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NUTRITIONAL ASPECTS OF COLLAGEN METABOLISM

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CONTENTS

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INTRODUCTION

Collagens are a family of proteins that constitute the major extracellular proteins found in the body. Most tissues and organs contain several types of collagen whose function is primarily structural [for reviews on collagen types, see (102)]. These types differ in both primary and higher order structure. The collagens polymerize extracellularly into a variety of structures that constitute the extracellular matrix [for reviews, see (151)]. Collagen associates both noncovalently and covalently with additional components of the extracellular matrix and with minerals to form specialized connective tissues including skin, tendon, and bone [see (49) for a review]. Collagen is remodeled continuously throughout growth and development. However, because it is extracellular, it must be depolymerized, degraded, and transported into cells to be hydrolyzed to amino acid residues for recycling into protein or for conversion to metabolites. Collagenous peptides enter the circulation and are transported either to the liver for breakdown to amino acids or to the kidney for excretion. Peptidases are present in both organs that cleave collagenous peptides. Since collagen is turned over continuously, a relatively constant amount of collagenous peptides containing the unique imino acid hydroxyproline is excreted every day and reflects the level of connective tissue metabolism and remodeling.

EXTRACELLULAR MATRIX

Collagen Types and Tissue Distribution

All collagens have two structural features in common: (*a*) collagens are trimers composed of three polypeptides, and (*b*) these trimers contain large amounts of the repeating amino acid sequence, X–Y–Gly, with a high content of proline in the X position and a high content of hydroxyproline in the Y position. This repeating amino acid sequence demands a helical conformation, and the three subunits of the protein fold into a triple-helical trimeric structure. The collagens are ubiquitous, and several types (discussed below) are found in most connective tissues. To date, at least fourteen collagen types have been identified [for review see (152)]. Types I, II, III, V, and XI are fibril-forming collagens and occur in almost all connective tissues; however, cartilage contains only types II and XI and not types I or III (152). Another group of collagens are recognized as *f*ibril-*a*ssociated *c*ollagens with *i*nterrupted triple helices (FACITs) (57). These collagens are types IX, XII, and XIV and are not fiber forming, but they are associated with fibers formed from collagens in the first group (152). For example, type IX is found on the surface of type II fibrils in cartilage (57, 154), and type XII, which shares similarity with type IX, may be associated with type I fibrils (36). Recently,

another member of this group named type XIV has been identified based on homology to type IX and XII collagens (37). Types II, IX, X, and XI occur primarily in cartilage (152). Type X collagen is particularly restrictive in that it occurs exclusively in calcifying and hypertrophic cartilage during endochrondral bone formation (140).

In addition to the fibril-forming and FACIT collagens are those collagens that form specialized structures. Type IV forms a unique structure and is the major collagen of basement membrane (162). Type VII occurs in skin as anchoring fibrils of basement membranes in the dermal epidermal junction (19, 85). Type VIII, originally reported in endothelial cell cultures (135), is the major collagen in Desmemet's membrane (158), which separates the corneal endothelial cells from the stroma.

HETEROGENEITY OF COLLAGEN TYPES Since each subunit or polypeptide is the product of a different gene, at least 25 different genes encode the polypeptides of the 14 collagen types. Most collagen polypeptides share some homology with polypeptides of another collagen type. Type I collagen occurs most abundantly as a heterotrimer consisting of two α1(I) polypeptides or subunits, and one α2(I) polypeptide or subunit. These two polypeptides exhibit 66% homology in the triple-helical domain (106). Type III collagen occurs as a homotrimer with three identical α1(III) subunits. The α1(II) subunit is 65% homologous to α1(I) and 58% homologous to α2(I) in the triple-helical regions of the polypeptide (106). Homotrimeric collagens are types II, III, VII, X, and XII. Type I collagen exists both as a homotrimer α1(I) and as a heterotrimer α1(I)α2(I). Other collagens are heterotrimers containing two and even three distinct subunits. Types VI and IX contain three different subunits (149, 153).

