Preparing the Soil: Practical Cellular Biochemistry for Regenerative Medicine

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Abstract This paper represents a review of current cell physiology and biochemistry principles to optimize DNA protein synthesis and regenerative cellular functions. Supplementation with specific anabolic and metabolic substrates can augment the effects of platelet growth factors and the repair of all tissues.

Introduction

Medicine has evolved during the recent decades to focus on pharmaceutical interventions for specific diseases. We have become conditioned to utilize a "drug for disease" approach to medicine. Admittedly, it can simplify the practice of medicine and expedite patient management with quotidian care. However, we were extensively trained in all the basic sciences so that we could and would think scientifically when diagnosing and treating. The birth of regenerative medicine has rekindled in many of us a passion to understand the cell biology and biochemical science of the body's mechanisms of repair. The rationales for this chapter are twofold. First, with any new development in medicine there is an almost immediate antipathy and resistance from the conventionalists. Scientific justification provides an incontrovertible basis for these new procedures and interventions. Therefore, in this chapter I will highlight some of the basic biochemical principles of cell metabolism that will enhance the effectiveness of Platelet Rich Plasma (PRP) in regenerative medicine. Secondly, these principles can easily be incorporated into a supplement protocol for patients who are to undergo a procedure. It is analogous to preparing the soil for a garden. To expect any regenerative procedure to have optimal results in a patient depleted of the essential elements of

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cellular function is myopic. No respectable gardener would plant in a plot of sand and expect his tomatoes to take root and produce. Instead he prepares the soil. Similarly, restoring cellular health should be the first consideration in regenerative medicine.

A fundamental principle to remember in this regard is that ALL CELLS WORK THE SAME (with the exception of the erythrocyte). If our goal is to increase mitogenicity, fibroblast migration, and chondrocyte and osteoblast activity, we must increase the metabolic capability of cells and promote anabolic functions with hormones and cofactors. And, above all, it must not be an onerous protocol for the patient. Based on the above principle, then, whatever we do for the fibroblast will work for the osteoblast, the chondrocyte and the neuron. Therefore, this discussion will first address mitochondrial energy production and the chemicals which can be readily supplemented to maximize it. The subsequent sections will discuss nuclear receptors and their ligands and the interactions among these that promote anabolic functions and how these nuclear hormones can be easily supplemented. Incorporated in this will be a review of vitamin cofactors and biochemistry that can augment metabolism and tissue repair. This chapter is only a simple review or overview of practical principles which can be easily understood and implemented. It is by no means a comprehensive text on biochemistry.

I began studying regenerative medicine 14 years ago because my patients continued to return with new strokes, new decubitus, and new myocardial infarcts. It was clear that the current medical paradigm was not adequate to either heal or prevent. In the beginning, I employed basic cellular biochemistry concurrent with traditional medical therapies with encouraging results. I found that "it is better to regenerate than to compensate". I had no access to PRP or stem cell technology. I looked for any manipulation that was known to stimulate the body's stem cell production and repair. These manipulations included optimizing mitochondrial energy production by increasing beta oxidation of fatty acids and electron transport efficiency, controlling deleterious systemic inflammatory processes, balancing nuclear hormones to achieve an inhibitory or facilitory benefit, and utilizing cofactors. My primary area of interest is neurogenesis, but because "all cells work the same", I also found that using these principles, long standing wounds and nonunion fractures healed and cardiac output increased and blood pressure and cholesterol decreased and muscles strengthened.

Platelet rich plasma and stem cell science has now added an exciting dimension to tissue repair. However, studies are reporting conflicting results regarding benefit and efficacy and this has generated considerable doubt and skepticism among traditionalists. I propose that these variable outcomes are due to the wide variability of patient chemistry. Perhaps the soil needs some preparation.

The following (Fig. 1) is an example of what just implementing basic biochemical principles can do with wound healing. PRP was unavailable at that time. The composite picture below shows the results of supplementation with coenzymeQ10, acetyl-L-carnitine, alpha lipoic acid, omega 3 fatty acids, arginine, glutamine, and Armour thyroid, in addition to the anabolic hormones, DHEA, testosterone, progesterone, estradiol, and somatotropin in a wound of 10 year duration.

