The Influence of Nutrition on Collagen Metabolism and Stability

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Introduction

Collagen is the major connective tissue protein and accounts for about 30% of the total protein in the mammalian body. Together with proteoglycans and elastin, it forms an extracellular matrix providing structural support and a mechanism for transmitting force for virtually all organs including muscle. The collagen content of most skeletal muscles is relatively low (about 2% or less) compared to other tissues such as skin, bone and cartilage; however, collagen plays a major role in determining the textural properties of meat (Light, 1987; Bailey, 1988). The collagen characteristics related to muscle texture include tensile strength, thermal shrinkage and melting temperature, and degree of solubility under various conditions. These characteristics are a function of collagen synthesis and changes occurring after synthesis. It has been difficult to establish the relationship between nutritional factors and collagen metabolism and structure. Tinker and Rucker (1985) suggested that the intra- and extracellular nature of metabolic events governing collagen synthesis, the long biological half-life of some collagen proteins, the influence of non-nutritional factors and the relationship of time to changes in collagen make elucidating the contribution of a nutritional factor(s) to collagen metabolism difficult. Nonetheless, nutritional factors do affect metabolism, synthesis and functional properties of collagen; the functional properties of collagen have a significant effect on meat texture (Light, 1987; Bailey, 1988). With efficient production of lean but tender muscle a priority, the role nutrition plays in collagen development requires attention.

The objective of this presentation is to examine selected nutritional factors and the mechanisms by which they may affect synthesis, degradation, accretion, and physical properties of collagen.

Collagen Biosynthesis

Collagen biosynthesis is a complex process which is influenced directly and indirectly by nutritional factors (for reviews, see Prockop et al., 1976; Bornstein and Traub, 1979; Bailey and Etherington, 1980; Miller and Gray, 1982; Last and Reiser, 1984; Bailey, 1987; Miller, 1987; Nimni and Harkness, 1988). The major events in the production of collagen are outlined in Figure 1. The processes involved in the formation of a collagen matrix can be divided into events which take place intra- and extracellularly. Transcription, translation and certain post-translation modifications of collagen occur within the cell while actual fibril formation and crosslinking occur after collagen is secreted from the cell. The triple helical collagen molecule consists of three polypeptide chains, called α-chains, each of which is comprised of a repeating tripeptide $(GLY\text{-}X\text{-}Y)_n$, where X is frequently proline and Y 4-hydroxyproline. The collagen molecule can be of homogenous composition (identical α-chains) as in type III collagen or consist of combinations of different α-chains as in type I collagen (Miller, 1987). At least twelve genetically and structurally distinct collagen isoforms (types) arising from at least 20 unique procollagen α chain genes have been identified in animal tissues (Nimni and Harkness, 1988; Raghow and Thompson, 1989). Collagen types are tissue-specific. Type I collagen is the major form in skin, muscle, tendon and bone and is usually associated with lesser amounts of type III collagen (Bailey and Etherington, 1980; Bailey, 1987). Type II collagen is the predominant form in cartilage (Miller and Gay, 1982; Miller, 1987), while types IV and V collagen are the main constituents of basement membranes (Miller, 1987).

Regulation of Intracellular Events

A fundamental difference between collagen types is manifested in variations in their primary structures which are determined by transcription of the different genes and translation of mRNAs specific for each collagen subunit (α chain) and type. Rate of collagen synthesis is controlled directly by both levels and translation rate of collagen — specific mRNAs into the precursor procollagen α chains. The translation cycle is dependent on polyribosomal assembly and intracellular tRNA and amino acid pool (proline) size (Bailey and Etherington, 1980; Boedtker et al., 1983). Both stability of the polyribosomal complexes and the intracellular pool of amino acids are influenced by dietary energy and protein levels (Tinker and Rucker, 1985).

The literature pertaining to molecular mechanisms controlling the expression of collagen genes was recently reviewed by Raghow and Thompson (1989). Regulation is complex and occurs at several different levels, involving transcriptional, post-transcriptional or translational mechanisms either alone or in concert. Raghow and Thompson (1989) divided synthesis control into two broad categories: 1) primary control of collagen gene expression in response to development of a specific tissue, and 2) modulation of collagen synthesis and collagen gene expression by various biological and chemical factors. Production of mainly types I

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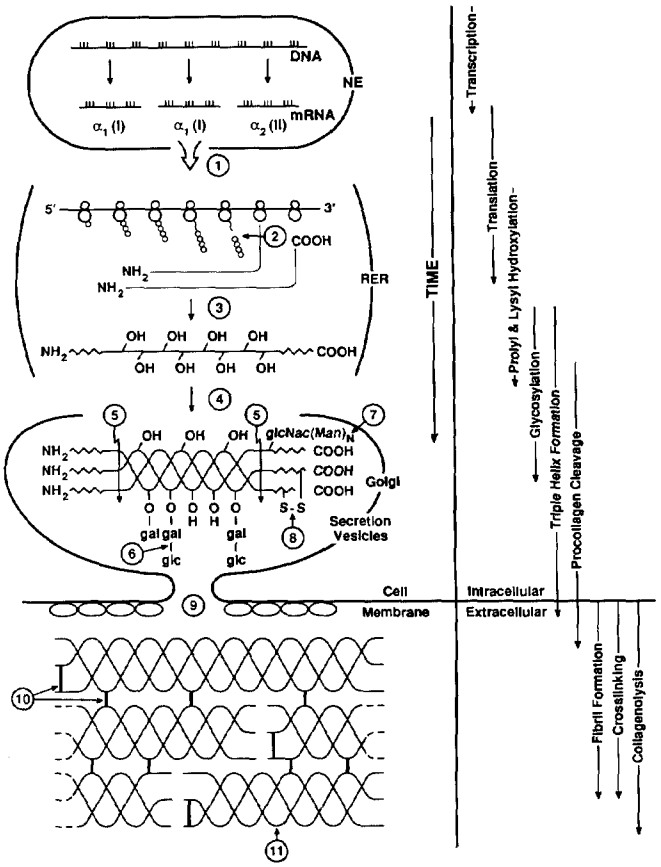
Figure 1



