are 51–57% homologous [9]. Notably, there is complex intracellular compartmentation for the NOS isoforms in animal cells and tissues [28] (Table 1). In general, eNOS, nNOS and iNOS are localized primarily in plasma membrane and cytoplasm, mitochondria and cytoplasm, and cytoplasm, respectively. nNOS and eNOS are expressed constitutively at low levels

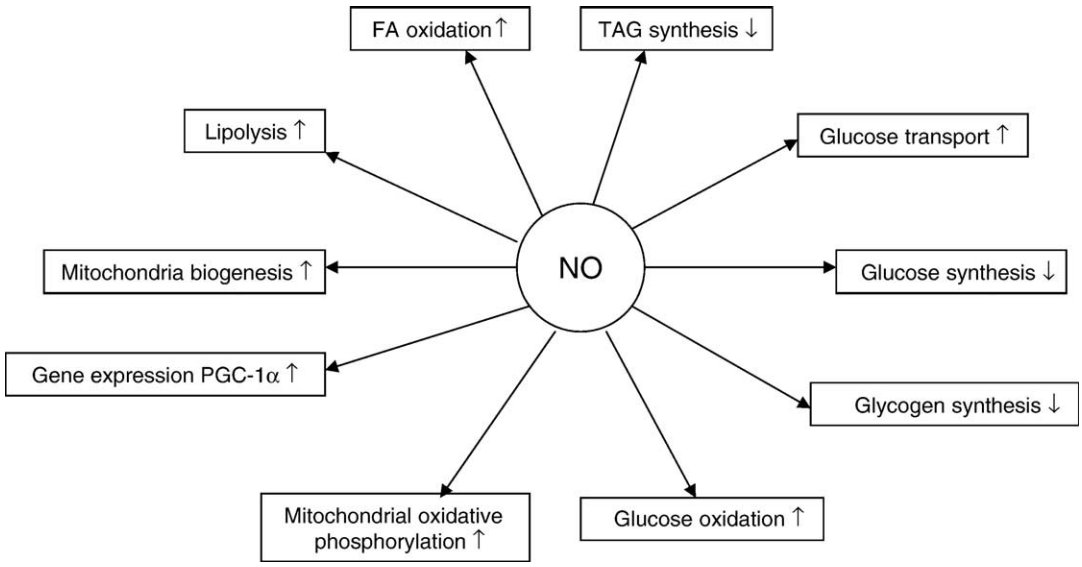

Fig. 3. Overall effects of physiological levels of NO on energy substrate metabolism. Nitric oxide increases fatty acids oxidation by reducing malonyl-CoA availability and decreases TAG synthesis by inhibiting GPAT activity. In addition, NO increases the basal lipolysis and mediates leptin-stimulated lipolysis. Nitric oxide also stimulates glucose transport in muscle and adipose tissue and increases glucose oxidation while decreasing hepatic glycogen synthesis. Recent studies also showed that NO increases mitochondrial biogenesis via activating PGC-1α expression.

in a variety of cell types and tissues, whereas iNOS is normally not expressed at a significant level in cells or tissues [5]. When induced by certain immunological stimuli (including inflammatory cytokines or bacterial endotoxin), iNOS is highly expressed in many cell types and produces a large amount of NO. Due to its high affinity for calcium and tight binding with calmodulin, iNOS is fully active in the absence of exogenous Ca^{2+} or calmodulin. All of the NOS isoforms can be induced under certain stimuli through transcriptional and translational mechanisms [5]. The intracellular compartmentation of NOS isoforms may play a role in regulating NO synthesis in animal cells. The NOS family of isoforms is the most recent addition to a group of only four other enzymes (phenylalanine hydroxylase, tyrosine hydroxylase, tryptophan hydroxylase and alkylglycerol monooxygenase) that are known to utilize a biopterin cofactor. Arginine, BH4 and heme promote formation of and also stabilize the active dimeric form of all isoforms of the NOS [9].

A small quantity of NO is produced by eNOS and nNOS, relative to the amount of NO generated by iNOS. For example, NO production by unstimulated bovine venular endothelial cells is 0.24 nmol per 10^6 cells/h [6], which is only approximately 7% of that by LPS-activated RAW 264.7 macrophages (3.3 nmol per 10^6 cells/h) [29]. Similarly, in vivo systemic production of NO is low in rats (e.g., 15 μmol/kg body weight per 24 h) but increases 15-fold in response to immunological challenge [7]. Compelling evidence shows that NO synthesis is regulated not only by the amount and/or phosphorylation of the NOS protein, but also by the availability of cofactors (particularly NADPH, BH4 and Ca^{2+}) and arginine [8]. Although the Km values of NOS

Table 1
Compartmentalization of the NOS isoforms in animal tissues

Organ	nNOS (Type 1)	iNOS (Type 2)	eNOS (Type 3)	Refs.
Brain	Cytoplasm, mitochondria, RER, postsynaptic membrane	Primarily cytoplasm	Cytoplasm, plasma membrane caveolae	[9,12,13]
Brown adipose	Weakly expressed in cytoplasm	Cytoplasm, nucleus	Cytoplasm, nucleus, plasma membrane caveolae	[13,14]
Heart	Sarcoplasmic reticulum, mitochondria	Primarily cytoplasm	Plasmalemmal and T-tubular caveolae, sarcoplasm	[13,15]
Kidneys	Mitochondria	Primarily cytoplasm	Cytoplasm, plasma membrane caveolae	[9,13]
Liver	Mitochondria	Primarily cytoplasm in periportal hepatocytes	Cytoplasm, plasma membrane, caveolae in hepatocytes and blood vessels	[13,16]
Skeletal muscle	Mitochondria, sarcoplasm, sarcolemmal membrane	Sarcoplasm, sarcolemmal membrane	Sarcoplasm, SR, sarcolemmal membrane caveolae	[12,17–25]
White adipose	Weakly expressed in cytoplasm	Cytoplasm, plasma membrane	Cytoplasm, plasma membrane caveolae	[26,27]

RER, rough endoplasmic reticulum; SR, sarcoplasmic reticulum.

for arginine are 3 to 20 μM, depending on its isoform, increasing extracellular arginine concentrations from 0.05 to 5 mM dose dependently increases NO synthesis in a variety of cells, including activated macrophages and endothelial cells [5]. It is now clear that arginine increases the transcription of iNOS in macrophages [4] and BH4 synthesis in endothelial cells [10], whereby arginine stimulates NO production in both cell types. Additionally, arginine-dependent NO synthesis is affected by various amino acids, including glutamine, lysine, glutamate, phenylalanine and taurine [8].

2.2. Nitric oxide synthase expression in insulin-sensitive tissues

Because of an important role for skeletal muscle, adipose tissue, heart and liver in NO synthesis [12,17–21] and the metabolism of energy substrates [3,22], here we review briefly the pertinent literature related to NOS expression in these insulin-sensitive tissues. In mammalian skeletal and cardiac muscle, all three isoforms of the NOS are constitutively expressed [12,17–21]. The NOS isoforms are present mainly in sarcolemma (plasma membrane), sarcoplasm (cytoplasm), sarcoplasmic reticulum and mitochondria of muscle fibers [23,28]. In addition to myocytes, all three NOS isoforms can be detected in capillaries and arterioles of the skeletal musculature [28]. The expression and activity of the NOS in skeletal muscle are regulated by several factors, which include fiber type, contraction, developmental stage and pathological state [21,24,25]. Brown adipose tissue expresses both eNOS and iNOS in the cytoplasm and nucleus, as well as eNOS in the plasma membrane [14]. In contrast, both eNOS and iNOS appear to be absent from the nucleus but are present in the cytoplasm and plasma membrane in white adipose tissue [26,27]. Interestingly, nNOS is weakly expressed only in the cytoplasm of brown and white adipose tissues and is absent from mitochondria or plasma membrane [14,26,27]. In liver, eNOS is uniformly distributed in hepatocytes and in the endothelium of hepatic arteries, terminal hepatic venules, and sinusoids, and the epithelium of biliary ducts, whereas iNOS is constitutively expressed primarily in the cytoplasm of periportal hepatocytes [16]. Although still under debate, most biochemical and genetic studies have shown that nNOS is the isoform found in the mitochondria of brain, heart, kidneys, liver and skeletal muscle [13]. Thus, the insulin-sensitive tissues are significant sites for NO production in the body, and the compartmentation of NO synthesis is likely related to specific cell functions.