Several collagen subunits occur in more than one form owing to alternative associations of subunits [for review, see (1)]. Type V collagen contains either two or three different subunits: $[\alpha1(V)]_3$; $[\alpha1(V)]_2\alpha2(V)$, or $\alpha1(V)\alpha2(V)\alpha3(V)$ (46). One of the subunits of type XI collagen, α3(XI), appears to be similar or identical to the α1(II) polypeptide of type II collagen (44). To date, five different polypeptides or subunits have been identified for type IV collagen (59, 70, 114). Although many different combinations of these subunits could exist, only a few such combinations have been isolated, the most common form being $[\alpha1(IV)]_2\alpha2(IV)$ (86).

VARIATION IN TYPES I AND III COLLAGEN GENE EXPRESSION Since the collagen genes are developmentally regulated, a particular cell or tissue expresses only a subset of its collagen genes at any one time, depending on the cell's or tissue's state of development [for reviews, see (139)]. The regulation of gene expression is well demonstrated for types I and III (1).

Type I collagen occurs in greatest amounts in embryonic bone (109) and tendons (141) where it constitutes 65 and 30%, respectively, of the total protein. Type I collagen is also found in lung, skin, and muscle, although the amounts of type I found in these tissues are much less than in bone and tendon. Type I collagen accounts for 2.9% of total protein in embryonic sheep skin (declining to 1.6% at birth) and 3.7% of the protein in embryonic sheep lung (declining to 2.4% at birth) (150). Type III collagen is often found in association with type I collagen in most connective tissues (83). Type III occurs most abundantly in extensible tissues such as fetal skin and blood vessels. It accounts for a large part of the collagen of embryonic skin (126). Recently, some type III collagen and its mRNA have been found in the less extensible tissues of bone and tendon (84, 138).

REGULATION OF SYNTHESIS Regulation of synthesis is achieved through a variety of modulators [for review, see (1)], including steroids (26, 27, 61, 127), drugs (87), interferon (131), growth factors (98, 128), cytokines (38, 40, 56, 78, 101, 125), and various metabolites including acetaldehyde (17, 69). Interferon has been used both to affect fibronectin synthesis (29) and to suppress collagen production in scleroderma fibroblasts in culture (79, 132); it may also have an effect on other fibrotic conditions (30) and wound healing (58).

Glucocorticoids decrease procollagen synthesis in fibroblast cell cultures as demonstrated by a decrease in total mRNA for type I procollagen. The decrease occurs in cellular, nuclear, cytoplasmic, and polysomal steady-state levels of mRNA for proα1 and proα2 polypeptides (27). More recent work by Cockayne & Cutroneo has shown that glucocorticoids may employ a mechanism that alters the level of binding of transcription factors to the proα2 collagen gene (26). Various tissues may respond differently; for example, estradiol was shown to increase mRNA levels for type I procollagen in osteoblastic cells of bone (42, 43).

Fibrosis that results from increased synthesis or decreased turnover of collagen is often the end result of tissue damage by drugs or other toxic agents (2, 25) or alcohol (4, 69). Carbon tetrachloride treatment of rats results in hepatic fibrosis (25) and the synthesis of a fibrogenic factor in liver (23).

Collagen Turnover

All tissues in the body contain collagen either serving as a structural element or performing a role in the pericellular matrix. Wherever collagen degradation has been measured, investigators have found that collagenous structures in general undergo continuous remodeling in turnover. As much as 3–5% of skin collagen may turn over per day (95). Early attempts to separate matrix collagen into pools destined for turnover at different rates were complicated

(95) and did not take into account either reutilization rates of labeled amino acids or the more recent finding that most secretory proteins are subject to a variable amount of intracellular turnover during their biosynthesis (7). Collagen was one of the first secretory proteins shown to be subject to intracellular turnover during its biosynthesis because of the ease with which degraded collagenous peptides containing hydroxyproline can be measured (12). Jackson & Heininger (72) used radioactive proline as a metabolic tracer and showed that collagen turned over at a rate of 2.6% per day and that proline was reused.