Intra- and extracellular events in the biosynthesis of collagen: 1) transcription of collagen-specific mRNAs; 2) translation; 3) prolyl and lysyl hydroxylation; 4) helix formation; 5) limited proteolysis; 6,7) glycosylations; 8) disulfide bond formation; 9) secretion and fibril formation; 10) crosslinking; and 11) fiber maturation and collagenolysis.
Adapted from Tinker and Rucker, 1985.

and III collagen in myoblasts (Gerstenfeld et al., 1984) or fibroblasts (Liau et al., 1985) and synthesis of predominantly type II collagen by chondrocytes in the formation of cartilage (Mayne and von der Mark, 1983) are examples of the cell-specific, tissue-specific expressions of collagen genes. Both rate of collagen synthesis and expression of collagen genes can be altered by various agents. Growth factors, vitamins and steroid hormones represent three classes of nutritional or biological factors which exert control over collagen biosynthesis. Some specific growth factors have been demonstrated to markedly increase collagen biosynthesis. Embryonic brain extract induced a 5- to 10-fold increase in collagen synthesis in rat muscle cells in culture (Kalcheim et al., 1982). Ignotz and Massagne (1986) reported that an increase of collagen in the extracellular matrix was a general response to transforming growth factor β. A hepatic fibrogenic factor stimulated the transcription of types I, III and V procollagen genes in liver cells several fold (Choe et al., 1987). Vitamin deficiencies tend to decrease collagen synthesis and to alter the type and mechanical properties of the collagen produced (Tinker and Rucker, 1985). Specific regulation of collagen gene expression has been demonstrated for two fat-soluble vitamins. Retinoids (Oikarinen et al., 1985) and vitamin D_3 (Rowe and Kream, 1982; Genovese et al., 1984) inhibited the synthesis of type I collagen in skin and bone, respectively, by reducing the level of procollagen mRNA. A thiamine deficiency decreased collagen synthesis and decreased the ratio of type III/I collagen in skin, suggesting that expression of a collagen gene was affected (Alvarez and Gilbreath, 1982). The role of vitamins in regulation of collagen metabolism is complex and clearly involves factors other than direct regulation of collagen gene expression. For example, the increased solubility and decreased mechanical strength of collagen associated with deficiencies of riboflavin (Prasad et al., 1983) and pyridoxine (Meyers et al., 1986) were apparently related to reduced calorie intake and growth rate, and impaired collagen crosslinking.

Steroid hormones have a pronounced effect on collagen biosynthesis (for review, see Cutroneo et al., 1986). The effect of glucocorticoids on collagen metabolism has been well-characterized. In virtually all tissues examined including skin, bone, lung, liver and granuloma, glucocorticoids selectively decrease collagen synthesis. The antianabolic effect of corticosteroids on collagen metabolism is related to a decrease in synthesis and steady-state levels of procollagen mRNAs (Cockayne et al., 1986). The mechanism by which rate of transcription of procollagen gene sequences, and, therefore, mRNA levels, is regulated is not entirely understood. Interaction of DNA-binding proteins with a procollagen gene which is altered by glucocorticoid administation may represent the primary point of transcriptional regulation (Cockayne and Cutroneo, 1988).

Sex hormones also influence collagen metabolism in a variety of tissues (see Cutroneo et al., 1986 for review). Estrogen and estradiol generally have an inhibitory effect on collagen synthesis in bone, tendon and granuloma tissues, although in some instances in bone no effect or an anabolic effect on collagen synthesis has been demonstrated. The effects of estrogens on bone collagen synthesis may be confounded by its inhibition of collagen degradation and its enhancement of bone collagen maturation by acceleration of the crosslinking process. Androgens have a strong anabolic effect on collagen synthesis in most tissues (Cutroneo et al., 1986). The mechanisms by which the steroid sex hormones influence collagen metabolism may involve regulation of gene expression as well as post-translational modifications.

Factors which mediate collagen gene expression can have a profound effect on the composition and structural properties of the collagen matrix through regulation of synthesis rate and expression of phenotype. Much of the evidence for regulation of collagen biosynthesis at the transcriptional or translational levels and information concerning molecular mechanisms has evolved from in-vitro experiments and has not addressed regulation of muscle collagen biosynthesis.

The post-translational modifications to the procollagen peptide which occur within the cell are hydroxylation of specific prolyl and lysyl residues, glycosylation of certain hydroxylysine residues by galactose or glucosylgalactose, triple helix formation, and proteolytic cleavage of the procollagen -COOH and -NH_2 terminal propeptides (Figures 1). Hydroxylation of prolyl and lysyl residues, essential for

stabilization of the collagen triple helix and crosslinking, is catalyzed by prolyl and lysyl hydroxylases (Berg et al., 1979). Both prolyl and lysyl hydroxylases require ascorbic acid, ferrous iron and α-ketoglutarate as consubstrates. A dietary deficiency of ascorbic acid results in scurvy, a condition characterized by defective prolyl and lysyl hydroxylation and crosslinking (Barnes, 1969; Bailey and Etherington, 1980). However, the reduced caloric intake and weight loss and reduced growth rate which is also characteristic of scorbutic conditions results in decreased collagen synthesis which is unrelated to defective or decreased hydroxylation (Chojkier et al., 1983).

The role that glycosylation plays in collagen function is not completely understood but may be related to increasing stability through modulation of collagen hydration or participation in non-enzymatic crosslinking reactions (Brodsky et al., 1988). Enzymatic glycosylation reactions are catalyzed by two specific transferases, both of which require sugar in the form of uridine diphosphate glycoside and a bivalent cation, usually manganese, for activity (Berg and Prockop, 1973; Jiminez et al., 1973). Direct or indirect nutritional effects on intracellular, enzymatic collagen glycosylation have not been demonstrated (Tinker and Rucker, 1985).