2.3. Nitric oxide targets

The target cells for NO action can be the NO-producing cell itself, the neighboring cells via its direct release or the distant cells via transport by glutathione and red blood cells [25]. The soluble guanylyl cyclase is a major receptor for physiological levels of NO [30]. The membrane-permeable NO binds to the prosthetic heme group of the enzyme, thereby activating the production of cGMP from guanosine triphosphate in target cells. Cyclic guanosine-3′, 5′-monophosphate is an intracellular signaling molecule, which plays an important role in regulating various cellular events through activation of cGMP-dependent protein kinase G (PKG), cGMP-gated ion channels and cGMP-dependent phosphodiesterases [13,30,31]. As a highly reactive free radical molecule, high levels of NO produced by iNOS can oxidize biomolecules (including proteins, fatty acids and DNA), thereby damaging cell membranes, inhibiting vital biochemical reactions (e.g., the mitochondrial Krebs cycle and respiratory chain) and even causing cell death [32]. Therefore, effects of NO on cell metabolism critically depend on its concentrations at the reaction site.

3. Interorgan metabolism of energy substrates

3.1. Small intestine

The metabolism of dietary energy substrates in animals and humans requires the cooperation of multiple organs and tissues, particularly the small intestine, liver, skeletal muscle, heart, kidneys, brain and adipose tissue (Fig. 4). The small intestine is responsible for the terminal digestion of fat, protein and carbohydrate in monogastric animals, whereas fermentation of these substances occurs in the rumen of ruminants to produce short-chain fatty acids (acetate, propionate and butyrate). The small intestine is also the primary site for the absorption of fatty acids, glucose, amino acids and other nutrients in both monogastric and ruminant animals [33]. Indeed, in most mammals (including humans, pigs, cattle and rats), extensive catabolism of dietary glutamine, glutamate and aspartate takes place in enterocytes as major fuels [33]. Glutamine, glutamate and proline are substrates for the net production of both citrulline and arginine in neonates and of citrulline in adults [5]. The circulating citrulline is taken up by extraintestinal and extrahepatic tissues (primarily the kidneys) and by almost all cell types (except for hepatocytes) for conversion into arginine by argininosuccinate synthase and argininosuccinate lyase [5]. The small intestine also plays an important role in the catabolism of many dietary essential and nonessential amino acids (including branched-chain amino acids, lysine, phenylalanine, methionine, glycine and serine), although the responsible cell types have not yet been identified [33]. Interestingly, oxidation of glucose and long-chain fatty acids (LCFAs) is limited in the small intestine under fed conditions. After feeding, the small intestine releases amino acids, glucose and lipoproteins (mainly as chylomicrons and very low-density lipoprotein, VLDL) [34]. Amino acids and glucose enter the portal vein through the mucosal vasculature and then reach the liver. In mammals, triacylglycerols (TAGs) packaged as soluble chylomicrons and VLDL leave the small intestine via exocytosis, are transported through lymph

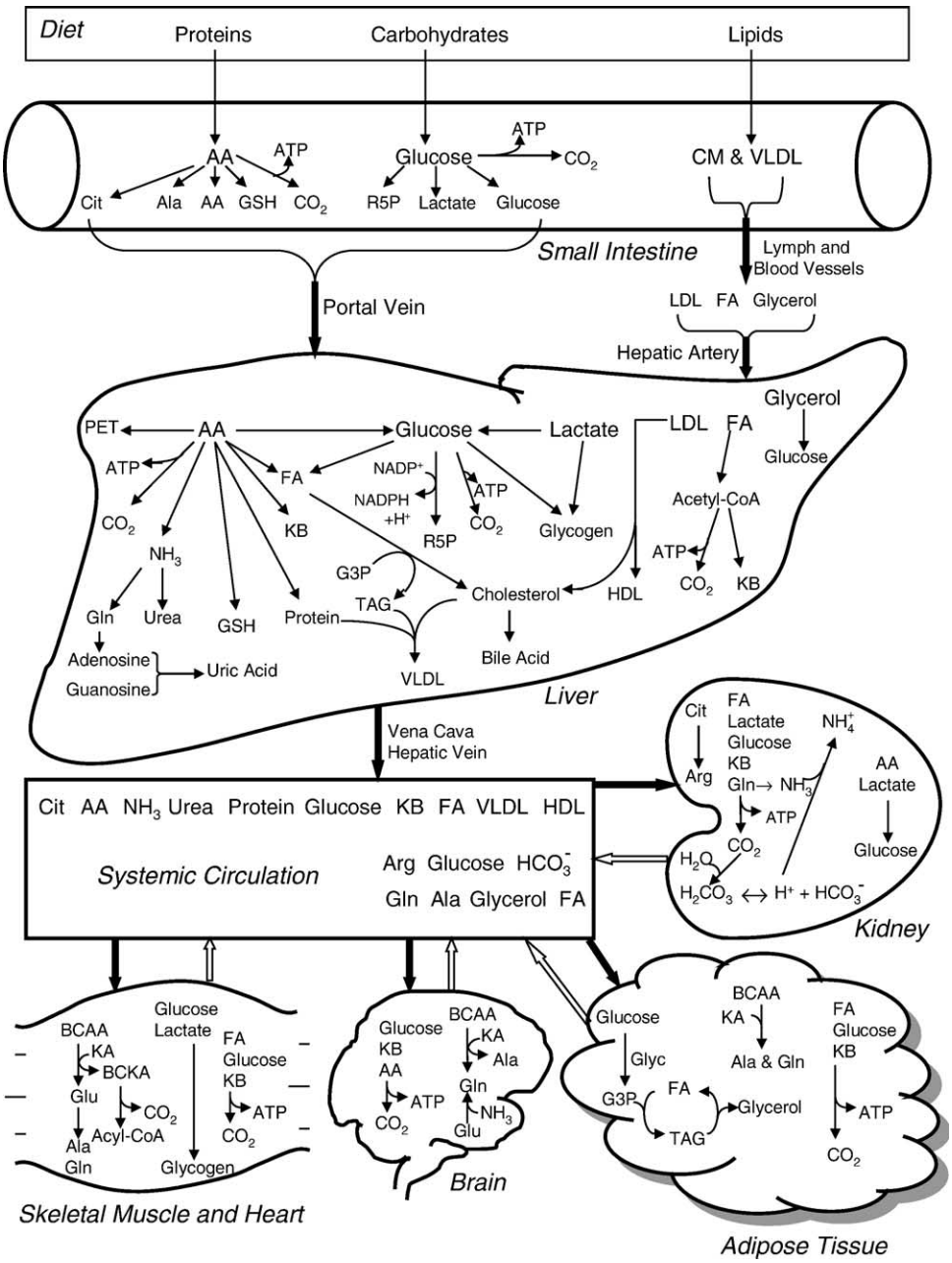

Fig. 4. Interorgan metabolism of dietary energy substrates in animals and humans. Proteins, carbohydrates and lipids are utilized for the provision of energy via multiple metabolic pathways and organs. Products of the digestion of these energy substrates reach the liver through lymphatic and blood vessels, and the metabolites of amino acids, glucose and fatty acids produced in various tissues enter the systemic circulation. Abbreviations: AA, amino acids; Ala, alanine; Arg, arginine; BCAA, branched-chain amino acids; BCKA, branched-chain α-ketoacid; Cit, citrulline; CM, chylomicrons; FA, fatty acids; Gln, glutamine; Glu, glutamate; GSH, glutathione; G3P, glycerol-3 phosphate; KA, α-ketoacids (pyruvate and α-ketoglutarate); KB, ketone bodies, PET, peptide; and R5P, ribulose-5-phosphate.

vessels and enter blood circulation through the thoracic duct and cranial vena cava. In birds, because the intestinal lymphatic system is poorly developed and because VLDL contains a much lower content of TAG than the mammalian chylomicrons and VLDL, the VLDL synthesized in the avian small intestine is absorbed directly into the portal vein. Absorption of LCFAs and release of chylomicrons by the small intestine of ruminants are much lower in comparison with monogastric animals because the lower content of dietary fat (<5% of dry matter intake) is necessary for not interfering with ruminal microbial metabolism [35]. Lipoprotein lipase (LPL), which is present on the wall of blood capillaries in peripheral tissues, hydrolyzes the TAG of chylomicrons and VLDL to form free fatty acids, glycerol and low-density lipoproteins (LDL). Low-density lipoprotein is the major carrier of cholesterol in humans, pigs, rabbits, sheep and guinea pigs [35]. During fasting, ketone bodies produced from the liver are the major energy substrates for

Table 2
Species differences in de novo fatty acid synthesis in liver and white adipose tissue

Species	Liver	Adipose tissue	Refs.
Cat	Yes	Yes	[35]
Chicken	Yes	No	[35]
Dog	Yes	Yes	[35]
Human	Yes	No	[37,38]
Mouse	Yes	Yes	[35,39]
Pig	No	Yes	[40]
Rabbits	Yes	Yes	[35]
Rat (adult)	Yes	No	[11,22]
Rat (young)	Yes	Yes	[22]
Ruminants	No	Yes	[35]

Ruminants: cattle, goats and sheep.

the small intestine of most animals except for the pigs that have limited hepatic ketogenesis and thus very low plasma levels of these fuels.