INTRACELLULAR ASSEMBLY AND TURNOVER The biosynthesis of collagen occurs on membrane-bound polysomes and, simultaneously with its translation, collagen is transferred to the endoplasmic reticulum where it undergoes a number of posttranslational modification reactions that result in trimer formation (8). Like many secretory proteins, collagen is subject to a number of proofreading enzymatic reactions within the endoplasmic reticulum, and possibly the golgi apparatus, that cause abnormally modified collagen to undergo intracellular degradation to small peptides (7). These peptides are secreted from the cell through the default pathway leading from the endoplasm reticulum through the golgi to secretory vesicles and exocytosis. Proteins within the endoplasmic reticulum that bind to malfolded proteins such as immunoglobin heavy-chain binding protein (BIP) have been shown to bind to malfolded immunoglobulin heavy chains (60, 82) and may bind mutated collagen molecules (P. Byers, personal communication). Folding of proteins in vitro is a slow and inefficient process (55). The longer the time a protein exists in a malfolded, unstable state, the greater the chance for degradation or nonspecific aggregation, in vivo (133). Consequently, investigators predict that accessory factors will be identified as catalysts in the folding process. A heat shock glycoprotein, hsp47, was discovered because of its ability to bind both gelatin and native type I collagen (115). Its collagen-binding properties were found to decrease at pH 6.3 (134). Hsp47 was immunolocalized to the endoplasmic reticulum (134). Hsp47 possesses an RDEL sequence at its carboxyl terminal region (68) similar to the KDEL sequence of other ER resident proteins. Antibodies to hsp47 have shown that hsp47 and collagen are immunoprecipitated together (115a); possibly hsp47 may assist in collagen folding.

The enzymes responsible for the intracellular degradation of collagen either within the endoplasmic reticulum or within the golgi apparatus have not been identified (7). Although the exact site of intracellular degradation is unknown, evidence indicates that a fraction of collagen may be transferred to the lysosomal compartment for degradation by lysosomal enzymes (10). A variable fraction (10–40%) of newly synthesized collagen is subject to in-

tracellular degradation, depending on the conditions (12). A required cofactor of collagen synthesis is ascorbic acid, which is essential for the hydroxylation of prolyl residues in newly synthesized collagen. Ascorbic acid deficiency in most systems results in underhydroxylated collagen that is unable to fold into a stable triple-helical conformation and is, therefore, subject to intracellular degradation (11). Genetic diseases that result in mutations within the triple-helical region of type I collagen result in collagen that is also incapable of assuming a triple helix and is subject to this pathway of intracellular degradation of newly synthesized collagen (157).

EXTRACELLULAR TURNOVER Collagen present in the extracellular matrix, either in tissues such as skin, bone, or tendon or in organs such as the liver and kidneys, is subject to turnover and remodeling catalyzed primarily by metalloproteinases with an involvement of serine proteinases. A family of metalloproteinases has been shown to degrade extracellular matrix macromolecules and collagen in particular (100). This family of metalloproteinases share common features in that its members are synthesized and secreted from cells as precursors that are inactive and must be proteolytically activated either by serine proteinases (63) or other metalloproteinases [for review, see (9, 74)]. For example, the major type I collagen-degrading enzyme, commonly referred to as fibroblast collagenase, is activated by plasmin and superactivated by stromelysin (112, 146), another member of the metalloproteinase family. The major collagenase synthesized by neutrophils is activated by cathepsin G, a serine protease (20) that also activates stromelysin (123). The family of metalloproteinases that includes collagenase is also under cytokine regulation in that its members are induced by interleukin I (100). Remodeling of connective tissues within bone, tendon, and skin occurs as a result of either hormonal regulation or mechanical or electrical stimulation. For example, bone remodeling is altered as a result of mechanical tension, compression, and electrical field effects (105). Mechanical effects are also important for cartilage that depends on diffusion of nutrients for maintenance (121), since it has no vasculature.

AMINO ACID DEFICIENCY

Starvation

During advanced stages of starvation such as amino acid deficiency in which the animal goes into negative nitrogen balance, the two major sources of protein in the body are the muscle and connective tissues. The loss of bulk in these two organs may reflect the relative turnover of proteins within muscle and connective tissues; muscle tissue provides a ready source of amino acids as a result of intracellular catabolism of muscle proteins. Extracellular matrix,

on the other hand, requires extracellular proteolysis for turnover. Since the turnover time for extracellular matrices is in general less than for muscle, the relative contribution of amino acids in the starved state is primarily from muscle, followed by connective tissues. Additional extracellular matrix components including fibronectin and proteoglycans are degraded during starvation (15, 21, 45, 71).

