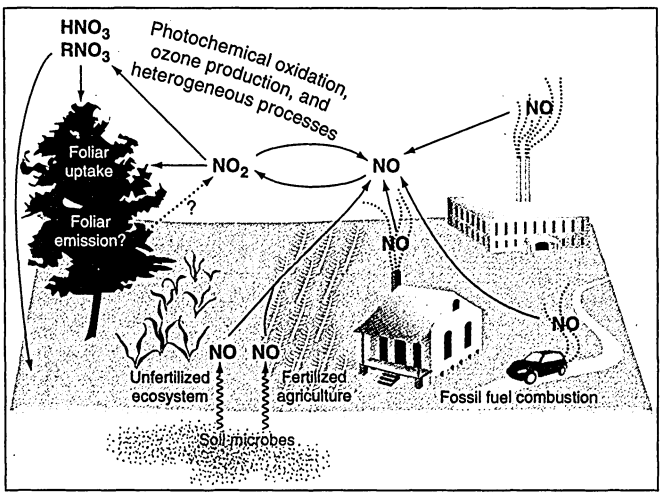
needed to reconcile the measured NO soil emission fluxes with the NO concentrations measured in the canopy air. Because of low sensitivity of the analytical methods available for bottom-up studies, it has been difficult to obtain data at the low concentrations typical of ambient nonurban NO_2 concentrations (0.05 to 1 ppbv) and thus to confirm the assumption of first-order uptake kinetics and the existence of a compensation point.



Sources and sinks. This scheme shows the main NO_x sources and sinks. The magnitude of NO_2 uptake by vegetation is not known, and the existence of an NO_2 source from vegetation is contentious.

The discrepancy between top-down and bottom-up approaches has important implications for our understanding of both atmospheric chemistry and plant function. Atmospheric NO_2 concentrations in nonurban surface air are typically much less than 1 ppbv, and the NO_2 compensation points determined in the bottom-up studies would thus imply that vegetation canopies are large NO_2 sources, with 24-hour average NO_2 emission fluxes on the order of 2×10^{10} molecules cm^{-2} s^{-1} (*16*). In comparison, soil emissions of NO are typically 10^8 to 10^{11} molecules cm^{-2} s^{-1} (*9*). Inclusion of such a large vegetation source in atmospheric chemistry models would require a hitherto unrecognized NO_x sink to balance the budget.

Resolving the differences in sign and magnitude of leaf-atmosphere NO_2 exchange will require both top-down and bottom-up approaches. Leaf-level measurements will have to be made with techniques that are sensitive at very low NO_2 concentrations. More studies of the key metabolites involved with NO_2 assimilation are needed. NO_2^-, NO_3^-, and chloroplast pH measurements during nitrate reduction may help explain the source of NO_2 within leaves. Combined with quantitative biochemical modeling (*17*), such measurements will improve the accuracy of NO_2 concentration-uptake curves and help determine the true value of the NO_2 compensation point—if such a point does indeed exist. As for top-down approaches, simultaneous measurements of NO fluxes from soils, NO_2 fluxes across leaf surfaces, NO_x fluxes above canopies, and NO_x concentrations in canopy air across a range of ambient NO_2 concentrations are necessary to test the models developed from controlled environment leaf-level studies. Without such measurements, the role of leaf-level exchange and the importance of plant physiological regulation for NO_2 exchange between the surface and the atmosphere cannot be quantified. This issue must be resolved to close the budget of this important atmospheric species.

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PERSPECTIVES: BIOMEDICINE

Protein Loss in Cancer Cachexia

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Patients with chronic diseases such as AIDS or cancer (particularly those with tumors of the pancreas, stomach, colon, and lung) often experience a life-threatening muscle wasting syndrome known as cachexia. Cachexia is characterized by a dramatic loss of triglycerides from adipose tissue and proteins from skeletal muscle. Although it superficially resembles starvation, it is refractory to nutritional intervention. Loss of skeletal muscle mass results in lowered mobility and, hence, a poorer quality of life for the patient, while erosion of respiratory muscle eventually leads to death from pneumonia. Cachexia is associated with reduced survival time irrespective of tumor mass or the presence of metastases, and it also interferes with cancer therapy. Knowledge of the molecular pathways leading to cachexia is required if an effective treatment is to be developed. The report by Guttridge *et al.* (*1*) on page 2363 of this issue identifies the master transcription factor NF-κB as an inhibitor of skeletal muscle cell differentiation and a mediator of cytokine-induced muscle wasting in mice. These findings provide potential new targets for therapeutic intervention to treat cachexia.