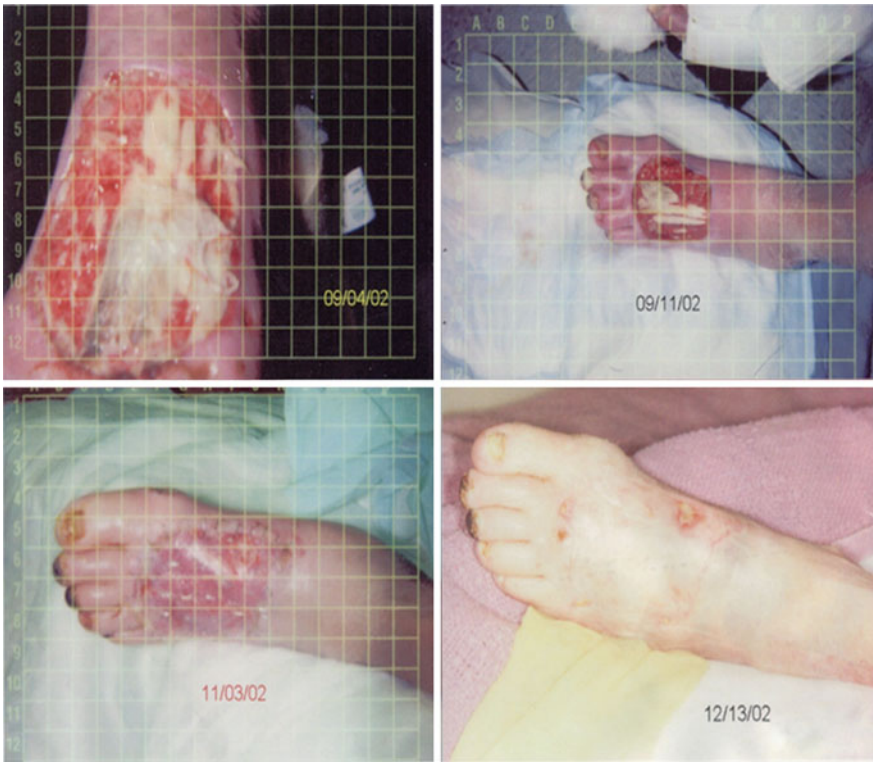

Fig. 1 Ischemic wound in 80 year old female healed with restoration of proper cellular biochemistry and anabolic function

This is the case of an 80 year old female with a 10 year old wound which was precipitated by bumping her foot on her wheelchair. Because of her occluded macro and microvasculature, and her inability to get oxygen and nutrients to this tissue, she was unable to heal. Her pain levels were severe. She refused to allow her doctors to amputate her leg.

Within a week after beginning the protocol she was beginning to demonstrate dramatic changes in the tissue. Beefy granulation tissue was developing by 9/11/02. This continued for another several weeks and by 11/03/02 her tissue was able to support a skin graft which subsequently had 100 % "take". Within another 6 weeks, she had a normal foot again. Total time from starting the protocol to wound healing was 3 months

Thyroid supplementation increases mitochondrial number in all cells and metabolic capability and activity. CoQ10 increases the energy production within the mitochondria of the cells. The acetyl-L-carnitine increases the ability of the tissue to "shift gears" and implement fatty acid metabolism in lieu of the tissue's dependence on oxygen and glucose and allows tissue maintenance and healing in the face of compromised blood flow. Alpha lipoic acid both increases tissue energy

production and reduces the free radical activity and oxidation potential of the ischemic tissue. Arginine and glutamine provide the protein substrates for new tissue. Arginine also converts to nitric oxide to increase the blood flow to the tissue by vasodilating all vessels that can still support some blood flow. Omega 3 fatty acids provide an energy substrate as well as raw materials from which to construct new tissue. The anabolic hormones serve to make new tissue.

The potential benefit of this approach in combination with the now available regenerative medicine procedures such as PRP should be at least additive, if not exponential. The following is a more comprehensive biochemistry review of these basics.

First Steps

Controlling Systemic Inflammation

C-Reactive Protein

All disease processes begin with oxidation, inflammation, infection, or an immune system gone awry, and usually a combination of these "Big 4". While the principle of PRP depends on initiation of inflammation in order for growth factors to be released, this should only be a controlled, self-limited local process. The rest of the body must not be in an inflammatory state if effective healing is to occur. With recurrent injury, prolonged exercise or diabetes, repeated antigen exposure such as infection, ethanol and smoking, the body never leaves the inflammatory preparatory phase for healing. Inflammatory cytokines, including interleukin-1, tumor necrosis factor-α, and the matrix metalloproteinases are destructive. Good barometers of the inflammatory state of the organism are C reactive protein (CRP) and homocysteine. Evaluation of a patient's CRP and homocysteine levels should be a first step. If these levels are elevated, they must be addressed.