Starvation is a life-threatening condition. The responses of the organism are to protect the critical functions necessary for survival. Collagen is one of the proteins affected by fasting. Within 24 hr of fasting, collagen synthesis decreases to 50% of normal in the articular cartilage of guinea pigs, and this reduction continues to 8–12% of control levels after 96 hr (142). Although starvation may represent total fasting, most chronic conditions are not associated with such severe restriction. Other studies have investigated the response of collagen metabolism to partial, rather than complete, energy restriction. Significant reductions in collagen production have been reported in food-restricted rats as a function of both duration and degree of food deprivation. Even rats that gained weight decreased collagen production in articular cartilage. In contrast, noncollagen protein production was reduced only in articular cartilage from rats that lost weight, but it did not progressively decrease as the caloric intake continued to decrease (143). These data suggest that collagen production is sensitive to changes in food intake in both short-term diseases, such as in surgical patients and patients with broken bones, as well as in more long-term diseases such as osteoporosis. The data also suggest that malnutrition may have profound effects on collagen production (143). The level of urinary hydroxyproline was higher in fasting than in nonfasting deer (35) and may serve as a marker for nutritional balance. The effects of mechanics on bone calcium loss have been documented in postmenopausal women (116) who showed that walking affects bone density.

Changes in collagen production as well as in other connective tissues have also been reported in childen and animals that have undergone protein/calorie malnutrition. The amounts of collagen in skin biopsies taken from children with clinical protein energy malnutrition were lower than those of controls (148). During pregnancy, not only is the collagen content of bones changed but the composition of the bones changes in fetal rats whose dams were protein or energy deficient (107). The turnover of soluble collagen in skin is decreased in protein-malnourished rats, but the degradation and conversion into insoluble collagen occurs in the same proportions as in the rapidly growing rats (110). Whether the changes in collagen production result from changes in caloric intake (food deprivation or starvation) and/or protein malnutrition, evidence from many systems has shown that collagen production is a labile process, influenced by many factors. Certain diseases such as diabetes are accompanied by changes in nutritional status that result in

undernourishment. Animals with streptozotocin-induced diabetes showed diminished growth with increased specific lung volume and weight. Collagen and elastin content were reduced (119) and there was decreased degradation of lung collagen (120). Undernourished, nondiabetic animals showed increased degradation of lung collagen (119).

Nutritional Emphysema

Several studies, including a recent review (88), have observed the effects of injury induced in the lung by prolonged nutritional alterations. The composition of the diet, age, and species of the animal determines the effects of nutritional interventions on lung structure and functions. The term "emphysema" may be misleading. Although the lung architecture is not normal, it is unclear whether or not there is actual tissue destruction. This is particularly true in models that use moderate to severe calorie restriction to study the effects of starvation on lung growth in juvenile rodents (62). In this model the lung connective tissue content is lower than in age-matched controls (89, 113, 137). Whether the decrease in lung collagen content results from the actual depletion of connective tissue or from lack of collagen production during lack of growth is not clear. The collagen and elastin may be depleted in meeting the nutritional requirements of the animals for survival (62, 136, 137). However, collagen and elastin levels are not lower than those found prior to the initiation of the nutritional intervention (81, 89). These findings suggest that in growing animals starvation prevents the normal accumulation of lung structural proteins rather than promoting their net loss.

In a model in which the animal is slowly growing or nongrowing, a net loss of connective tissue content, specifically collagen, has been observed. These findings are different from those cited above for the younger, growing rats in which similar food restriction results in less than normal connective tissue accumulation, but not the loss of these proteins (90). Thus, age as well as dietary restrictions determine the response of connective tissues, including collagen, to the specific insult.