3.2. Liver

Although the liver represents only 2–3% of the total body weight, it accounts for approximately 20% of the total energy expenditure under basal conditions [34]. Gluconeogenesis (formation of glucose from noncarbohydrate precursors), ketogenesis (production of acetoacetate and β-hydroxybutyrate), amino acid oxidation, urea cycle and syntheses of cholesterol, bile acids and VLDL occur primarily in the liver. The liver also synthesizes and releases high-density lipoproteins (HDLs) into the circulation. Importantly, this organ is a major site for the oxidation of fatty acids in all animals studied, including humans, pigs, cattle, rats, mice and chickens [35]. During fasting, the rates of gluconeogenesis (from amino acids, lactate and glycerol in all animals and also from propionate in ruminants), glycogenolysis and ketogenesis (from acetyl-CoA) in liver increase markedly to supply glucose and ketone bodies to the brain, peripheral tissues and cells of the immune system as well as glucose for red blood cells [34]. Because of the blood–brain barrier, plasma LCFAs cannot be utilized for oxidation by the brain [35], and instead, ketone bodies serve as major energy substrates for this tissue when plasma glucose level is reduced (e.g., <4.5 mM in adult humans) during fasting [36]. In the fed state, glucose, which enters the portal circulation from the small intestine (with exception of roughage-fed ruminants), is either stored as glycogen or metabolized via the pentose cycle and fatty acid synthesis in the liver, or enters the systemic circulation for utilization by extrahepatic cells and tissues [36]. In humans, rats, cats, dogs, rabbits, mice, and avian species (e.g., chickens and ducks), liver is a major site for de novo fatty acid synthesis from glucose and amino acids, whereas in pigs and ruminants, this synthetic event is virtually absent from the liver (Table 2).

3.3. Skeletal muscle and heart

Skeletal muscle is quantitatively the largest organ in the body, representing about 40–45% of the total body mass or 30% of the resting metabolic rate in adults [41]. Skeletal and cardiac muscles are the major sites for the oxidation of fatty acids and glucose, the transamination of branched-chain amino acids (leucine, isoleucine and valine) and the release of glutamine and alanine (major glucogenic substrates) [36]. Glutamine and alanine derive their α-amino groups from branched-chain amino acids, and their carbon skeletons primarily from glucose. Thus, muscle plays an important role in the regulation of plasma levels of glucose and fatty acids. The fatty acids utilized by the skeletal muscle and heart can be derived from either the lipolysis of TAG in adipose tissue or the hydrolysis of the circulating TAG-rich chylomicrons and VLDL by LPL [42]. Among animal tissues, LPL is expressed mainly in muscle and adipose tissue, where the enzyme binds to the capillary endothelium [37]. Interestingly, the muscle and adipose tissue LPL are regulated differentially by hormones and nutritional factors. Fasting increases LPL activity in the muscle to facilitate TAG hydrolysis but decreases LPL activity in adipose tissue to reduce its uptake of TAG and thus fat storage [38]. Acetyl-CoA, a common intermediate of glucose, fatty acid and amino acid oxidation, either enters the Krebs cycle for complete oxidation or is utilized for lipogenesis in skeletal and cardiac muscles, depending on the intracellular energy status and activity of acetyl-CoA carboxylase (ACC) [34]. In skeletal and cardiac muscles, as in the liver, malonyl-CoA, a product of the ACC reaction, is an allosteric inhibitor of carnitine palmitoyl transferase-I (CPT-I), the enzyme that transfers the long-chain fatty acyl-CoA into mitochondria for β-oxidation [42]. Intracellular concentrations of malonyl-CoA are regulated not only by its synthesis from acetyl-CoA but also by its decarboxylation. Therefore, conditions that result in reduced or increased intracellular levels of malonyl-CoA favor LCFA oxidation or lipogenesis, respectively [42,43]. Thus, the level of malonyl-CoA is decreased in skeletal and cardiac muscles during exercise or fasting, thereby promoting fatty acid oxidation in these tissues [42].

3.4. Adipose tissues

There are white and brown adipose tissues in animals, the amounts varying with species, developmental stage and environment [44]. Most mammals are born with significant amounts of both white and brown adipose tissues, with an exception of pigs that possess no brown adipose tissue at birth or in adult life [40]. With an increase in age, the amounts of white adipose tissue normally increase. For example, in pigs, fat content is only 1% at term birth and increases to 12% at 14 days of age [45]. In contrast, the amounts of brown adipose tissue normally decrease in rodents with increasing age and virtually disappear shortly after birth in large mammals. Thus, brown adipose tissue is present at relatively low levels in adult rats and is virtually absent from adult humans [44]. Interestingly, under certain conditions (e.g., an increase in NO production, PPAR-γ coactivator 1α (PGC-1α) expression

or eukaryotic-translation-initiation-factor-4E binding protein), white adipose tissue may be converted into brown adipose tissue that expresses high levels of uncoupling protein (UCP)-1 [3]. Because of the presence of large amounts of mitochondria and UCPs, brown adipose tissue is a major site for heat production in newborn mammals and may play an important role in the oxidation of energy substrates in adults.

White adipose tissue is the major site responsible for the substantial change in whole body fat and plays an important role in energy balance. Adipose tissue fat accretion depends on the balance between the rates of accumulation and breakdown of TAG in adipocytes [37]. Significant oxidation of glucose and fatty acids occurs in adipose tissues of animals [11,22] and humans [36]. Whether de novo fatty acid synthesis occurs in adipose tissue depends on animal species and age/obesity (Table 2). For example, fatty acid synthesis is nearly absent from the adipose tissue of humans, chickens or adult rats. Interestingly, white adipose tissue is the major site for de novo fatty acid synthesis in pigs and ruminants under normal fed conditions. Both the white adipose tissue and the liver are primary sites for de novo lipogenesis in dogs, cats, rabbits and young rodents [35]. In some monogastric animals (e.g., pigs and young rats) and ruminants (e.g., cattle and sheep), glucose and acetate are the major precursors for lipogenesis in adipose tissue, respectively [35,36]. As noted above, in fed animals, adipose LPL hydrolyzes chylomicrons and VLDL to produce free fatty acids for TAG synthesis and storage in adipocytes. There are even fat depot-specific differences in LPL expression in humans in response to hormonal and nutritional changes [38]. Interestingly, Kratky et al. [39] recently reported that there was an alternative lipase called endothelial lipase in LPL-deficient mouse adipose tissue that could provide free fatty acids to adipocytes for reesterification. This endothelial lipase appears to be a phospholipase that utilizes HDL as its main substrate [39].