Non-enzymatic glycosylation (also called glycation) of both fibrous and basement membrane collagens occurs (Cohen and Yu-Wu, 1983; Monnier et al., 1984; Bailey and Kent, 1989). Non-enzymatic glycosylation is the first step in the Maillard or browning reaction and is a condensation reaction between carbohydrate and Σ-amino groups of lysine and hydroxylysine residues of proteins. In proteins with a long half-life such as collagen, non-enzymatic glycosylation and subsequent covalent crosslink formation occurs with aging or in response to elevated blood sugar levels (Pongor et al., 1984; Tanaka et al., 1988). Such non-enzymatic glycosylations and cross-link formation represent post-translational modifications which occur slowly and in addition to normal lysyl oxidase-mediated crosslinking (Bailey and Kent, 1989).

The formation of the triple helix appears to be a spontaneous reaction requiring the alignment of -NH_2 and -COOH terminal peptides and disulfide bond formation between them (Goldberg, 1985; Bruckner et al., 1981; Fessler et al., 1981). Packaging of triple helical molecules into secretion vesicles and secretion from the cell are accompanied by proteolytic cleavage of -NH_2 and -COOH terminal propeptides which further produces helical collagen molecules with non-helical telopeptides at the -NH_2 and -COOH terminus (Bornstein and Traub, 1979).

The excised -NH_2 and -COOH terminal propeptides or their fragments influence collagen synthesis via a feedback inhibition mechanism. Wiestner et al. (1979) first reported that -NH_2 terminal propeptides from types I and III procollagens produced inhibition of fibroblast collagen synthesis (types I and III) without affecting degradation or hydroxylation of collagen, synthesis of type II collagen or synthesis of non-collagen proteins. Subsequent experiments demonstrated that the -NH_2 terminal propeptide and certain of its fragments exerted control on collagen synthesis by inhibiting translation of procollagen mRNAs rather than by affecting mRNA levels (Horlein et al., 1981; Raghow and Thompson, 1989). The -COOH terminal propeptide was also shown to have an inhibitory effect on collagen synthesis. The mechanism of synthesis inhibition was post-transcriptional since collagen mRNAs were not diminished (Aycock et al., 1986). The specific effects that excised -NH_2 and -COOH terminal propeptides and their fragments have on collagen synthesis suggest that the biosynthetic process (and, therefore, growth rate) may possess a mechanism to regulate both rate of collagen synthesis and type of collagen produced.

Regulation of Extracellular Events

The important extracellular events in collagen biosynthesis, crosslinking, fibril formation and collagenolysis involve organization of collagen molecules into an extracellular matrix as well as matrix degradation. After secretion from the cell, specific lysine and hydroxylysine residues of the collagen α chains are oxidatively deaminated by the extracellular enzyme, lysyl oxidase. The resulting reactive aldehydes, lysine and hydroxylysine residues spontaneously condense to form covalent crosslinks of varying composition and stability (Eyre et al., 1984; Eyre, 1987; Yamauchi and Mechanic, 1988). Lysyl oxidase requires copper and pyridoxal as cofactors; lysyl oxidase activity and proper crosslinking are dependent on adequate dietary copper (Siegel, 1979; Kagan, 1986). Numerous studies have documented the relationship between copper deficiency and impaired collagen crosslinking (Carnes, 1968; Opsahl et al., 1982), as well as between increased dietary copper levels and increased lysyl oxidase activity (Rayton and Harris, 1979; Opsahl et al., 1982). Dietary copper levels resulting in impaired collagen crosslinking and defective collagen maturation are extremely low (1-2 mg Cu/kg of diet) and are significantly lower than dietary copper required for normal growth (Opsahl et al., 1982; Tinker and Rucker, 1985). Cattle maintained on a synthetic, low copper diet (1 mg Cu/kg of diet) exhibited reduced weight gain and feed conversion as well as lower lysyl oxidase activity and disorganization of connective tissue fibrils (Davies et al., 1984). Low dietary copper may not necessarily contribute to differences in muscle collagen or in meat texture. Bouton et al. (1988) measured the mechanical properties of cooked muscle from two groups of young cattle with wide variations in liver copper concentrations (8 vs 132 mg/kg of dry liver) and noted no differences in shear values or adhesion measurements. The effect of limited copper intake would probably be noted first in reduced growth rate and collagen synthesis rather than in depressed lysyl oxidase activity. Similarly, other factors which affect protein synthesis and growth may not affect the crosslinking process itself. McClain et al. (1973) reported that zinc deficiency resulted in increased crosslinking in skin of rats. Tinker and Rucker (1985) observed that differences in crosslinking in zinc deficiency resulted from decreased de novo collagen synthesis and a proportionate increase in crosslink content in the skin of deficient animals. A series of recent studies (Fields et al., 1983; Failla et al., 1988; McCormick et al., 1989) have clearly demonstrated that the source of dietary carbohydrate effects copper metabolism in rats. Effects of copper deficiency were exacerbated when fructose, glucose or sucrose rather than starch comprised the primary dietary carbohydrate. McClain (1976, 1977) first reported at the Reciprocal Meat Conference that source of dietary carbohy-

drate influenced collagen crosslinking in muscle. He observed that when fructose rather than glucose served as the dietary carbohydrate, yields of salt- and acid-soluble collagen were higher and crosslinked components lower.