CONTRIBUTION OF COLLAGEN TO NUTRITION

The contribution of connective tissue to overall body metabolism, specifically the contribution of collagen, is complicated [for a review, see (155)]. Body composition changes with aging: muscle mass and skeletal mass decrease with age while fat mass increases (28, 41). After menopause, both muscle mass and skeletal mass decrease (116); obesity may reduce bone mineral loss by increased weight bearing on the spine (3). Recent studies show that normally a significant basal level of collagen turnover occurs in lung and other tissues (95). This turnover takes place in the absence of either tissue

remodeling or pathological processes. How turnover relates to the contribution of collagen to overall body metabolism or to the actual deposition of pathological amounts of the protein in tissues is unclear. Certainly, pathological conditions such as fibrosis lead to abnormal amount or types of collagen in many organs (see above).

One clinical manifestation of abnormal collagen turnover affected by malnutrition is inhibition of growth which, in turn, influences the well-being of the individual. An increased demand for collagen synthesis may occur in growing individuals or animals with acute injuries. Specific effects of malnutrition on collagen turnover may be age-dependent, and the young that are still growing may be more susceptible to such changes. In adolescents with Crohn's disease, collagen turnover responded to nutritional supplementation. Collagen was restored to several tissues of the body, suggesting that collagen synthesis had been decreased (111). In animal studies, weanling rats fed isocaloric diets containing varying amounts of casein experienced a weight gain related to the amount of protein in the diet, thus demonstrating that collagen metabolism is dependent on the animals' nutrition. (156). This finding holds true for both fetuses and dams. Once again, the type as well as the amount of collagen in an organ of a growing animal depends on the amount of protein and the caloric intake of the animal (77, 108, 159). Clearly, collagen is not the stable protein it was thought to be twenty years ago, but rather is a labile protein susceptible to various nutritional alterations. How specific alterations contribute to the overall metabolism of the individual remains to be determined. These contributions will be dependent upon tissue, age, the type of nutritional alteration, and whether they are of short-term or long-term duration.

ROLE OF VITAMINS IN COLLAGEN METABOLISM

Vitamin A

GENE INDUCTION Vitamin A is known to have profound effects on epithelial cells. It has been used to cause the differentiation of F9 teratocytes, including induction of type IV collagen and laminin (144), and to cause the formation of cleft palates in newborn mice. All-*trans*-retinoic acid induces striking digit pattern duplications when locally applied to the developing chick limb bud (147). In other cases, retinoic acid has been shown to suppress differentiation of the epidermis. Retinoic acid can inhibit several differentiation-associated keratins (93). Excess vitamin A can induce metaplasia of both embryonic and adult keratinizing epithelia. In hamster cheek pouch epithelium given a topical application of vitamin A, the formation of keratin was inhibited and the epithelium lacked all features of keratinization (66). Chronic vitamin A intoxication leads to liver injury similar to that

caused by alcoholic cirrhosis (5). Vitamin A deficiency has resulted in the synthesis of increased fibronectin in cultured hepatocytes. The addition of vitamin A reversed this effect, suggesting that vitamin A may have a direct effect on an extracellular matrix protein (91). There may be a relationship between retinol and fibrosis (14).

The work of Oikarinen et al (122) and Hein et al (65) indicated that various retinoids inhibited collagen production by human fibroblast cultures. Using concentrations of retinoids of 10^{-5} M or higher, they reported that all-*trans*- and 13-*cis*-retinoic acid inhibited collagen gene expression at the level of mRNA. This concentration is too high to suggest that inhibition occurs via retinoid-binding proteins (124) or the family of retinoid receptor proteins (16, 54); rather, it may occur through a different mechanism (see below). Several studies are consistent with retinoids inducing connective tissue proteins (94). Rat calvaria cultures transformed with sv40 have been induced with retinoic acid for type I procollagen and the noncollagen protein osteopontin (64).

EMBRYONIC DEVELOPMENT Vitamin A has a dramatic effect on embryonic development; it can induce digit pattern duplications when applied locally to the developing chick limb bud (39). Thaller & Eichele have done extensive work on the identification and spatial distribution of retinoids and their effects in the developing chick limb bud (147) and have shown that retinoic acid may be a local chemical mediator of morphogenesis.