Loss of skeletal muscle proteins reflects an imbalance between the rate of protein breakdown (catabolism) and the rate of resynthesis of depleted proteins. The ubiquitin-dependent proteolytic pathway breaks down most skeletal muscle proteins in a variety of wasting conditions (*2*). In this pathway, proteins are marked for degradation by the attachment of ubiquitin, which requires the activity of enzymes E1, E2, and E3. The polyubiquitinated protein is then degraded in a multisubunit complex, the 26*S* proteasome—a tubelike structure consisting of a stack of four rings, two outer α rings and two inner β rings (see the figure). The proteasome releases short oligopeptides containing six to nine amino acid residues, which are rapidly degraded into amino acids by cytosolic peptidases.

When ubiquitin-proteasome proteolysis is accelerated in muscle, usually there is a concurrent increase in the production of mRNAs encoding enzymes in this pathway (*2*). Preventing the transcription of just one proteasome α subunit (C2) with antisense oligonucleotides reduces the number of proteasomes, proteolytic activity, and consequently protein degradation (*3*). Recent

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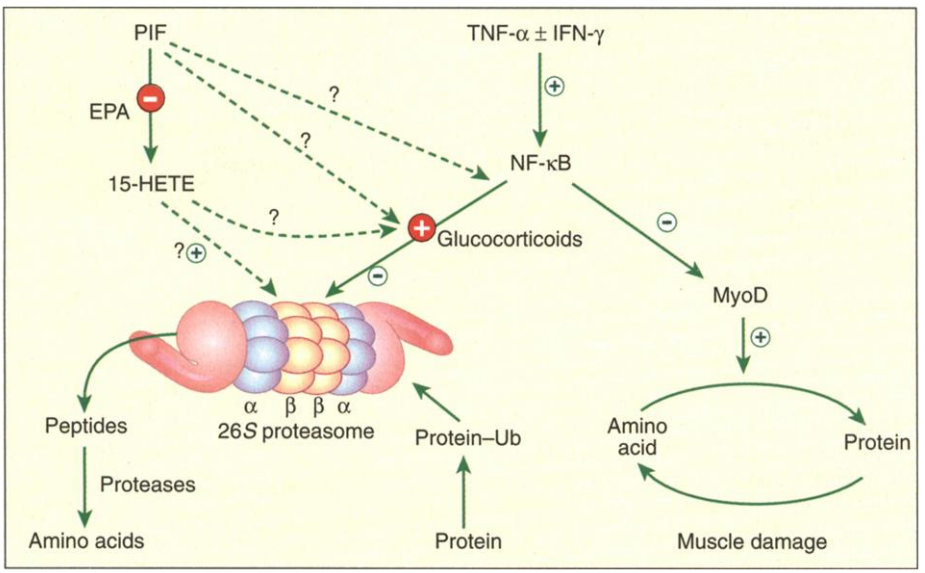
data suggest that NF-κB appears to suppress the transcription of another proteasome α subunit (C3) in muscle cells (*4*). Glucocorticoids (which increase protein catabolism) oppose this suppression by antagonizing the interaction of NF-κB with an NF-κB response element in the C3 subunit promoter region; they also increase cytosolic levels of IκBα, an inhibitor of NF-κB activity. Some cytokines such as tumor necrosis factor–α (TNF-α) and interferon-γ (IFN-γ) induce NF-κB activation and suppress C3 subunit expression, which would be expected to reduce protein degradation. Although both TNF-α (*5*) and interleukin-6 (IL-6) (*6*) increase production of ubiquitin, neither induces a change in proteasome subunit expression. These results are consistent with the inability of cytokines to induce muscle protein catabolism directly (*7*) and suggest that loss of lean body mass in animals administered cytokines may result from a defective muscle repair process.