During food deprivation, adipose tissue TAG is hydrolyzed by HSL and monoacylglycerol lipase into glycerol and free fatty acids. Hormone-sensitive lipase, often considered to be a key regulatory enzyme in lipolysis, is present in the cytoplasm under basal unstimulated conditions. Interestingly, another lipase, adipose triglyceride lipase localized in lipid droplets, was recently discovered to play a role in initiating the hydrolysis of TAG to produce diacylglycerol, the preferred substrate for HSL [46]. The adipose triglyceride lipase is highly expressed in the white adipose tissue of humans and mice, but its expression in domestic animals remains to be established. Finally, monoglyceride lipase hydrolyzes monoacylglycerol to form glycerol and free fatty acids, thereby completing the hydrolysis of TAG [37,47]. Owing to a lack of glycerol kinase in adipose tissue, glycerol is not utilized by this tissue but is released from adipocytes into the circulation. The TAG-derived glycerol can be used for hepatic and renal gluconeogenesis. Long-chain free fatty acids released from TAG hydrolysis can be oxidized to provide adenosine triphosphate (ATP) in adipocytes or are released from the cells. Because of their hydrophobic properties, LCFAs are transported in blood by albumin and oxidized by most tissues to provide ATP except in the brain [48]. Because white adipose tissue represents a significant portion (15–40% depending on sex and obesity) of total body mass, the metabolism of adipose tissue likely contributes significantly to the whole body homeostasis of glucose and fatty acids [41,49]. Thus, selective overexpression of the glucose transporter 4 (GLUT-4) in adipose tissue enhances glucose transport by adipocytes and improves whole-body insulin sensitivity in mice [49].

4. Regulation of lipolysis and fatty acid oxidation

4.1. Lipolysis and its regulation

Lipolysis is an early critical event in the utilization of the stored TAG in adipose tissue and other tissues. Lipolysis is tightly regulated by hormones and perilipin. Perilipins are proteins that coat the surface of lipid droplets in adipocytes and have a protective effect on lipolysis in their non-phosphorylated state [50]. The perilipin is part of the PAT-family proteins, which include perilipin, adipophilin (adipose differentiation-related protein) and TIP47 (the tail-interacting protein of 47 kDa [50]). These proteins are associated with the neutral lipid droplets and share similarities in their nucleotide sequences [51]. At present, the precise roles of adipophilin and TIP47 in fat metabolism are not known. Upon activation of the lipolysis cascade by adrenergic or other agonists, both HSL and perilipins are phosphorylated by cyclic adenosine-3′,5′-monophosphate (cAMP)-dependent protein kinase A (PKA), and HSL is also phosphorylated by PKG and AMPK (Fig. 5). Phosphorylation activates HSL and also promotes the interaction of perilipins (particularly perilipin A) with HSL. Such an association triggers the translocation of HSL from the cytosolic compartment to the lipid droplet surface to initiate lipolysis [51]. Glucagon (in young rodents but not in humans) and β-adrenergic agonists (including catecholamines) increase intracellular cAMP levels by activating adenylyl cyclase, whereas insulin reduces intracellular cAMP concentrations by stimulating phosphodiesterase-3B activity [50]. Likewise, guanylyl-cyclase activators (e.g., NO and CO) and Viagra (sildenafil citrate) increase intracellular cGMP levels in many cell types (including vascular smooth muscle cells and endothelial cells) by increasing cGMP production and inhibiting phosphodiesterase-5 activity, respectively (Fig. 5).

4.2. Pathways for fatty acid oxidation

The mitochondrial β-oxidation pathway is quantitatively the major pathway for oxidation of short ($<C_8$)-, medium (C_8–C_{12})- and long (C_{14}–C_{20})-chain fatty acids in animals.

Long-chain fatty acyl-CoA enters mitochondria through the CPT system. Mitochondrial β-oxidation of long-chain fatty acyl-CoA yields acetyl-CoA, which is oxidized via the mitochondrial Krebs cycle and electron transport system to yield CO_2, water and ATP. β-Oxidation of long-chain ($C_{14–20}$) and very long-chain ($>C_{20}$) fatty acids, as well as dicarboxylic acids, branched-chain fatty acids and bile acid derivatives, can also occur in peroxisomes, which is independent of the carnitine transport system. Peroxisomal systems I and II are responsible for β-oxidation of straight very-long chain fatty acids and branched very long-chain fatty acids, respectively. The short-chain fatty acids produced via the peroxisomal systems enter mitochondria for complete oxidation. The mitochondrial β-oxidation of fatty acids is enhanced in response to mitochondrial biogenesis induced by transcription factors, such as nuclear respiration factor (NRF)-1, NRF-2, PPAR-α, and mitochondrial transcription factor A (mtTFA). Peroxisome proliferator-activated receptor-γ coactivator 1α up-regulates the expression of these transcription factors and UCPs in a cGMP-dependent mechanism [52]. Thus, PGC-1α is a master regulator of mitochondrial biogenesis and oxidative phosphorylation. Like PPAR-α, PPAR-β (also called PPAR-δ) enhances fatty acid oxidation in tissues (e.g., liver, heart and skeletal muscle) through increases in expression of CPT-I and enzymes of the β-oxidation pathway [53]. Peroxisome proliferator-activated receptor-γ is expressed predominantly in adipose tissue and plays an important role in adipogenesis and lipid storage through increases in LPL expression and TAG synthesis. Synthetic ligands for PPAR-α, PPAR-β and PPAR-γ include hypolipidemic drugs (fibrates), HDL and hypoglycemic drugs (thiazolidinediones, TZDs), respectively [53]. Natural/biological PPAR ligands include long-chain and very long-chain fatty acids, high-fat diet, adrenal steroid dehydroepiandrosterone and eicosanoids derived from arachidonic acid via the lipoxygenase and