REGULATION OF COLLAGEN SYNTHESIS Vitamin A has been reported to inhibit (51, 122) collagen synthesis in human dermal skin fibroblasts. Oikarinen et al (122) concluded that the reduction of collagen synthesis occurs at the level of mRNA production for the proα1(I) polypeptide chain. Geesin et al (51) reported that at high concentrations of retinoids both collagen synthesis and ascorbate-mediated lipid peroxidation were inhibited. These investigators concluded that the effect of vitamin A on collagen synthesis may be concentration dependent and that high, nonphysiological doses of retinoids affect ascorbate-induced lipid peroxidation, which in turn inhibits ascorbate-induced collagen synthesis (51).

Vitamin C

The dramatic effect of ascorbic acid on collagen synthesis was recognized, although not understood, as early as 1795 when the British Navy included citrus juice in the rations of its sailors to prevent scurvy. The measurement of vitamin C in human tissues continues as functional measures are proposed (73). The effect of ascorbate is not specifically limited to collagen but extends to glycosaminoglycan synthesis of proteoglycans (13). The importance of ascorbic acid in maintaining collagen production is supported by studies in

which diabetic rats that have deficiencies in wound healing were shown to be deficient in ascorbic acid. When ascorbic acid was added to the diet, wound healing improved (103).

ANTIOXIDANT In the early 1960s, ascorbic acid was shown to serve as a cofactor for the enzyme prolyl hydroxylase (126). This enzyme converts prolyl residues in collagen to hydroxyproline. The latter enables collagen to assume a triple-helical conformation at body temperature and to polymerize into functional collagen fibers. Prolyl hydroxylase is an iron-containing enzyme, and the iron must be in the ferrous state to form the catalytic complex. Ascorbic acid is believed to be the antioxidant that maintains the iron in the reduced ferrous state (92). Ascorbic acid may also serve as part of tissues' antioxidant defense by protecting collagen fibrils from damage caused by intensely energetic free radicals. Highly reactive free radicals produced either endogenously or by the metabolism of drugs or by radiation cause tissue damage. Vitamin C is a member of a group of micronutrients that may protect against tissue damage (99).

INDUCTION OF COLLAGEN GENES Lipid peroxidation induces a wide range of cellular effects; it has been associated with alterations in second messenger pathways and cell proliferation. Chojkier et al (24) concluded that ascorbic acid induces lipid peroxidation and reactive aldehyde production in cultured human fibroblasts and that this process is necessary for stimulation of collagen synthesis. In their work, ascorbic acid and lipid peroxidation products stimulated equally the net production of collagen relative to noncollagen proteins, and the transcription of procollagen α1(I)mRNA levels. Geesin et al (51, 52) reported that two different retinoids, at similar concentrations, inhibited both ascorbate-stimulated lipid peroxidation and collagen synthesis. They concluded that the ability of the retinoids to inhibit the oxidant effect of ascorbate, and not their receptor-mediated activity, is responsible for their effect on collagen synthesis.

In addition, ascorbic acid stimulates collagen synthesis by increasing the transcription rate of collagen genes and increasing the proportion of membrane-bound polysomes in collagen-synthesizing cells (80). Many investigators have noted that ascorbic acid stimulates increased transcription of collagen genes in different cultured cell types (24, 50, 97). Kao et al (80) reported that when tendon fibroblast cultures were exposed to ascorbic acid for more than six hours the fraction of membrane-bound polysomes increased significantly. Recently, Geesin et al (52, 53) provided evidence that linked ascorbate-induced lipid peroxidation and collagen synthesis. The alteration of cell membranes by lipid peroxidation may cause changes similar to those caused by growth factor treatment in which the cell responds to cell mem-

brane-mediated signaling events. Geesin et al (53) also studied the effects of various growth factors on collagen synthesis by dermal fibroblasts; the two factors that significantly stimulated collagen synthesis, transforming growth factor-β (TGF-β) and fibroblast growth factor (FGF), were most sensitive to lipid peroxidation.

As discussed above, ascorbic acid was identified in the early 1960s as a cofactor for the enzyme prolyl hydroxylase in the hydroxylation of prolyl residues, an essential posttranslational modification of collagen. This reaction is essential for the formation and stabilization of the triple helical conformation and for polymerization of the trimers into functional collagen fibers.