CRP elevations have been well-documented in all inflammatory, autoimmune diseases and infections and are reflective of even occult destructive processes. CRP is a hepatic protein generated in response to cytokines (Interleukin 6, TNF alpha, etc.) which are the physiologic response to a variety of stimuli; smoking, insulin, toxins, heavy metals, fatty tissues, oxidized LDL, fungi, viruses, parasites, and tissue injury. It is worthwhile to note that in our western culture, a primary cause is high glycemic carbohydrate consumption, especially when combined with high fructose corn sweeteners (Fig. 2), which generates elevated insulin levels, which increases liver fat via fatty acid synthase, which produces IL-6, which in turn activates acute phase reactants in the liver (Fig. 3). These acute phase reactants also include mannose binding protein, mannose binding lectin, serum amyloid A, haptoglobin, complement, ferritin and ceruloplasmin, fibrinogen and other coagulation promoters. These are all proinflammatory reactants and

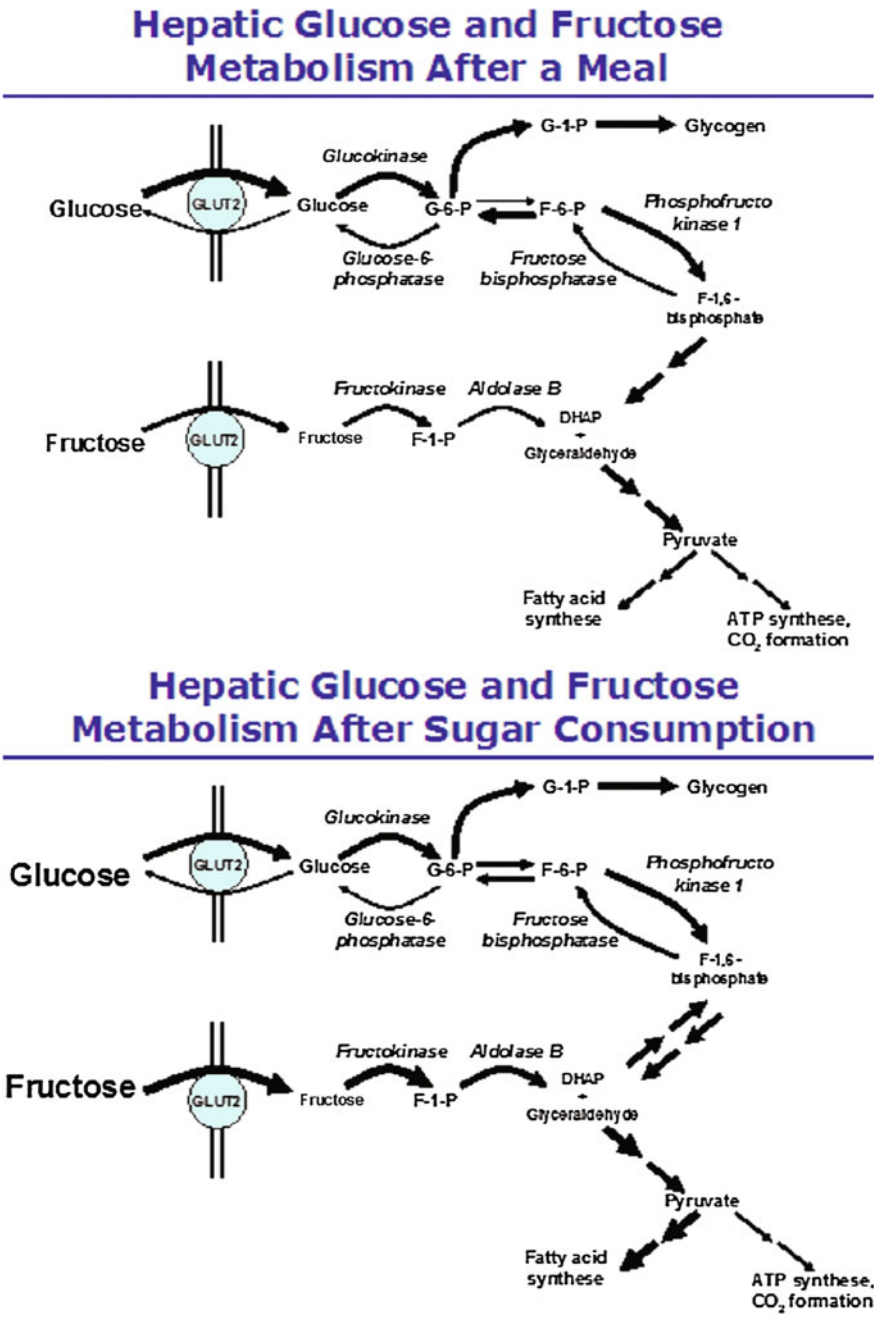

Fig. 2 Concurrent intake of both glucose and high fructose sweeteners increases fatty acid synthase and hepatic fat. This promotes inflammatory responses with elevations of CRP and IL-6

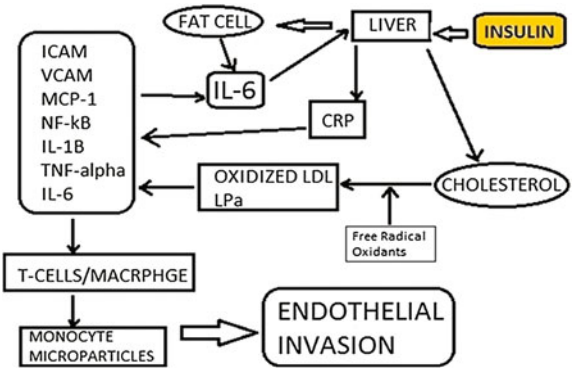

Fig. 3 Vicious cycle of inflammation beginning with hyperinsulinemia

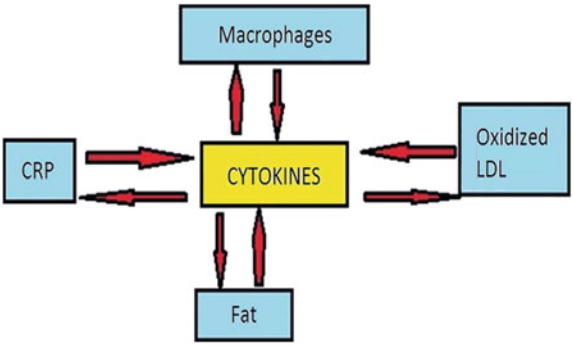

Fig. 4 Perpetual inflammation

represent activation of the lectin, classical, and alternative complement pathways. So the entire organism is geared up for battle. This is certainly not fertile soil for regeneration.