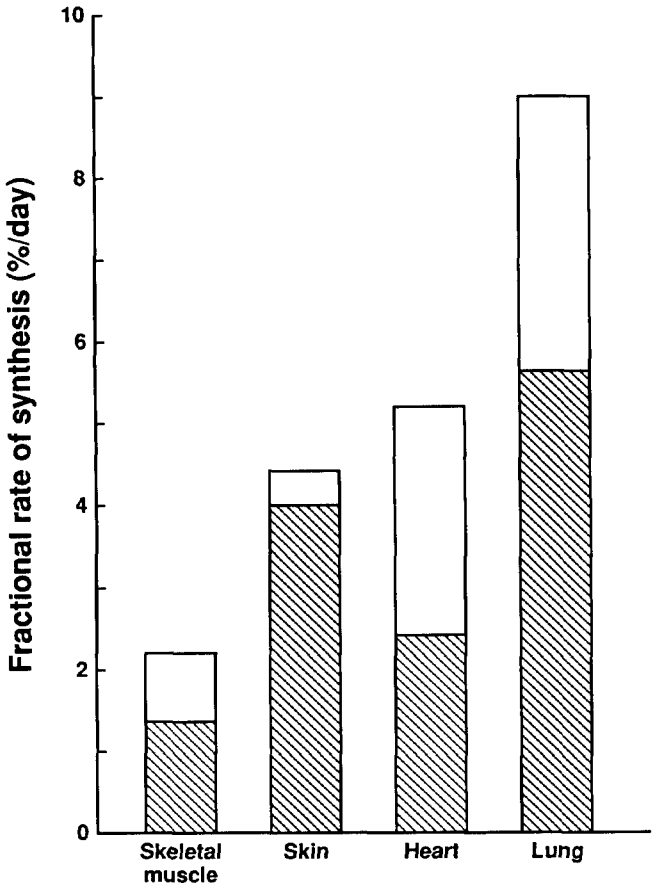
In addition to the necessary cofactors, a second requirement for lysyl oxidase activity and crosslinking is alignment of collagen molecules into the characteristic quarter stagger conformation of the fibril (Siegel, 1979; Bailey and Light, 1985). It has been proposed that a portion of the helical region of one collagen molecule serves as the binding domain for lysyl oxidase as it oxidatively deaminates lysyl residues in the telopeptide region of an adjacent collagen molecule (Bailey and Light, 1985). Interestingly, glucose concentrations in the physiological range for human blood (5 mM) and above were reported to inhibit both collagen fibril formation and lysyl oxidase activity in vitro (Lien et al., 1984). Non-enzymatic glycosylation of collagen was thought not to have played a role in the inhibition of fibril formation in vitro since the reaction occurs slowly. The mechanism by which carbohydrate source influences collagen crosslinking is complex and not well understood. The disruptive effect of simple sugars on fibril formation and lysyl oxidase activity is a plausible explanation for observed decreases in crosslinking.

The characteristic macromolecule of the extracellular matrix is comprised of collagen microfibrils which aggregate to form collagen fibrils of varying diameter (Craig and Parry, 1981; Parry and Craig, 1988). Various covalent and noncovalent interactions and interaction with non-collagen components of the extracellular matrix aid in organization, stabilization and cell adhesion of the collagen fibril (Last and Reiser, 1984). Growth, size and diameter of collagen fibrils are influenced by tissue type, age, stage of development and collagen type (Parry and Craig, 1988).

Degradation of fibrillar collagen occurs extracellularly through the action of collagenase and neutral proteinases. Types I and III collagen are more susceptible to degradation than type II collagen and heavily crosslinked collagen is more resistant to solubilization by collagenase than less crosslinked collagen (for reviews, see Bailey and Etherington, 1980; 1987; Jeffery, 1986). Collagenase is a metallo-enzyme and requires both CA^{2+} and Zn^{2+} for activation (Werb, 1974). Nutritional zinc deficiency and reduced collagenase activity were reported to lower collagen degradation in chick bone (Starcher et al., 1980). Corticosteroids (Koob et al., 1980) and estrogen (Cutroneo et al., 1986) are inhibitors of collagenase synthesis in a variety of tissues and probably regulate synthesis at the transcriptional level (Bailey and Etherington, 1987).

Although collagenase and neutral proteinase can degrade extracellular collagen, in-vivo studies have indicated that degradation of collagen already incorporated into fibrils is negligible. During periods of restricted growth followed by refeeding, there was no significant decrease in the level of labelled hydroxyproline in the skin of young rats (Etherington, 1977; Etherington and Bailey, 1982). In neonatal rats, fibrillar collagen degradation was estimated to be less than 1% per day during normal growth. In a study of crosslink turnover in lung tissue in growing rats, Last et al. (1989) concluded that extracellular collagen was not degraded at a measurable rate. Actual degradation of collagen is much higher than values for fibrillar collagen degradation reflect, but the degradation occurs intracellularly and consists of breakdown of newly synthesized collagen by non-lysosomal and lysosomal proteinases (Berg, 1986). Bienkowski (1984) estimated that about 15% of the collagen synthesized by fibroblasts is broken down rapidly within the cell, a process termed basal degradation. McAnulty and Laurent (1987) examined collagen synthesis and degradation in vivo in various tissues of adult rats. Figure 2 depicts the fractional synthesis rate of collagen per day in skeletal muscle, skin, heart and lung tissue. Synthesis rates are lower in skeletal muscle than in other tissues higher in collagen (about 2.2% for muscle vs. 9.0% for lung) but that fraction of collagen which is degraded rapidly and probably intracellularly is substantial. These data are consistent with in-vitro studies which demonstrated that a proportion of newly synthesized collagen was rapidly degraded. They also indicated that collagen is rapidly synthesized and degraded in skeletal muscle. Growth and the net accumulation of collagen is dependent on turnover (degradation) of collagen as well as synthesis. Not clearly understood is how newly synthesized collagen is incorporated into existing fibrils within the extracellular matrix (Etherington, 1987; Nimni and Harkens, 1988).

Figure 2



Collagen metabolism in tissues of the adult rat. Bars represent fractional rates of synthesis and are divided in 1) that fraction of collagen which is degraded rapidly and probably intracellular (open portion), and 2) that fraction which is either degraded extracellularly or, if tissues are still growing, may represent collagen deposition (hatched portion).
Adapted from Laurent, 1987.

Growth Rate

It is generally acknowledged that for optimum tenderness the best time to slaughter animals is after a period of rapid growth (Etherington, 1987). This principle has been cited by small and large producers alike, and livestock finishing systems are predicated upon it.