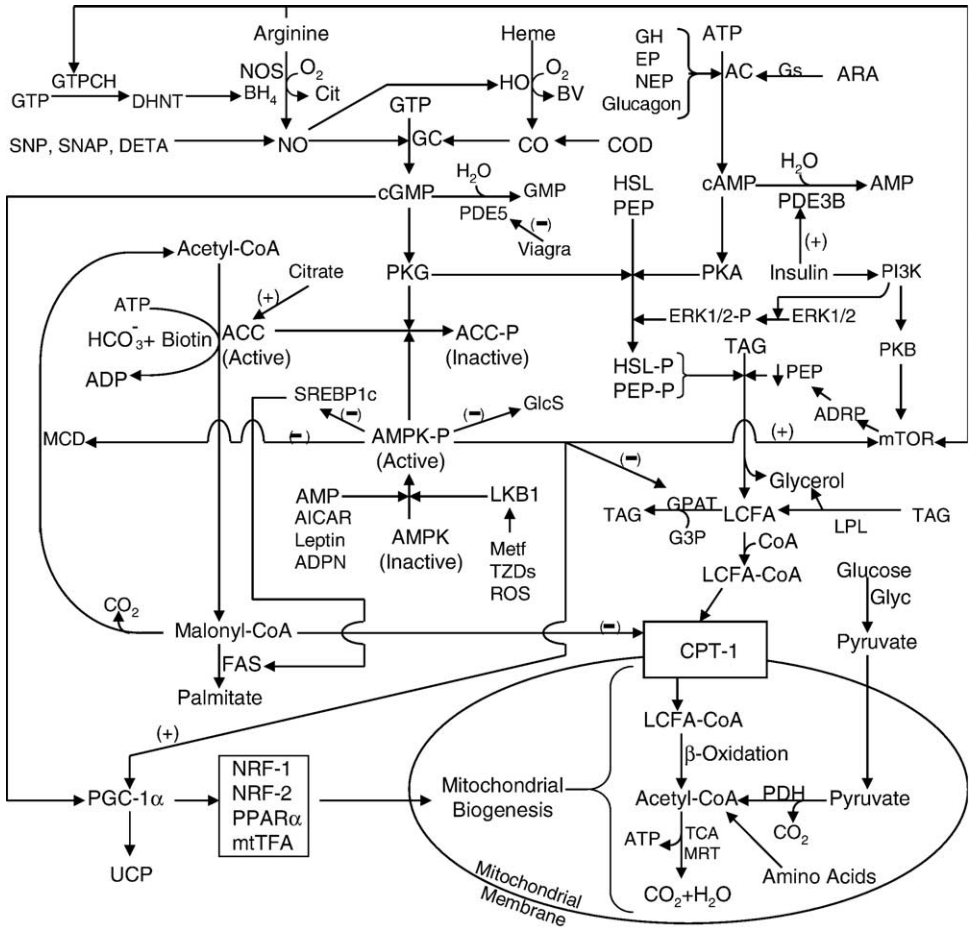

Fig. 5. Putative mechanisms for the role of NO in regulating substrate oxidation and lipolysis in animals and humans. Physiological levels of NO enhances oxidation of fatty acids, glucose and amino acids through cGMP-dependent multiple pathways. Abbreviations: AC, adenylyl cyclase; ADPN, adipocyte differentiation-related protein (adipophilin); βARA, β-adrenergic receptor agonists; Cit, citrulline; BV, biliverdin; cAMP, cyclic AMP; COD, CO donor (e.g., $[Ru(CO)_3Cl_2]_2$); DETA, diethylenetriamine–NO adduct; DHNT, 7,8-dihydroneopterin triphosphate; EP, epinephrine; ERK1/2, extracellular signal-regulated kinases 1 and 2; FAS, fatty acid synthase; GC, guanylyl cyclase; GH, growth hormone; Gs, stimulatory G-protein; GlcS, glucose synthesis; Glyc, glycolysis; GMP, guanosine-3′,5′-monophosphate; GTPCH, guanosine triphosphate cyclohydrolase-I; HO, heme oxygenase; LKB1, tumor suppressor protein kinase; Metf, metformin; MRT, mitochondrial respiration chain; mTOR, mammalian target of rapamycin; PDE3B, phosphodiesterase-3B; NPE, norepinephrine; PDE5, phosphodiesterase-5; PDH, pyruvate dehydrogenase; PEP, perilipins; PKA, cAMP-dependent protein kinase A; PKB, protein kinase B; ROS, reactive oxygen species; SREBP, sterol regulatory element binding proteins; Viagra, sildenafil citrate.

cyclooxygenase pathways [54]. The activated PPAR interacts with retinoid X receptor (RXR) to form PPAR/RXR heterodimers, which bind to the peroxisome proliferator response elements localized in numerous gene promotors to increase expression of specific genes. Like any metabolic pathways, although protein phosphorylation and allosteric regulation of key enzymes provide a rapid mechanism for regulation of lipolysis and fatty acid oxidation via changes in the specific activity of an enzyme, gene expression is the most effective mechanism for long-term regulation of these events through alterations in the amount of enzyme protein.

5. Nitric oxide and AMPK

5.1. Adenosine-3′,5′-monophosphate-activated protein kinase and substrate oxidation

Adenosine-3′,5′-monophosphate-activated protein kinase plays a crucial role in regulating glucose and fatty acid metabolism in animals. It is a heterotrimeric enzyme consisting of three subunits: a catalytic α subunit as well as regulatory β and γ units [55]. Adenosine-3′,5′-monophosphate-activated protein kinase acts as a sensor for cellular energy and is activated by an increased [AMP]/[ATP] ratio. Activation of AMPK occurs via phosphorylation by an established upstream AMPK kinase, LKB1. Adiponectin and leptin exert their physiological effects on cells (e.g., adipocytes) through activation of AMPK [55]. The overall effect of AMPK activation is to switch off the ATP-consuming pathways such as lipogenesis or gluconeogenesis while switching on the ATP-producing pathways such as fatty acid and glucose oxidation. Available evidence suggests that AMPK activity is rapidly altered through protein phosphorylation and dephosphorylation, but a change in its protein level may be regulated at the gene transcriptional level and/or protein degradation.

Multiple mechanisms are responsible for the action of AMPK on the metabolism of energy substrates. The activation of AMPK stimulates GLUT-4 translocation and glucose transport in both skeletal muscle and cardiac muscle [56–58]. This results in an increase in whole-body glucose utilization. In addition, activated AMPK phosphorylates and thus inactivates ACC. As a result, the intracellular malonyl-CoA level is reduced, which allows for an increase in fatty acid oxidation [59]. Adenosine-3′,5′-monophosphate-activated protein kinase also activates malonyl-CoA decarboxylase, the enzyme responsible for the degradation of malonyl-CoA [60]. Glycerol-3-phosphate acyltransferase, the enzyme that catalyzes the first reaction of TAG synthesis, is another target regulated by AMPK. In liver, adipose tissue and muscle, AMPK inhibits glycerol-3-phosphate acyltransferase activity [60,61]. Adenosine-3′,5′-monophosphate-activated protein kinase may also enhance expression of PGC-1α in cells [52], thereby promoting mitochondrial biogenesis and oxidative phosphorylation. Finally, AMPK may increase mammalian target of rapamycin activity and expression, which results in increased synthesis of adipophilin in adipocytes [62]. This may result in reduced expression of perilipin, and therefore, adipophilin can bind to the neutral lipid droplets to facilitate TAG hydrolysis. Thus, through multiple mechanisms, AMPK stimulates the catabolism and oxidation of energy substrates.

5.2. Nitric oxide and AMPK

There is an emerging body of evidence indicating that there is a relationship between NO and AMPK expression/activity in cells [63–70]. Such a relationship cooperatively promotes glucose and fatty acid oxidation. There is ample evidence showing that AMPK regulates NO production in cells. For example, eNOS is phosphorylated by AMPK at position Ser^{1177} [64]. Thus, an increase in AMPK activity under various physiological and pathological conditions can lead to an increase in NO synthesis by eNOS. Indeed, the activation of AMPK in human skeletal muscle during exercise was associated with an increase in the phosphorylation and activation of eNOS [65]. This will increase blood flow and nutrient uptake, thereby favoring the oxidation of both glucose and fatty acids by skeletal muscle. In addition to physiological stimuli, AMPK can be activated by pharmacological agents. For example, AMPK activator, 5-aminoimidazole-4-carboxamide-1-B-D-ribofuranoside (AICAR), enhances NOS activity and glucose transport in muscle cells [66]. It is noteworthy that both in vivo investigations [67] and in vitro studies involving isolated skeletal [66] or cardiac muscles [58] demonstrated that NOS inhibitors diminished AICAR-stimulated glucose uptake, indicating an important role for NO in mediating the role of AMPK in promoting glucose uptake. Most recently, activation of AMPK has been shown to decrease iNOS expression in myocytes, macrophages and adipocytes [69], suggesting an anti-inflammatory role for AMPK in cells.

Although AMPK can regulate NO synthesis, NO can modulate AMPK activity through two mechanisms: a change in gene expression and AMPK activation via peroxynitrite. For example, dietary supplementation of arginine to ZDF rats increased AMPK mRNA levels in adipose tissue approximately twofold [11]. Recently, a novel pathway to activate AMPK has been shown in animal cells without a change in [AMP]/[ATP] ratio [68]. Peroxynitrite ($ONOO^-$), generated from the spontaneous reaction of NO with superoxide anion (O_2^-), activates AMPK through a c-Src-mediated and phosphatidylinositol 3-kinase (PI3K)-dependent pathway in cultured bovine aortic endothelial cells and in mouse aorta and hearts [68]. Although $ONOO^-$ at high levels (>100 μM) can cause oxidative stress, $ONOO^-$ at 1–10 μM activates AMPK [70]. It is noteworthy that the antidiabetic drug metformin enhances AMPK activity through this distinct pathway by increasing $ONOO^-$ production within a physiological range [70]. Conversely, an inhibition of $ONOO^-$ formation either by scavenging O_2^- or by using NOS inhibitor L-NAME diminished metformin-induced AMPK activation.

Importantly, in eNOS knockout mice, metformin had no effect on AMPK activity in endothelial cells, which emphasizes the importance of endogenous NO synthesis in AMPK activation. The PI3K activation by $ONOO^-$ may specifically increase the association of AMPK with its upstream kinase LKB1 without changing the activity of LKB1 [70]. These findings support the conclusion that physiological levels of free radicals are signaling molecules involved in metabolic regulation [71].