CRP binds to the phosphocholine on the surface of dead cells and bacteria and activates the entire complement system. This then induces endothelial adhesion proteins ICAM1, VCAM1, angiotensin1, monocyte chemokines, endothelial NF-kB, endothelial IL-1B, IL-6, and TNFalpha. The significance of these adhesion molecules is extensively discussed in the PRP literature. Interestingly, these adhesion molecules then precipitate more IL-6, which then produces more CRP, which produces more inflammatory cytokines and adhesion molecules. This inflammatory cycle will continue. As an aside, the hepatic inflammation also generates cholesterol which, if there are excessive systemic free radicals, will result in increased oxidation of LDL which will, in turn, produce more cytokines and adhesion molecules thereby increasing the entire inflammatory environment (Fig. 4).

The activation of CRP therefore produces; leukocyte adherence and chemotaxis, extravasation into the inflamed endothelial intima, cellular inflammation, foam cell accumulation, decreased smooth muscle nitric oxide with a consequential decrease in vascular relaxation, increased vascular contractile signals

from angiotensin II, and hypertension. This very basic understanding of CRP and inflammation underscores how ill-conceived is simple cholesterol reduction therapy for preventing cardiovascular disease. It is also an example of why, without an understanding of basic cellular chemistry, 'a drug for a disease' approach to medicine is both naïve and ineffective.