Vitamin D

Recently, it has become apparent that vitamin D should be considered a steroid hormone. Its synthesis in the skin upon exposure to ultraviolet light obviates the dietary necessity for vitamin D. Vitamin D is metabolized in the liver to 25-hydroxyvitamin D_3, which is then metabolized in the kidney. The close regulation of renal production of 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], the most potent of the naturally occurring derivatives of vitamin D, is suggestive of its hormonal nature. Additional support for this viewpoint is the presence of high affinity nuclear receptors for vitamin D in such tissues as the kidney and intestine. One of the target tissues for $1,25(OH)_2D_3$ is the intestine, which depends on this hormone for the absorption of dietary calcium [for a review of the effects of vitamin D on bone and intestinal cell differentiation, see (145)]. Vitamin D deficiency results in increased nonmineralized bone matrix, resulting in rickets in children and osteomalasia in adults. Evidence suggests an increased collagenase burden in the growth plate of rachitic rats (31). Vitamin D deficiency may be related to abnormal lung development (48).

Administration of $1,25(OH)_2D_3$ stimulates osteoclastic bone remodeling. It also promotes absorption of calcium and phosphorus across the intestinal epithelial cells. Exactly how mineralization is regulated is still not known. However, bone gla protein and matrix gla protein, which bind calcium, are involved in mineralization. Both are stimulated by $1,25(OH)_2D_3$ (47, 96, 161). Another bone protein, osteopontin, is also stimulated by $1,25(OH)_2D_3$ (160). Bone mineralization may have indirect effects on collagen metabolism, as a more rapid turnover is expected for less mineralized matrix. Specific effects of $1,25(OH)_2D_3$ on collagen synthesis are controversial (145).

The target tissues of vitamin D's endocrine action are the kidney, intestine, and bone (145). Recent evidence supports the interaction of $1,25(OH)_2D_3$ with the hematopoietic and immune systems. In hematopoietic cells, $1,25(OH)_2D_3$ promotes the differentiation of promonocytes into monocytes, then macrophages, and finally into osteoclasts, cells that mediate bone resorption. $1,25(OH)_2D_3$ stimulates increased production of osteoclast cells. Also,

gamma interferon produced by activated T-lymphocytes stimulates activated macrophages to produce $25(OH)_2D_3$-1-hydroxylase, the enzyme essential for production of $1,25(OH)_2D_3$. Thus, the bone marrow contains a vitamin D paracrine system that produces and uses $1,25(OH)_2D_3$ (130).

The events leading to the calcification of the matrix of growth cartilage and/or the induction of new bone formation are poorly understood. Deposits of mineral occur extracellularly in a transforming matrix that contains collagens, glycoproteins, proteoglycans, and other proteins. Dietary intake of calcium, phosphate, vitamin D, protein, and copper is important in the process of bone mineralization. Vitamin D deficiency has been implicated in abnormalities of proteoglycans and in collagen metabolism in growing bones and lungs of rats (6, 48). Vitamin D metabolites are necessary for maximal stimulation of calcification of cartilage matrix (67). Although the exact mechanism by which vitamin D influences collagen metabolism remains unclear, apparently the vitamin is necessary for the normal production of this connective tissue. Not only is collagen influenced by 1,25-dihydroxy vitamin D_3, but noncollagen bone proteins such as osteocalcin are required for the assembly of bone and are induced by this vitamin (161).

Comparative studies indicate that in the growth plate cartilage from normal and vitamin D-phosphate deficient rats, different levels of collagenase are present. The growth plate cartilage from the vitamin D-deficient rats contains excess collagenase compared to the amount of tissue inhibitor of metalloproteinase (TIMP). As a result of the imbalance between the enzyme and the inhibitor, collagen is degraded. This process allows for thinning of the longitudinal septa and expansion of the hypertrophic cells in growth plates of rachitic rats. (31, 32). Thus, vitamin D deficiency can potentiate collagen breakdown. On the other hand, in rachitic chick epiphyseal growth cartilage, the hypertrophic chondrocytes are likely to be responsible for the increased levels of type X collagen that provide a maximum area of calcifiable matrix (129). All types of collagen may not be affected equally in deficiencies of vitamin D. In the transition from proliferation to hypertrophic cell zones in the growth plate, there is an increase in chondrocyte volume and production of type X collagen, but a decrease in overall collagen content, mediated by a proteinase-inhibitor imbalance.