A variety of cytokines, including TNF-α, IL-1β, IL-6, and IFN-γ, have been implicated in the induction of cachexia in animal models (*8*). Exactly how these cytokines induce cachexia is unclear, but TNF-α is known to be a potent activator of NF-κB. Now, Guttridge *et al.* (*1*) report that activation of NF-κB by TNF-α in differentiating murine muscle cells (myocytes) inhibits their differentiation by suppressing production of MyoD mRNA at the posttranscriptional level. MyoD is a transcription factor that is essential for skeletal muscle differentiation and for repair of damaged tissue, and it may be particularly important for the replenishing of wasted muscle (*9*). Thus, NF-κB seems to have two opposing effects on skeletal muscle protein homeostasis: it halts protein degradation by blocking production of the C3 proteasome subunit, and yet it promotes cachexia by preventing muscle cell differentiation during muscle repair. However, skeletal muscle explants incubated with TNF-α alone do not show protein breakdown (*7*), which suggests that other factors are required for muscle wasting. Accordingly, the investigators found that once differentiated into myotubes, muscle cells were completely refractory to TNF-α–induced changes in MyoD expression. IFN-γ alone also had no effect, but the combination of TNF-α and IFN-γ induced significant reductions in both MyoD and myosin expression in both cultured myotubes and mouse skeletal muscle. Other cytokines such as IL-1β or IL-6 were ineffective, either alone or in combination with TNF-α. Although MyoD is expressed at low levels in adult skeletal muscle, its expression is induced in muscle stem cells in response to injury, suggesting that these cells require MyoD to proliferate and to differentiate during muscle repair. These findings indicate that cytokines working through NF-κB may prevent the rebuilding of damaged skeletal muscle.



Muscle breakdown. Signaling pathways that regulate protein homeostasis in skeletal muscle. Cytokines such as TNF-α together with IFN-γ activate the transcription factor NF-κB. This leads to decreased expression of MyoD, a transcription factor that may be important for replenishing wasted muscle. Activated NF-κB also acts as a repressor of proteasome subunit expression and hence suppresses protein degradation, an activity that is antagonized by glucocorticoids. (The proteasome is a multisubunit complex involved in the breakdown of ubiquitinated proteins.) Tumor factors such as PIF increase production of proteasome subunits through the intermediary 15-HETE. It is not known whether this is a direct or indirect effect (dashed arrows). Eicosapentaenoic acid (EPA) inhibits 15-HETE production in response to PIF and prevents muscle wasting in cancer patients.

What factors are involved in activating protein catabolism in skeletal muscle? A proteolysis-inducing factor (PIF) has been observed in serum samples from cachectic mice and cancer patients (see the figure). PIF is a sulfated glycoprotein produced by tumors that induces protein catabolism in isolated muscle cells (*10*). It appears to activate the ubiquitin-proteasome pathway directly, possibly through an intermediate molecule, 15-hydroxyeicosatetraenoic acid (15-HETE).

How can all of this information help in the treatment of cachexia? One approach is to block NF-κB activity, and this has been shown to inhibit cachexia in an animal model (*11*). Another approach is to block signaling pathways induced by PIF (which may or may not involve NF-κB) that lead to proteasome activation. For example, eicosapentaenoic acid is an effective inhibitor of 15-HETE formation in muscle cells treated with PIF (see the figure). This polyunsaturated fatty acid is effective in the attenuation of cachexia not only in murine models, but also in patients with unresectable pancreatic cancer with the result that lean body mass is preserved (*12*).

The Guttridge study and others provide valuable information about the factors regulating protein homeostasis in skeletal muscle. However, we still do not know precisely how the imbalance between protein catabolism and resynthesis of depleted proteins leads to cachexia. Is NF-κB indeed a common intermediate in protein catabolism and protein resynthesis pathways as the Guttridge work suggests? Do cachectic factors directly induce expression of ubiquitin-proteasome pathway components, and, if so, what are the signaling molecules involved? Armed with the answers to these questions, treatment of cachexia should be possible, with concurrent improvements in both the quality of life and survival time of cancer patients.

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