Knowing this, then, how do we interrupt this cycle of inflammation and reduce CRP? There are numerous ways to do this, of course, but one answer is omega 3 and 6 fatty acids (PUFA's). These fatty acids compete for the enzymes that form eicosanoids from free fatty acids and tissue arachidonic acid via cyclooxygenases, COX1 and 2, thereby interfering with the production of the inflammatory prostaglandins, prostacyclins, thromboxanes and leukotrienes. While the biochemistry of this process is beyond the scope of this paper, reducing these inflammatory products with PUFA's will dramatically decrease levels of CRP and the general inflammatory state of the organism. It is also not surprising that, as is extensively documented in the cardiovascular literature, PUFA's reduce the incidence of atherosclerotic disease (He et al. 2002; Hu et al. 2002; Dewailly et al. 2002; Angerer et al. 2002).

PUFA's as one would expect are anti-angiogenic and inhibit VEGF, PDGF, PDECGF, COX, Prostaglandin E2, nitric oxide, NF-kbeta, and metalloproteinases (Spencer et al. 2009). One would think that this would diminish the effect of PRP. However, PUFA's both facilitate leukocyte migration into tissue via the metabolism of omega 6 FA and arachidonic acid to prostaglandin D2, and then regulate this migration by omega 3 FA which has been metabolized to prostaglandin D3. So this inflammatory neutrophil process is stimulated by cytokine release and by prostaglandin D2 and is then regulated by prostaglandin D3 (Tull et al. 2009). This further highlights the importance of these bioactive lipids in regulation of the inflammatory response.

Omega 3 and 6 fatty acid supplementation should be a part of any regenerative medicine protocol. PUFA's will, however, decrease platelet aggregation so increased bleeding from procedures is possible.

Homocysteine

High levels of homocysteine are known to inhibit osteogenesis resulting in osteoporosis and increased fracture risk and correlates with low B vitamins 6 and 12 and folate, the biochemical significance of which will be discussed later (Dhonukshe-Rutten et al. 2005; Herrman et al. 2005). Inhibition of the fibrillin-fibronectin dimerization by homocysteine impedes elastin and cross-linking of collagen and elastin. Fibrillins constitute the major backbone of multifunctional microfibrils in elastic and nonelastic extracellular matrices. Because it is always present in decreased wound healing and vasculopathies, elevated homocysteine is implicated as a major etiologic factor in these pathologies (Hubmacher et al. 2010; Hubmacher et al. 2011; Sebatier et al. 2009). Therefore, reducing homocysteine should enhance the effects of regenerative therapies in bone and soft tissue. How can this be accomplished?

Homocysteine is an amino acid which is formed from dietary methionine by the cleaving of a methyl moiety from methionine. Under normal circumstances, homocysteine levels are maintained by its re-methylation to methionine. High levels of homocysteine result in elevated asymmetric dimethylarginine which interferes with vasodilatation from the normal nitric oxide synthesis from arginine and indirectly increases inflammatory molecules via increased IL-6 from increased levels of oxidized LDL (see Figs. 2 and 3) Homocysteine levels are normally reduced by re-methylation to methionine by methylfolate and methylcobalamin. However, the folic acid and B12 must be methylated first. This methylation reaction of the B vitamins is dependent on the normal function of the MTHFR (methylene-tetrahydofolate reductase) gene. From my unpublished observations, this gene has a much higher rate of mutation than one would expect. There are two mutations that are surprisingly common, the A1298C and the C677T. The C677T mutation cannot methylate folic acid and as a result, in these patients homocysteine levels are elevated with the above potential pathologic consequences. Note that serum levels of folate will be normal in these patients. The ramifications of this mutation are significant. Fetal brain development depends on methylfolate and since the rates of mutation are high, it is recommended that obstetricians obtain this simple genetic test for mutations of the MTHFR gene in their patients.

For adequate connective tissue organization following regenerative procedures, it is important to obtain adequate levels of methylfolate and methylcobalamin to reduce homocysteine levels. If genetic testing is not possible or is otherwise prohibitive, simple supplementation with methylfolate 5–7 mg. per day would be recommended.