6. Nitric oxide and mitochondrial function

6.1. Nitric oxide and mitochondrial respiration

Recently, much attention has been directed toward the identification of mitochondrial NOS and the effect of NO on mitochondrial respiration [72–75]. There is substantial evidence showing the presence of nNOS in the mitochondria (also known as mitochondrial NOS or mtNOS) of many mammalian tissues, such as brain, liver, muscle, heart and kidney [12]. Thus, it is reasonable to expect that NO plays a role in mitochondrial oxidation and oxygen sensitivity of respiration in tissues. Nitric oxide regulates mitochondrial respiration through distinct mechanisms. First, physiological levels of NO mediate blood flow, therefore increasing the supply of metabolic substrates and oxygen to mitochondria. Second, high levels of NO may directly modulate the activity of the mitochondrial electron transport system [74]. For example, at concentrations that were likely above physiological levels of NO in mitochondria, exogenous NO was shown to reversibly bind the oxygen-binding site of cytochrome *c* oxidase (the terminal enzyme in the mitochondrial respiratory chain), thus inhibiting electron transport and oxygen consumption. The result was a reduced supply of ATP from substrate oxidation and enhanced glycolysis as a significant alternative ATP-producing pathway [52,74,75]. This mechanism may play an important role in acute oxygen sensing and in adaptation to low-oxygen conditions when NO levels are high. However, under normal physiological conditions, acetyl-CoA produced from energy substrates is readily oxidized in mitochondria [34], and thus, it is unlikely that endogenous NO generated by nNOS and eNOS inhibits mitochondrial respiration in animal cells when iNOS is expressed at a low level.

6.2. Nitric oxide and mitochondrial biogenesis

Nitric oxide has been shown to have a beneficial effect on mitochondrial biogenesis, that is, mitochondrial proliferation and activation [52]. Nitric oxide-induced mitochondrial biogenesis is a key to long-term regulation of cellular metabolism [76]. In a variety of mammalian cell lines, such as brown adipocytes, 3T3-L1 cells, skeletal muscle L6 cells and immortalized HeLa cells, NO triggers mitochondrial biogenesis and enhances coupled respiration and ATP concentrations [52,75]. This process is mediated via a cGMP-dependent signaling pathway that activates the expression of PGC-1α, a master regulator of mitochondrial biogenesis and oxidative phosphorylation [52]. Peroxisome proliferator-activated receptor-γ coactivator 1α is a key transcriptional cofactor regulating expression of PPAR-α, which in turn stimulates the expression of NRF-1 and mtTFA [77]. Collectively, these transcription factors regulate expression of nuclear and mitochondrial genes encoding proteins that are involved in the respiratory chain as well as mitochondrial DNA transcription and replication [78,79]. Thus, eNOS knockout mice exhibited a reduced level of mitochondrial content in many tissues, such as brain, liver and muscle, which is accompanied by reduced O_2 consumption, reduced steady-state ATP levels, defective energy expenditure and increased body fat gain [52,75].

The actions of NO on mitochondrial function are coordinated to meet the energy requirement of the cell. The mitochondrion is an important site for the oxidation of glucose, fatty acids and amino acids, where the released chemical energy is either coupled with ATP synthesis or dissipated as heat. Therefore, mitochondria play a crucial role in regulating energy balance [78]. Because NO stimulates the formation of metabolically active new mitochondria, the endogenous or exogenous provision of NO is of great interest regarding its effect on the treatment of obesity. In rat skeletal muscle, incubation with sodium nitroprusside (SNP, a NO donor) increased the oxidation of glucose, pyruvate, palmitate and leucine via a cGMP-dependent pathway [80,81]. Likewise, dietary supplementation of arginine to ZDF rats increased expression of PGC-1α as well as the oxidation of glucose and fatty acids in abdominal fat tissue [63]. These findings provide a promising solution to reducing fat mass through modulation of the arginine–NO pathway in animals and humans and emphasize the metabolic basis for the pharmacotherapy of arginine [82].

7. The role for the arginine–NO pathway in glucose metabolism

7.1. Nitric oxide and gluconeogenesis/glycogen synthesis

There is evidence suggesting a role for NO in regulating hepatic gluconeogenesis. The conversion of lactate and pyruvate into glucose in rat hepatocytes is inhibited by NO donors in a dose-dependent manner [83]. The mechanism may involve an inhibition of the activity and/or expression of phosphoenolpyruvate carboxykinase [83], which is now known to be mediated by AMPK [55]. On the other hand, glycogen synthesis from glucose is inhibited by NO in hepatocytes [84] and 3T3-L1 cells [85] due to a decreased activity of glycogen synthase [84]. Thus, elevated levels of NO are expected to increase the release of glucose from the liver under conditions of iNOS induction. Interestingly, NO also reduces glycolysis in hepatocytes by decreasing the activities of key enzymes such as glucokinase [86] and glyceraldehyde-3-phosphate dehydrogenase [87] in coordinate action to spare glucose utilization. However, the net effect of NO on hepatic glucose metabolism likely

depends on the balance between the two opposing effects (synthesis and utilization). Excitingly, we recently found that dietary supplementation of arginine, which increased NO synthesis within physiological ranges in various tissues [9], normalized plasma glucose levels in streptozotocin-induced diabetic rats [88] and attenuated hyperglycemia in ZDF rats [11].

7.2. Nitric oxide and glucose transport

Glucose transport across the cell membrane by GLUT-4 is a key regulatory step for glucose utilization in skeletal muscle, heart and adipose tissue. Insulin is a major hormone regulating glucose uptake by these tissues. It stimulates the phosphorylation of insulin receptor substrate and the activation of PI3K. In skeletal muscle, in addition to insulin, contraction or exercise also stimulates glucose uptake [89,90]. However, unlike the insulin-signaling pathway, exercise/contraction has no effect on insulin receptor phosphorylation or PI3K activity [91]. Two different intracellular pools of GLUT-4 may exist in skeletal muscle and respond to the two different signaling pathways [91]. When activated, GLUT-4 undergoes translocation from an intracellular tubulovesicular reservoir to the plasma membrane of skeletal muscle cells [92,93] or adipocytes [94]. Compelling evidence shows that NO mediates the stimulatory effect of exercise on glucose transport by skeletal muscle. For example, NO donors dose dependently increased glucose transport in isolated skeletal muscle [91,95,96]. Exercise increases eNOS gene expression [97], eNOS protein levels [95] and eNOS activity [98] in skeletal muscle, as well as vascular NO production [97,99,100]. Conversely, an inhibition of NOS activity reduced both basal and exercise-stimulated glucose transport in muscle [95]. Furthermore, Roberts et al. [101] reported that exercise increased sarcolemma GLUT-4 content in rat skeletal muscle, and this effect was blunted by administration of N^G-nitro-L-arginine methyl ester (L-NAME), a competitive NOS inhibitor. Thus, the infusion of a NOS inhibitor during cycling exercise reduced leg glucose uptake, which was overcome by coinfusion with L-arginine [96]. In addition to muscle, NO stimulates glucose transport in 3T3-L1 adipocytes through GLUT-4 translocation to the plasma membrane via a mechanism independent of the insulin-signaling pathway [102].

Nitric oxide may mediate the effect of insulin in stimulating glucose transport in skeletal muscle and adipose tissue. Administration of L-NAME impaired insulin secretion and resulted in reduced glucose tolerance in rats [103–105] and humans [106]. Another competitive NOS inhibitor, N^G-monomethyl-L-arginine (L-NMMA), also attenuated the insulin-mediated increase in glucose uptake by muscles, including heart, soleus muscle, extensor digitorum longus muscle and gastrocnemius muscle [103]. Infusion of L-NMMA to rats also inhibited glucose uptake in many adipose tissue depots, such as brown interscapular, retroperitoneal and gonadal adipose tissues [103]. This is consistent with the recent observation that there is an inverse relationship between plasma levels of L-NMMA and glucose disposal in obese or aging subjects [107]. In cultured 3T3-L1 adipocytes, addition of SNP increased glucose uptake, whereas treatment with a NO scavenger or guanylate cyclase inhibitor reduced the SNP-stimulated glucose uptake to basal levels [102]. Of note, some studies indicated that NOS inhibitors had no effect on insulin-stimulated glucose transport in incubated skeletal muscle [95,101], but NO production was not directly measured in these short-term experiments. The disparity between these in vitro and in vivo findings may be explained by (1) microvascular involvement, which occurs in vivo but is virtually absent from an incubated tissue [108], and (2) NO concentrations in the target site. Thus, the roles of NO in enhancing glucose uptake by insulin-sensitive tissues appear to involve both blood flow-dependent and independent mechanisms. Nitric oxide is a major vasodilator, and thus, an increase in physiological levels of NO is expected to increase the delivery of glucose to muscle cells and adipocytes [109,110]. Consequently, an inhibition of NO synthesis by L-NMMA reduced endothelium-dependent blood flow and inhibited insulin-stimulated glucose uptake in humans [110].