High plane of nutrition and rapid growth rate result in greater collagen synthesis. The enlarged pool of new collagen which is less crosslinked and therefore soluble and heat-labile is believed to dilute the older, tougher existing collagen, resulting in a more tender muscle (Etherington, 1987). Studies with cattle and sheep indicate that while at certain times more rapidly growing animals produce muscle that is more tender, meat from the less rapidly growing animals is often just as tender or more so.

Aberle et al. (1981) and Fishell et al. (1985) slaughtered young steers of the same age but grown at different rates. Slower growth rate was associated with lower muscle tenderness scores, higher collagen transition temperatures, lower proportions of soluble collagen and slightly higher total collagen amounts. Similar results were observed when muscle and collagen characteristics of mature cows fed a high-energy diet were compared to those on a maintenance energy diet (Miller et al., 1987). Conversely, Wu et al. (1981) reported that there were neither larger nor consistent differences between steers fed high-energy or roughage diets. Hall and Hunt (1982) found that feeding A-maturity steers a high-energy concentrate diet compared to roughage prior to slaughter did not increase the proportion of salt- or acid-soluble collagen or improve palatability or shear force values, particularly when feeding times were extended. They concluded that percent soluble collagen, i.e., newly synthesized collagen, alone did not account for much of the tenderness variability and that growth rate enhanced by a high plane of nutrition may have contributed to collagen maturation. Crouse et al. (1985) reported that among cattle fed low- and high-energy diets, muscle from cattle raised on the low-energy diet was more tender while containing a lesser proportion of soluble collagen. Total insoluble collagen in muscle of cattle on either high- or low-energy diets was the same. Miller et al. (1989) compared characteristics of intramuscular collagen among growing rams and wethers. Growth rate and collagen synthesis and degradation, while not a function of nutritional status, was markedly affected by sex condition. Compared to wethers, the more rapidly growing rams possessed more newly synthesized collagen with lower thermal transition temperature and greater solubility. However, total intramuscular collagen and total insoluble collagen was greater in rams than in wethers and the percent soluble collagen was lower. The variability in collagen synthesis and characteristics would probably be reflected in variable meat texture and palatability.

The mechanisms by which plane of nutrition and growth rate affect collagen metabolism are not entirely understood. Attempts to predict muscle collagen characteristics on the basis of diet, growth rate, age and other interactions are often unsuccessful. Recent studies examining collagen synthesis and degradation, expression of collagen type, and crosslinking may serve to further elucidate the relationship between nutrition and growth and collagen metabolism.

Collagen Synthesis and Degradation

Collagen synthesis and degradation in vivo are studied in a wide range of tissues by the use of amino acid labelling techniques. For example, small animals are dosed with isotopically labelled amino acids, usually proline or lysine, and the rate of their incorporation into hydroxyproline or hydroxylysine is used as a measure of collagen synthesis. Disappearance of label from tissue collagen has often been assumed to represent collagen degradation or turnover. Results of earlier in-vivo studies on collagen synthesis and degradation have been criticized for their failure to take into account very rapid collagen degradation and/or re-utilization of the labelled anion acid. The resulting estimates of collagen synthesis and degradation, therefore, could have been significantly underestimated (Laurent, 1987).

Normal vs. Restricted Growth

Molnar et al. (1986, 1987, 1988) studied the effect of growth retardation in rats on the pool size of acid-soluble and insoluble skin collagen. In these studies, $^{18}O_2$ incorporation into collagen hydroxyproline and its loss over time provided measures of collagen synthesis and degradation, respectively. The use of $^{18}O_2$ was first described by Jackson and Heiniger (1974). Because $^{18}O_2$ cannot be re-incorporated into collagen or other proteins, problems associated with labelled amino acid reutilization are avoided and reliable collagen synthesis and turnover estimates achieved. Values for synthesis degradation and maturation rates of skin collagens of normal and protein-deficient rats are shown in Table 1. In the well-nourished animal, the soluble (total) collagen synthesis rate, the rate at which soluble collagen matures (crosslinks) into insoluble collagen and the rate of degradation (maturation + degradation rate = soluble collagen efflux rate) were much higher than in the malnourished animals. Although the size of the soluble collagen pool and the movement of collagen out of the soluble pool were greater for rapidly growing animals, the fractions of soluble collagen degraded (53%) or maturing into insoluble collagen (46%) were the same for both healthy and malnourished rats. These data indicate that the potential for higher concentrations of mature insoluble skin collagen is substantially greater

Table 1. Synthesis and Degradation Rates of Soluble and Insoluble Skin Collagens.

Rats	*Soluble collagen synthesis rate*	*Total soluble collagen efflux*	*Soluble collagen degradation rate*	*Insoluble collagen synthesis rate*
Healthy	99 ± 8	70 ± 8	37 ± 8	32 (46)
Malnourished	11 ± 2	11 ± 2	6 ± 5	5 (45)

Adapted from Molnar et al., 1988

in the rapidly growing animal than in the growth-restricted animal. At the same time, the rapidly growing animal will have more soluble collagen and greater collagen degradation that are usually associated with youthful, less structurally mature collagen. Whether the muscle collagen pool behaves in like fashion is not known. The results of studies with meat animals cited previously indicate that, in some cases, rapid growth and increased collagen synthesis may not be diluting the pool of mature insoluble collagen but instead actually increasing it.

Rapid or Compensatory Growth

Laurent and colleagues used in-vivo infusion of proline together with a flooding dose of cold (unlabelled proline) to estimate synthesis and degradation of collagen in chicken and rat muscle during growth and hypertrophy and in the steady state. During stretch-induced growth in chicken skeletal muscle, total collagen content of the muscle increased but not as rapidly as non-collagen intracellular protein (Laurent et al., 1978; Sparrow, 1982). However, intramuscular collagen synthesis (perimysium and endomysium) actually parallelled production of non-collagen protein with the overall decrease in total collagen attributed to decreased production of epimysial collagen (Laurent et al., 1978; Sparrow, 1982).