Mitochondrial Energy

Carnitine

Oxidative phosphorylation in the mitochondria produces the ATP required for cellular energy and metabolism. Without this the body would be inanimate. Optimizing energy production in cells will increase their mitosis as well as their function. This means that fibroblasts, chondrocytes, tenocytes, myocytes, osteoblasts and neurons can then reproduce, migrate, and repair. Energy sources for this process include both free fatty acids (FFA) and glucose. This is important because two readily available supplements can be utilized to augment ATP production in the mitochondria and, by extension, augment cellular function. These are L-carnitine and coenzymeQ10. (I prefer to use acetyl-L-carnitine because of its fat solubility and ability to easily cross the blood-brain barrier.) The goal is to get FFA and glucose both to and across the mitochondrial membranes and into the matrix in a form suitable to donate electrons. If we avoid a discussion of all the enzymatic reactions and delta E's and delta G's, it becomes much easier to follow. Let's address the beta-oxidation of FFA's first.

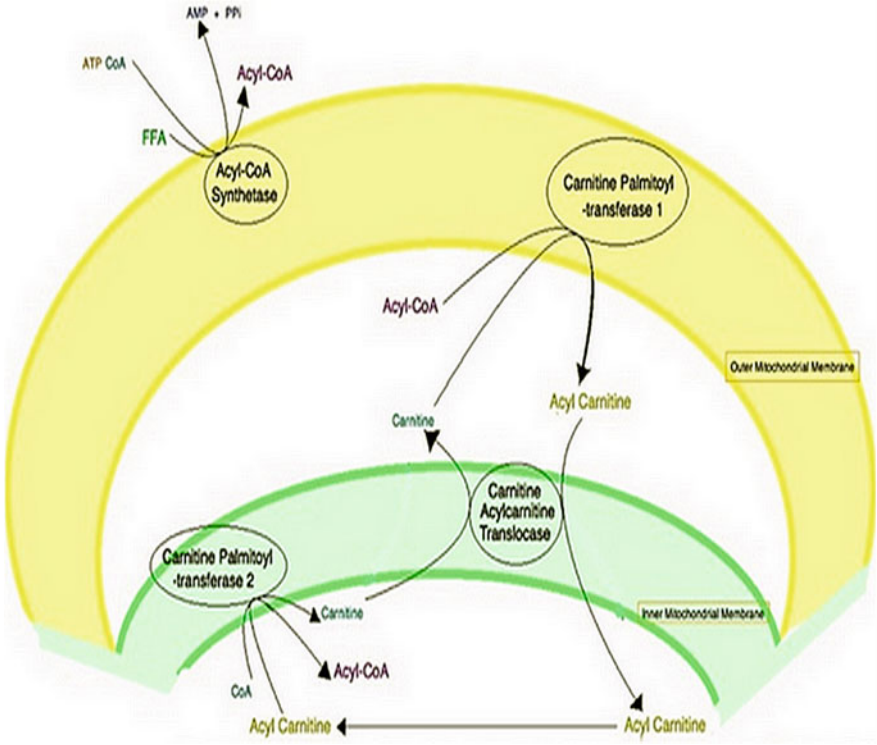

Fig. 5 Beta oxidation of FFA. The carnitine shuttle

FFA's are first activated in the cytoplasm with CoA to become acyl-CoA. This is transformed into acyl-carnitine at the outer mitochondrial membrane and is now able to be transported across. Once inside, it is converted back to acyl-CoA, releasing the carnitine to resume its transport function. This is called the Carnitine Shuttle (Fig. 5). Fat cannot be used for energy in the mitochondria without carnitine which is a rate limiting step for fatty acid metabolism. The relevance of this to regenerative medicine is readily apparent. All tissues must alternate between fatty acid metabolism and glucose as availability of each vary minute to minute. The cardiac muscle, for example, derives as much as 60 % of its energy from fatty acid metabolism. In addition, the efficiency of this process is compromised in aging as the carnitine concentration declines.

Once the acyl-CoA is in the mitochondria there is a 4 step process in which the molecule is oxidized, hydrated, oxidized again, and cleaved. The result is the production of a mole of each of the electron donors, FADH2 and NADH, and a mole of acetyl-CoA. The acetyl-CoA then enters the TCA cycle which yields another 3 mol of NADH, another 1 mol of FADH2 and a mole of ATP. All these products then enter the oxidative phosphorylation phase which now involves coenzymeQ10.