7.3. Nitric oxide and glycolysis/glucose oxidation

In addition to increasing glucose transport, NO also mediates glucose metabolism via glycolysis and Krebs cycle in skeletal muscle. For example, incubation of rat soleus muscle with SNP significantly increased lactate release and glucose oxidation in a dose-dependent manner (1–25 mM of SNP) [80,81,111]. This finding is interesting in that high levels of NO donors did not inhibit mitochondrial respiration in skeletal muscle. It is noteworthy that dietary arginine supplementation enhanced glucose oxidation in both retroperitoneal and epididymal fat tissues of ZDF rats [11]. These findings support the view that physiological levels of NO stimulate glucose oxidation in skeletal muscle and adipose tissue.

The mechanism for the action of NO on glucose metabolism in skeletal muscle and adipose tissue is mediated through cGMP production and cGMP-dependent protein kinase. Soluble guanylate cyclase activity is present in skeletal muscle [112,113] and adipose tissue [114]. Indeed, incubation with NO donors rapidly increased cGMP level in skeletal muscle, whereas the addition of a guanylate cyclase inhibitor blocked this effect [81,111]. The inhibitor also blocked NO donor-stimulated glucose transport and utilization [80,81]. Likewise, a cGMP analogue stimulates glucose metabolism in skeletal muscle [80]. In ZDF rats, an increase in cGMP level in response to an inhibition of its hydrolysis was associated with elevated glucose transport and glucose oxidation in soleus muscle [111]. However, in insulin-resistant obese Zucker rats, this inhibitor failed to increase cGMP level or improve glucose utilization [111]. These results suggest that the NO/cGMP pathway is altered in skeletal muscle of obese or diabetic subjects.

8. The role for the arginine–NO pathway in lipid metabolism

8.1. Nitric oxide synthesis by iNOS and obesity

Obesity is associated with an increase in expression of iNOS and overproduction of NO. Gene expression of iNOS in subcutaneous adipose tissue and adipocytes was higher in obese compared with nonobese humans [14,114]. Additionally, in adolescents, elevated serum NO_x levels (an indicator of systemic NO synthesis [115,116]) were strongly correlated with increased body adiposity [117]. Although eNOS expression is also enhanced in adipocytes of obese subjects, iNOS is primarily responsible for the increased production of NO in obesity-related metabolic diseases. In genetically obese and diabetic rodent models, such as *ob*/*ob* mice and ZDF rats, iNOS protein expression in both adipose tissue and skeletal muscle was higher in comparison with the lean nonobese wild-types [118,119]. Remarkably, treatment with an iNOS inhibitor [118] or selectively disrupting the gene encoding iNOS [119] improved insulin sensitivity in *ob*/*ob* mice and ZDF rats, thereby preventing these animals from diet-induced obesity. In support of these in vivo findings, high levels of NO in culture medium promoted the differentiation of preadipocytes of white adipocyte or brown adipocyte lineage into mature adipocytes [120,121]. We hypothesize that an inhibition of inducible NO synthesis may inhibit PPAR-γ expression and, thus, adipocytes differentiation and proliferation.

8.2. Nitric oxide and lipogenesis/lipolysis

The release of nonesterified fatty acids from adipose tissue plays an important role in the development of obesity and insulin resistance [45]. There is evidence showing that NO affects lipid metabolism in adipose tissue, including both lipogenesis and lipolysis. However, the current literature is rather confusing. Whether exogenous NO stimulates or inhibits lipid metabolism appears to depend on the types and doses of NO donors, tissue site and intracellular redox state [122–124]. For example, we found that 1 to 5 μM SNP stimulated lipolysis, but 25 to 500 μM SNP inhibited lipolysis in adipose tissue of obese humans, in a concentration-dependent manner (G. Wu and S.K. Fried, unpublished data). Of note, atrial natriuretic peptide induced lipolysis in human adipose tissue via the NO-cGMP pathway but had no effect in rat adipose tissue [125]. Furthermore, Gaudiot et al. [122] demonstrated that different NO donors had differential effects on basal or stimulated lipolysis in isolated adipocytes, and this modulation was mediated through an antioxidant-related effect [123]. Additionally, some studies showed that NO donors increased basal lipolysis [124] but inhibited isoprenaline-induced lipolysis in adipocytes [126]. It should be borne in mind that these in vitro studies involved NO donors at concentrations ranging from 20 μM to 2 mM [e.g., 0.5–2 mM *S*-nitroso-*N*-acetyl-penicillamine (SNAP) or 20 μM to 2 mM SNP], and therefore, the physiological significance of the findings is not clear at present. High levels of NO may oxidize and inactivate catecholamines nonenzymatically, thereby reducing the rate of stimulated lipolysis in adipocytes.

Although the responses of fat metabolism to exogenous NO donors appear to vary greatly, in vivo studies involving an inhibition of NO synthesis have consistently demonstrated that physiological levels of NO increase lipolysis in rats. For example, NO mediates the stimulatory effect of leptin on lipolysis in adipose tissue of rats [127]. In addition, dietary supplementation of arginine to adult ZDF rats, which increased NO synthesis, stimulated lipolysis and fatty acid oxidation in adipose tissue, contributing to a marked reduction in body fat mass [11].

8.3. Hypocholesterolemic effect of NO

Because hypercholesterolemia is a significant risk factor for endothelial dysfunction, there is increasing interest in its prevention through dietary and pharmacological means. As noted above, NO plays an important role in the regulation of fatty acid metabolism in liver. Compelling evidence shows that NO donors exhibit a hypocholesterolemic effect by mediating lipoprotein metabolism. For example, administration of a NO donor to rabbits caused a decrease in plasma LDL level [128], suggesting a role for NO in inhibiting LDL production. In support of this view, SNP reduced the release of apo B (the major protein component of LDL) from incubated hepatocytes [128]. Conversely, feeding the rats with NOS inhibitors (0.02% in the diet) resulted in marked increases in serum triglyceride, cholesterol and VLDL, as well as body fat [129]. Nitric oxide regulates hepatocyte lipid metabolism mainly by inhibiting de novo fatty acid synthesis and decreasing the activity of ACC [130]. As a result, malonyl-CoA level is decreased in response to NO, which activates CPT-I activity and stimulates fatty acid oxidation in the liver.

The effects of competitive NOS inhibitors on elevating serum fatty acids, LDL and VLDL could be reversed by the coadministration of arginine [131]. Thus, in streptozotocin-induced diabetic rats, treatment with 2% arginine in their diet attenuated the elevated serum levels of triglyceride and LDL by about 20% [132]. Likewise, arginine treatment improved lipid metabolism in genetically diabetic and fatty rats [133]. Most recently, Fu et al. [11] reported that a long-term arginine treatment reduced retroperitoneal fat mass by 45% and had no effect on the weight of other tissues in ZDF rats. Importantly, an increase in dietary arginine intake from 2.5 to 5–7.5 g/day is associated with a rise in the serum level of HDL and a decrease in systolic blood pressure in humans [134]. Thus, in addition to its beneficial effect on endothelial function [135], dietary arginine supplementation may reduce plasma levels of VLDL and triglycerides through NO production.

9. The role for the arginine–NO pathway in amino acid metabolism

9.1. Nitric oxide and amino acid oxidation/ureagenesis

Although arginine is the nitrogenous substrate for NO synthesis, little is known about the effects of physiological

levels of NO on the metabolism of amino acids in animal cells. Limited evidence suggests that high levels of exogenous NO may indirectly increase rates of amino acid oxidation in the liver on the basis of enhanced urea synthesis by rat hepatocytes perfused with 0.2 mM SNP [136]. Interestingly, Stadler et al. [87] reported that 0.05 to 5 mM SNAP did not affect urea production by rat hepatocytes incubated with 10 mM NH_4Cl (~100 times the physiological level of plasma NH_4^+) as the major source of exogenous nitrogen. Such a high level of ammonia might have maximized the capacity of hepatic ureagenesis, and thus, urea production could not be further increased in response to NO provision.