Collagen synthesis and degradation estimates during muscle hypertrophy are given in Table 2 (Laurent et al., 1985). There was a fivefold increase in collagen synthesis and degradation of newly synthesized collagen decreased by half during hypertrophy. The degradation of mature collagen increased approximately fourfold. Laurent et al. (1985) attributed the degradation of mature collagen to breakdown of collagen containing covalent crosslinks, though not necessarily mature crosslinks. Regression of hypertrophy led to a decrease in total collagen content, perhaps because the newly synthesized collagen deposited during rapid growth was not highly crosslinked, and thus subject to degradation (Sparrow, 1982). Histological examination revealed heavier, more prominent endomysial sheaths in hypertrophied muscle than in normal muscle (Sparrow, 1982).

The mechanism of collagen turnover in rapidly growing (hypertrophying) muscle appears to differ from that in skin or tissues experiencing normal or restricted growth. It is interesting that in hypertrophying muscle mature collagen (i.e. fibrillar collagen) is apparently turning over; most studies involving skin or lung tissues indicated that degradation of collagen incorporated into fibrils is negligible (Etherington and Bailey, 1982; Jefferys et al., 1985; Last and Reiser, 1989).

The increase in muscle size with growth gives rise to the question: How does the connective tissue network within muscle grow? The data reported by Laurent's group gives credence to the "remodeling" theory that during growth increased collagen synthesis coupled with a decreased degradation of newly synthesized collagen occurs; an increase in the degradation of mature collagen is necessary for growth to take place (Woessner, 1979: Laurent, 1985; Laurent et al., 1985). Alternatively, growth of the extracellular matrix with little or no degradation of existing collagen may occur, although the mechanism by which new collagen molecules integrate themselves amongst established molecules is not known (Etherington, 1977, 1987; Etherington and Bailey, 1982).

Fasting

Spanheimer and Peterkofsky (1985) examined the effect of fasting of guinea pigs on collagen and non-collagen protein synthesis and on mRNA levels for specific collagen proteins. Collagen production decreased soon after fasting began and after 96 hours of fasting collagen synthesis was reduced to 25% of non-fasting values in bone, cartilage and skeletal muscle (Table 3). Effects on non-collagen protein synthesis were much less severe, demonstrating a specific effect on collagen synthesis. There was no evidence of increased collagen degradation as a result of fasting. Procollagen mRNAs for α_1 (I), α_2 (I) and α_1 (II) procollagen chains were decreased in tissues of fated animals but there were no coincident decreases in mRNAs for non-collagen proteins. Acute fasting resulted in a rapid and specific decrease in collagen synthesis, mainly through modulation of mRNA levels. Spanheimer and Peterkofsky (1985) discounted the possibility that a deficiency of one or more major nutrients resulted in decreased collagen synthesis. They suggested, rather, that the response may have been mediated by certain steroid or peptide hormones which were affected by fasting. Glucorticoids levels increase during fasting and glucocorticoids have an inhibitory effect on collagen synthesis through reduction of collagen-specific mRNA; (Cutroneo et al., 1986; Raghow and Thompson, 1989). Insulin and the somatomedins stimulate collagen synthesis and levels of both are decreased during fasting (Covalis, 1980; Isley et al., 1982).

Collagen Type

Most tissues contain a mixture of collagen isoforms or types which are distinct genetically and structurally. Muscle epimysium and perimysium consist primarily of types I and III collagen with small amounts of type V present (Light and Champion, 1984; Bailey, 1988). Endomysium contains types

Table 2. Collagen Metabolism During Hypertrophy of Anterior Latissimus Dorsi Muscle of Chicken Measured by In-Vivo Labelling.

	Control	*Hypertrophy*
Tissue-free pool hydroxyproline, dpm	2,680 ± 910	5,240 ± 1,230
Total hydroxyproline in protein, dpm	2,310 ± 250	22,820 ± 3,410
Collagen synthesis rate, %/day	1.12 ± 0.3	5.0 ± 1.0
% Degradation of newly synthesized collagen	49 ± 7	19 ± 9

Adapted from Laurent et al., 1985

Table 3. Effect of Fasting on the Relative Rate of Collagen Production in Various Tissues Measured by In-Vivo Labelling.

Tissue	*Relative rate of collagen production*		
	Fasted (F)	*Control (C)*	*(F/C) x 100*
		%	%
Parietal bone	12.0 ± 1.2	51.5 ± 0.6	23
Long bone	6.0 ± 1.8	23.8 ± 0.9	25
Cartilage	4.2 ± 1.1	18.8 ± 1.5	22
Skin	0.5 ± 0.15	12.8 ± 1.1	4
Skeletal muscle	0.5 ± 0.1	2.0 ± 0.6	25
Lung	0.17 ± 0.07	1.6 ± 0.2	11

Adapted from Spanheimer and Peterkofsky, 1985

III and V in its fibrous portion, and the basement membrane of endomysium is of non-fibrous type IV collagen (Light and Champion, 1984; Bailey 1988). Types I and III collagen differ in amino acid composition, in degree of glycosylation and in their fibril-forming characteristics. Due to the presence of cysteine residues, type III collagen forms disulfide bonds within its triple helical region (Nimni and Harness, 1988). Type III collagen is less soluble (Diethardt and Tuma, 1971) and more resistant to enzymic proteolysis than type I collagen (Wu et al., 1982).

However, type III collagen forms fibers of smaller diameter than type I and a mixture of both types I and III collagen produces fibers with a diameter proportional to the concentration of the two collagen molecules (Lapiere et al., 1977; Parry and Craig, 1988). Some studies have correlated increased amounts of type III collagen in intramuscular collagen with increased muscle toughness (Bailey and Sims, 1977; Bailey et al., 1979) although others have found no clear relationship between type III collagen content and muscle texture (Light et al., 1985; Burson and Hunt, 1986). It is clear that fibrils consisting of type I collagen will differ chemically from fibrils containing type III molecules. The significance of shifts in type III/I collagen ratios in tissues, however, may be related to the regulatory function of type III molecules perform in the formation of large fibrils of type I collagen (Miller and Gay, 1982).