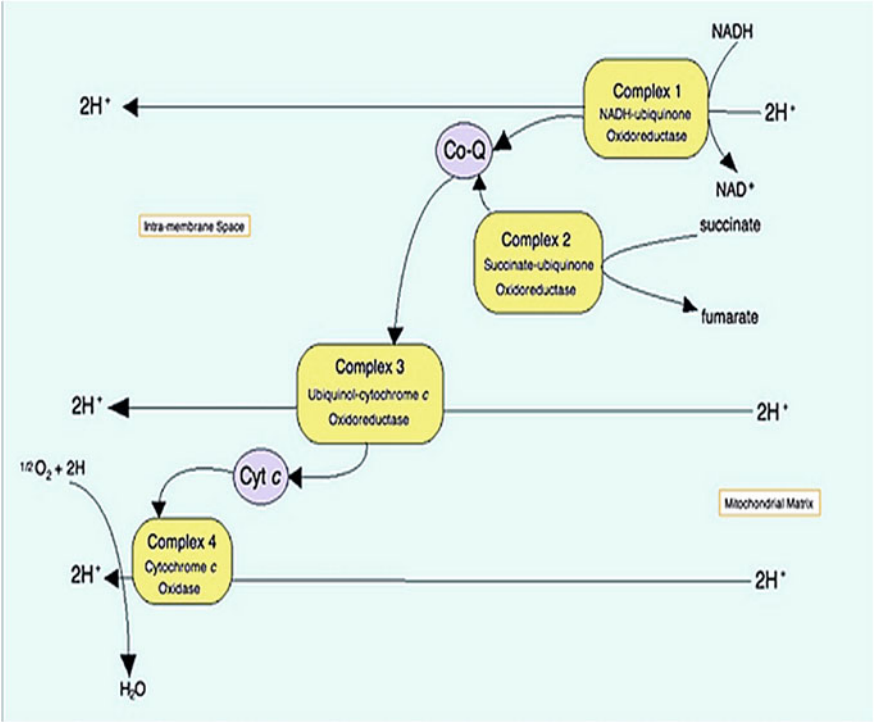

Fig. 6 Electron flow during oxidative phosphorylation

CoenzymeQ10

Inside the mitochondrial membrane and matrix, the electron donors NADH and FADH2 are also generated from glucose via glycerol-3-phosphate. CoenzymeQ10 transports electrons from both donors derived from both FFA's and glucose to cytochromes (enzyme complex III) from enzyme complexes I and II and ultimately drives ATP synthesis (Fig. 6).

This profound oversimplification of these reactions of oxidative phosphorylation underscores the pivotal roles of both carnitine and coenzymeQ10 in the generation of cellular energy. Increasing the availability of both of these will result in increased cellular function, whether that function is mitogenesis, fibroblast migration, osteogenesis, or neurogenesis. I will discuss the role of vitamin D, vitamin K2, and carnitine later in this chapter in the production of osteocalcin and osteogenesis. There is also an extensive literature studying the benefits of coQ10 and carnitine in congestive heart failure, angina, and renal failure. These benefits come as no surprise given the above biochemistry. By the way, carnitine synthesis in the liver and kidney depends on availability of vitamin C and methionine. Therefore, if homocysteine cannot be methylated to methionine as discussed above, then carnitine production can be compromised. It all fits together.