Recent studies indicate that PPAR-α is a negative regulator of the urea cycle via down-regulation of the expression of carbamoylphosphate synthase-I, ornithine carbamoyltransferase, argininosuccinate synthase and argininosuccinate lyase in the liver [53]. Thus, in contrast to a high level of NO that may occur under conditions such as sepsis and inflammation, physiological levels of NO may reduce the expression of urea-cycle enzymes and, thus, urea synthesis from ammonia indirectly through enhanced expression of PPAR-α. This action of NO is expected to inhibit amino acid oxidation in the liver, thereby increasing the entry of dietary amino acids into the systemic circulation. It is likely that NO exhibits differential effects on amino acid synthesis and catabolism, depending on target cells and tissues, its local concentration in the reaction site, interactions among signaling pathways and nutritional states.

9.2. Nitric oxide and intracellular protein turnover

In animal cells, amino acids are utilized for the synthesis of proteins, and proteins are continuously degraded to produce amino acids. This constitutes an intracellular protein turnover. At present, little is known about the effect of physiological levels of NO on protein turnover in cells. However, exposure of rat hepatocytes to high levels of exogenous NO has been reported to decrease protein synthesis [137], thereby increasing the availability of amino acids for catabolism. Interestingly, an increase in NO synthesis by iNOS mediates the effect of tumor necrosis factor-α on inhibiting protein synthesis and stimulating protein degradation in skeletal muscle [138]. A catabolic effect of high levels of NO on protein metabolism in liver and skeletal muscle is consistent with the enhanced hepatic ureagenesis under septic and inflammatory conditions. Whether physiological levels of NO regulate protein turnover warrants investigation. Of note, physiological levels of NO stimulate the release of anabolic hormones (e.g., insulin and growth hormone) in both birds and mammals [82] and, thus, are expected to promote a positive protein balance, particularly in young and growing animals.

9.3. Nitric oxide and polyamine synthesis

Polyamines play a critical role in regulating DNA and protein synthesis, as well as cell proliferation and differentiation [82]. In vascular smooth muscle cells, the presence of extracellular NO donors (30 to 300 μM) inactivated ornithine decarboxylase, thereby inhibiting polyamine synthesis from arginine/ornithine and cell proliferation in a dose-dependent manner [139]. This effect of NO is beneficial in preventing atherosclerosis in animals and humans. In contrast, culture of endothelial cells with a lower extracellular concentration of a NO donor (10 μM) or with sepiapterin (a precursor for BH4 synthesis) increased polyamine synthesis and cell proliferation [140]. Such an effect would play an important role in angiogenesis and wound healing under various physiological conditions.

10. Nitric oxide and the metabolic syndrome

Available evidence shows that the oxidation of fatty acids, glucose and amino acids involves the exquisite coordination of multiple organs in animals (Table 3). Since its initial description by Reaven [141], there has been considerable debate about the precise definition of the metabolic syndrome or Syndrome X [142]. Nonetheless, a cluster of key characteristics of the metabolic syndrome includes obesity, hyperglycemia (fasting serum glucose >6.1 mM), hyperinsulinemia, hyperlipidemia, hypertension and insulin resistance (an impaired response of cells or tissues to physiological concentrations of insulin). These factors, independently or collectively, contribute to a high risk for cardiovascular disease, a major cause of death in

Table 3
Major fuels for organs and tissues under various nutritional conditions in animals and humans[a]

Organ	Fed	Postabsorptive	Brief fasting	Prolonged fasting	Hypocaloric protein diets
Adipose tissue	FA, glucose	FA, glucose	FA, KB	FA, KB	FA, KB
Brain	Glucose	Glucose	KB, glucose	KB, glucose	KB, glucose
Heart	FA, glucose, lactate	FA, glucose, lactate	FA, KB	FA, KB	FA, KB
Kidneys	Lactate, FA, glucose	Lactate, FA, glucose	KB, FA, glutamine	KB, FA, glutamine	KB, FA, glutamine
Large intestine	Butyrate, glutamine	Glutamine, butyrate	KB, glutamine	KB	KB
Liver	AA, FA	FA, AA	FA	FA	FA
Lymphoid organs	Glucose, glutamine	Glucose, glutamine	Glucose, KB, glutamine	Glucose, KB, glutamine	Glucose, KB, glutamine
Skeletal muscle	FA, glucose	FA, glucose	FA, KB	FA, KB	FA, KB
Small intestine	Glutamine, Glu, Asp	Glutamine, glucose	KB, glutamine	KB, glutamine	KB, glutamine

AA, amino acids; Asp, luminal aspartate; Glu, luminal glutamate; FA, free fatty acids; KB, ketone bodies (acetoacetate and β-hydroxybutyrate).

[a] Adapted from Wu [33] as well as Wu and Marliss [36].

developed nations. Glucosamine, an inhibitor of NO synthesis by constitutive NOS [6], may contribute to the development of insulin resistance in the vasculature and skeletal muscle of obese or diabetic subjects. As noted in the preceding sections, physiological levels of NO can ameliorate all of these adverse features of the metabolic syndrome in animal models of obesity. Thus, administration of arginine can reduce plasma levels of glucose, homocysteine, fatty acids and triglycerides in streptozotocin-induced diabetic rats [88] and ZDF rats [11], and improve insulin sensitivity in obese humans [63,131,132,143,144]. A distinct advantage of arginine over drugs (e.g., metformin and TZDs) is that dietary arginine supplementation will not increase body fat mass. Another advantage of the arginine solution is to reverse endothelial dysfunction associated with major cardiovascular risk factors (hypercholesterolemia, smoking, hypertension, diabetes, obesity, insulin resistance and aging) [135] and to prevent ammonia toxicity and organ dysfunction brought about by hypoargininemia [82,145]. Arginine or its effective precursor (citrulline) may provide a potentially novel effective treatment for the prevention and treatment of the metabolic syndrome in humans and animals.

11. Conclusions and future research directions

Nitric oxide synthesized from arginine plays an important role in intermediary metabolism. The overproduction of NO by iNOS often results in many pathological conditions. However, physiological levels of NO have many beneficial effects on fuel homeostasis, including the stimulation of glucose and fatty acid oxidation as well as the inhibition of glucose, TAG and LDL synthesis (Fig. 3). These favorable effects are possibly mediated by increases in mitochondrial biogenesis and oxidative phosphorylation [52], as well as activation of specific transcription factors and signaling pathways [146]. We propose that the nutritional factors (e.g., ω-3 polyunsaturated fatty acids, antioxidants, and phytochemicals) that increase NO synthesis by eNOS/nNOS or NO bioavailability [8] will enhance substrate oxidation, energy expenditure and insulin sensitivity. Conversely, factors (e.g., high-fat diet, oxidative stress and protein malnutrition) that reduce NO synthesis by eNOS/nNOS or NO bioavailability [8] will likely lead to the development of the metabolic syndrome. Because iNOS contributes a large amount of NO to cells, selective inhibition of this NOS isoform may reduce oxidative stress and prevent a decrease in the catabolism and/or oxidation of energy substrates by various cell types (including adipocytes, hepatocytes, myocytes and endothelial cells in obese subjects). Future studies involving cell culture, animal models and humans are required to provide compelling experimental evidence to elucidate the importance and mechanisms for the roles of the arginine–NO pathway in regulating lipolysis, glucose and TAG synthesis, glucose and fatty acid oxidation, as well as expression of PGC-1α, AMPK and heme oxygenase in cells and tissues. Additionally, research is needed to define a possible role for NO in the regulation of UCP expression in brown adipose tissue, blood flow within brown and white adipose tissues, fat depot-specific metabolism of energy substrates, fat tissue-specific amino acid metabolism and whole-body energy expenditure. Collectively, these integrated biochemical, molecular and physiological studies will aid in providing new knowledge to design a novel, safe and effective way to prevent and treat obesity as well as metabolic syndrome in humans and companion animals (e.g., cats and dogs). Finally, another application of these findings is to reduce unfavorable fat mass (e.g., subcutaneous fat) in animals (e.g., cattle, fish, goats, pigs, poultry and sheep) of agricultural importance through dietary supplementation with arginine or its precursors. Such an outcome may enhance economic returns of livestock production by increasing lean muscle growth and reducing fat accretion in the carcass. The future holds great promise for the use of L-arginine or L-citrulline to both improve health in humans and enhance the efficiency of animal agriculture.

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