The proportions of a collagen isoform in a tissue can change in response to age and development and various hormonal and pharmacologic agents (Bornstein and Sage, 1980; Merlino et al., 1983; Raghow and Thompson, 1989). Such modulation of collagen synthesis is known as "switching." The predominant form of mature interstitial collagen derived from fibroblasts or myocytes is type I. It has generally been accepted that greater proportions of type III collagen occur in fetal or immature tissues. However, most of the studies describing shifts in type III/I collagen ratios have involved skin when type III collagen was present in greatest concentrations in fetal or immature tissues (Epstein et al., 1974; Ramshaw, 1986; Mays et al., 1988). In heart muscle and lung from rats, an age-related shift in collagen occurred but with type III collagen content increasing rather than decreasing (Mays et al., 1988).

Nutritional factors affect the synthesis of distinct collagen types in tissues. Deyl et al. (1981) reported that chronic (19 mo) food restriction and a low-protein diet resulted in a substantial increase in type III concentration in the skin. Molnar (1988) concluded on the basis of loss of $^{18}O_2$ label from the soluble skin collagen pool of malnourished rats, types I and III collagens were probably turning over at different rates. The turnover of types I and III collagen in skin appeared to be identical in well-nourished animals (Klein and Chandrarajan, 1977). Molnar et al. (1988) noted no increases in type III/I ratios in skin collagen of rats fed a low protein diet for 42 days but with refeeding and increased growth there was a marked increase in the Type III/I collagen ratio. After 6 weeks of catch-up growth, type III/I ratios resembled control values. Deyl et al. (1981) observed an increase in type I collagen following 6 months of refeeding after a period of restricted growth. Hypertrophy of the myocardium also resulted in shifts in collagen type proportions. Copper deficiency-induced hypertrophy in rats resulted in increased type III/I collagen ratios relative to non-hypertrophic animals (Dawson et al., 1982). Bleomycin-induced cardiac hypertrophy in the rabbit, however, produced increased proportions of type I collagen (Turner and Laurent, 1986). Information on the effect of age and development, hormonal or other agents on collagen type expression in skeletal muscle is scant. Burson et al. (1986) compared characteristics of intramuscular collagen from the *longissimus* muscle among bulls and steers and noted no differences for total collagen or proportion of type III collagen.

The contributions that genetically distinct forms of collagen make to muscle texture are not clearly understood. However, certain characteristics of type III collagen such as disulfide crosslinking and decreased solubility compared to type I suggest that its presence in muscle contributes to overall toughness (Light, 1987; Bailey, 1988). Nutritional factors, especially as they affect growth rate, can modulate the synthesis of types I and III collagen in certain tissues, notably skin. Specific nutritional effects on expression of collagen types I and III in skeletal muscle connective tissue depots have not been reported.

Collagen Crosslinking

The stability and tensile strength of collagen fibrils depends largely on the formation of covalent intra- and intermolecular crosslinks between collagen molecules. The chemistry of collagen crosslinks, the pathways of their formation and intermediate and mature crosslinking structures have been reviewed in the last ten years (Etherington and Bailey, 1980; Eyre et al., 1984; Eyre, 1987; Yamauchi and Mechanic, 1988).

As collagen ages, its complement of covalent crosslinks

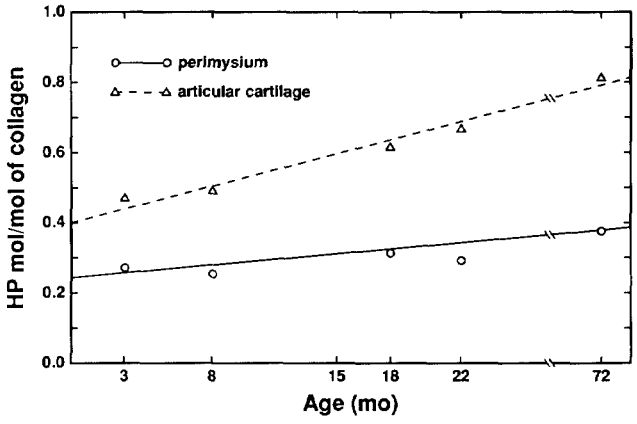
mature such that the less stable aldimine and ketoimine reducible crosslinks diminish and are replaced by more stable, non-reducible structures. Among the known, mature, non-reducible crosslinks are hydroxylysylpyridinoline (HP) (Fujimoto et al., 1977), histidinohydroxylysinonorleucine (HHL) (Yamauchi et al., 1987) and compound M (Barnard et al., 1987). The stable, non-reducible crosslinks appear to be condensation products of the reducible aldimine and ketoimine crosslinks although exact mechanisms of formation have not been elucidated. Evidence for a precursor-product relationship between reducible and non-reducible crosslinks exists since there is a stoichiometric relationship between the disappearance of reducible crosslinks and the formation of non-reducible crosslinks such as HP (Last et al., 1989) and HHL (Yamauchi et al., 1987).

Crosslink profile also varies with different tissues and is related to the genetic type of collagen (Eyre et al., 1984) as well as physiological function (Yamauchi and Mechanic, 1988).

The results of studies examining the effect of nutritional status and growth rate on collagen crosslinking have been contradictory. At issue is the question: Do fluctuations in dietary protein and energy and growth rate retard or accelerate the crosslink maturation process? Studies conducted with cattle and sheep discussed previously indicated that nutrition and growth do affect muscle crosslinking status as reflected by collagen solubility, thermal transition temperatures and shear force values. However, the data also strongly suggest the direction of the changes in crosslink maturation in muscle differs depending on growth rate, rate of new collagen synthesis, animal age, sex condition and the interaction of these factors.