MINERAL BALANCE AND BONE HOMEOSTASIS AS RELATED TO COLLAGEN METABOLISM

Calcitonin

Dietary calcium is the source of calcium for bone mineral (22). Calcitonin, a 32 amino acid peptide hormone secreted from thyroidal C-cells, is critical in maintaining calcium homeostasis because of its marked inhibition of osteoclastic bone resorption (117). Osteoclasts have been observed to shrink

in size and decrease bone resorption within minutes of calcitonin application (34). Blood calcium concentration is the most important regulator of blood calcitonin secretion. As the calcium level rises, there is a proportional rise in calcitonin secretion (33). Because of its inhibition of osteoclastic bone resorption, calcitonin has been used successfully to treat diseases characterized by bone resorption and hypercalcemia. Calcitonin has been widely used in Paget's disease; it is also used to treat some cases of osteoporosis and the hypercalcemia of malignancy.

Many questions remain about the roles calcitonin plays in maintaining calcium homeostasis and skeletal integrity. To date, no skeletal disease has been attributed conclusively to calcitonin abnormalities (34). Do diseases of skeletal and calcium homeostasis result from primary and/or secondary calcitonin secretion abnormalities?

Parathyroid Hormone

Parathyroid hormone (PTH) regulates the levels of calcium and phosphate in the blood by affecting the activity of specific cells of bone and kidney. In turn, blood calcium is the prime regulator of PTH secretion; a rise in blood calcium levels results in a decrease in PTH secretion. Thus, a mutual regulatory interaction of PTH and calcium keeps the blood calcium level constant despite fluctuations in diet, bone metabolism, and renal function. In bone, PTH administration results in an increase in osteoclast number and function. Paradoxically, PTH effects this by binding to bone-forming osteoblasts, which then secrete factor(s) that stimulates osteoclasts to resorb bone (104). It may suppress the level of matrix proteins such as osteopontin (118).

PTH binds to specific G-protein-coupled receptors in bone and kidney, thereby activating adenylate cyclase and phospholipase C. Recently, the PTH receptor was cloned and characterized (76). Its striking homology with the calcitonin receptor (75) and its lack of homology with other G-protein-linked receptors indicate that receptors for calcium-regulating hormones are related and represent a new family of G-protein-coupled receptors. The effects of PTH on bone are complex and still poorly understood. PTH administration leads to an increase in osteoclast cell number and activity. Release of calcium is accompanied by an increase in phosphate release and the release of other bone matrix components, such as collagen (18).

Parathyroid hormone and vitamin D interact in a number of important ways. In the kidney, PTH activates the enzyme responsible for synthesizing $1,25(OH)_2D_3$, which is a potent inducer of calcium absorption in the intestine. Calcium absorption can increase from 10 to 70% in response to $1,25(OH)_2D_3$. This effect can operate synergistically with PTH to raise blood calcium levels. Bone is poorly mineralized in the absence of vitamin D metabolites, and PTH cannot mobilize calcium efficiently from poorly mineralized bone. Because

vitamin D mediates the effect of PTH on blood calcium, it is used, in addition to oral calcium, to treat hypoparathyroidism.

CONCLUSION

The collagens are a family of proteins that constitute the major extracellular proteins of the body. They are ubiquitous proteins serving in either structural or pericellular matrix functions and are regulated during differentiation, growth, and remodeling of connective tissues. Collagen synthesis and degradation are altered by a large variety of metabolites, growth factors, hormones, cytokines, as well as mechanical stimuli. The regulation of collagen mass and specific types of collagen are related to the nutritional state of the animal and are affected by numerous disease processes because most disease processes involve changes in extracellular matrix either directly as in wound healing, inflammation, and fibrosis or indirectly as in starvation, diabetes, and abnormalities in mineral balance.

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