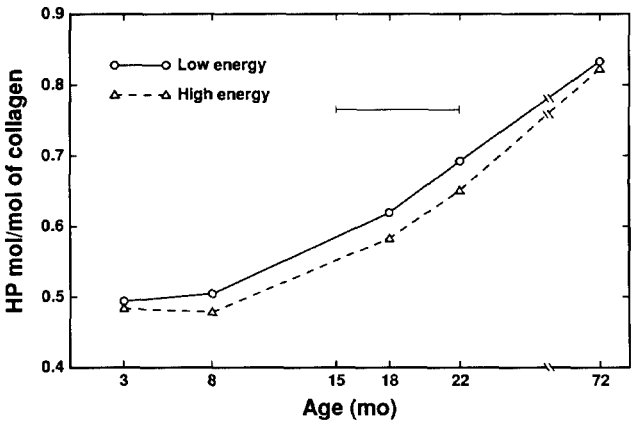
Studies on skin collagen metabolism in growth-restricted rats have produced conflicting evidence of the effect of arrested development on crosslinking. McClain et al. (1975) and McClain and Wiley (1977) observed a decrease in intramolecularly crosslinked β-chain components and an increase in the α-chain fraction in the soluble collagen, indicating fewer mature crosslinks. Likewise, Rao and Rao (1980) reported that restricted protein intake resulted in increased

Figure 3



Effect of age on hydroxypyridinoline content of ovine perimysium and articular cartilage. HP concentration was determined by reverse phase HPLC and fluorescence detection after hydrolysis.
From McCormick et al., 1989b.

Figure 4



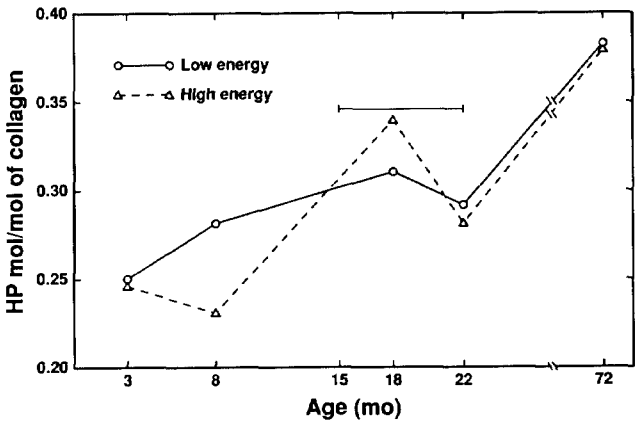
Effect of diet on hydroxypyridinoline content of ovine carticular cartilage. Horizontal bar represents period from 15 to 22 months during which animals on a low-energy diet were fed a high-energy diet.
From McCormick et al., 1989b.

labile reducible crosslink concentration, increased α-chain and decreased β-chain content and increased collagen solubility, indicating reduced collagen maturation. Etherington and Bailey (1982), on the other hand, observed that during arrested development rat skin collagen became increasingly insoluble and the reducible crosslink profile of the insoluble fraction resembled that of more mature collagen.

Experiments conducted in our laboratory indicated that concentrations of the non-reducible crosslink HP were affected by plane of nutrition and growth rate (McCormick et al., 1989). Articular cartilage and *longissimus* muscle perimysium from lambs and sheep fed high- and low-energy diets and sheep fed a high-energy diet after a period of undernutrition were analyzed for HP. The levels of HP in articular cartilage, primarily type II collagen, were significantly higher than in perimysium which consists mostly of types I and III collagen. HP concentrations in both tissues increased with age but substantial amounts of HP were present in both articular cartilage and perimysium of young (3 mo) animals (.46 and .26 mol/mol of collagen, respectively, Fig. 3).

Restricted energy intake and refeeding did not markedly affect HP levels in articular cartilage (Fig. 4). For animals maintained on a low-energy diet, perimysial collagen HP concentration increased until animals reached 18 mo of age and then declined at 22 mo, indicating that prolonged low-energy diets may interfere with or retard crosslinking (Fig. 5). These data support observations that restricted energy or protein intake compared to high-energy diets resulted in muscle collagen that is more soluble or muscle that is more tender (Hall and Hunt, 1982; Crouse et al., 1985; Johnson et al., 1989). For lambs slaughtered at 8 mo of age, a high-energy diet resulted in significantly low perimysial HP concentrations. However, for sheep maintained on a low-energy diet for 15 mo and slaughtered after 3 mo of feeding a high-energy diet, HP concentration increased significantly (Fig. 5). Additional time on a high-energy diet decreased perimysial HP levels in sheep 22 mo of age. For young animals, high-energy diets may cause decreased perimysial HP compared to low-energy diets by accelerating new collagen synthesis. However, the rapid growth and increased protein synthesis

Figure 5



Effect of diet on hydroxypyridinoline content of ovine perimysium. Horizontal bar represents period from 15 to 22 months during which animals on a low-energy diet were fed a high-energy diet. From McCormick et al., 1989b.

associated with refeeding also resulted in rapid and increased crosslinking in perimysial collagen. Extended high-energy feeding tended to reduce the non-reducible crosslink concentration. The changes observed in perimysial HP levels provide direct evidence that variations in the crosslinking pattern of muscle collagen result from changes in dietary regimen and growth rate as well as age and development.

Summary

The role of nutrition in regulation of collagen metabolism and the structural stability of the collagen matrix in muscle of meat animals is difficult to assess. Specific nutrient factors influence collagen protein synthesis through transcriptional and translational regulation of gene expression, altering both amount and type of collagen synthesized. Post-translational modification of collagen (especially crosslinking) is also affected. Modulation of collagen gene expression also occurs in response to growth factors and steroid hormones, concentrations of which are sometimes a function of nutritional status.

Growth rate and the accompanying changes in protein synthesis rate have marked effect on muscle collagen properties. Under some circumstances, the response to increased growth and new collagen synthesis is increased crosslinking, and accelerated maturation of crosslinks and decreased muscle tenderness. On the other hand, rapid growth and increased new collagen synthesis can result in more tender muscle. The processes of intra- and extracellular collagen degradation and collagen fibril growth as they relate to collagen synthesis, animal growth rate and meat texture are not clear.

Our understanding of collagen metabolism regulation and the role of collagen in determining meat texture has advanced significantly in the last ten years. In light of the current emphasis on increasing growth rate and muscle deposition in meat animals, more research is needed on the effect of enhanced and accelerated muscle growth on collagen synthesis.

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