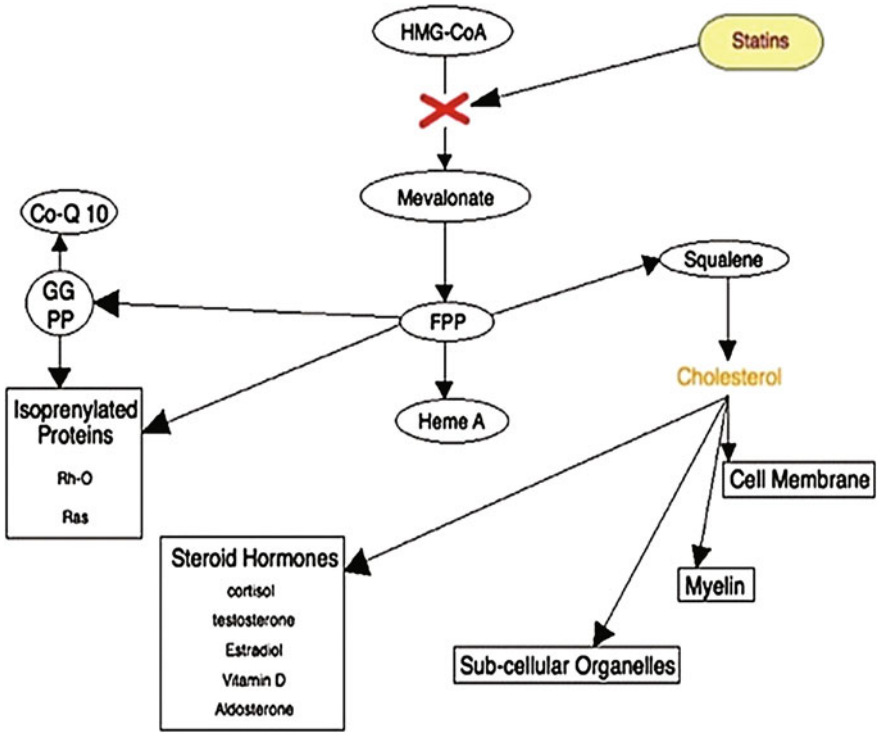

Fig. 7 Statin inhibition

No discussion of coenzymeQ10 is complete without mentioning statin drugs. Statins block the production of cholesterol by inhibiting the enzyme HMG-CoA reductase. However, they also affect everything downstream (Fig. 7). Production of cell membranes, myelin, all steroid hormones, subcellular organelles, and, of course, coenzymeQ10 are all affected. Statins inhibit wound healing, halt stem cell production, can cause rhabdomyolysis, weakness, and memory loss and at high doses can cause cardiomyopathy, diabetes, neuropathy, and impotence. Statins do, however, decrease cardiovascular events by 20–30 % but this is likely due to its reduction of IL-6, IL-8, and CRP (Mantuano et al. 2007). Its cardiovascular benefit, then, comes from its anti-inflammatory properties rather than from lowering cholesterol. PUFA's will also reduce these inflammatory cytokines without the potential deleterious effects.

My recommendation for regenerative procedures is discontinuing statin drugs and the supplementation of acetyl-L-carnitine 2 g per day and coQ10 400 mg per day. Protect the cardiovascular system with PUFA's.

Nuclear Receptors and Hormones

Thyroid

The review of mitochondrial energy production logically leads to the discussion of nuclear hormones and specifically thyroid. It is well known that thyroid increases metabolic rate via the mitochdria. Thyroid also increases the number of mitochondria. Goglia et.al. (2002) found that iodothyronines regulate energy metabolism by both rapid and more protracted mechanisms. T2 directly affects mitochondrial energy transduction while T3 acts more slowly on nuclear receptors to influence genes regulating cell metabolism and mitochondrial function. That is, T2 appears to be working directly on the mitochondria at local tissue and T3 at the nucleus. T4 is the primary thyroid produced by the thyroid gland and is deiodinated to T2 and T3 in the peripheral tissues. It is also understood that deiodinase activity declines with age. If this enzyme level has declined or is ineffective or if, in hypothyroid patients T4 alone is used to supplement, then peripheral energy production from the mitochondria will be inadequate and the regenerative capability of the patient will be compromised. Unfortunately, this is not well known by physicians and T4 supplementation is used almost exclusively. Combination thyroid supplementation that includes T2, 3 and 4 is biochemically a better choice for regenerative medicine. Before the advent of TSH assays, basic metabolic rate was used to determine thyroid function and could be used in conjunction with TSH and Free T3 and Free T4 levels, especially in aged or hypothyroid patients.

Thyroid, specifically T3, binds to nuclear receptors and then forms heterodimers with vitamin D receptors, retinoic acid receptors and retinoid X receptors where derivatives of vitamin A serve as ligands for the latter two. This, then, results in DNA synthesis and regulation of transcription. An example of this is the upregulation of the growth hormone gene from the pituitary by thyroid and retinoic acid response elements (Garcia-Villalba et al. 1996). The dynamics of these interactions are still unclear and under investigation. Conceptually though, when all receptors are bound with their ligands and functionally optimized mitochondria are also activated by thyroid, there is cross-talk between these endocrine systems and the cellular machinery fires up. This is an interesting interaction that has important implications for protein synthesis and mitogenesis. The contribution of vitamin A for connective tissue repair is well documented and that of vitamin D will be discussed later in this chapter.

The many thyroid actions are well known, but of additional interest here is its angiogenic effect which together with thyroid-induced increased cardiac output and vasodilatation, provides support for mitogenesis in wound healing, especially in patients with significant microvascular disease. Thyroid is also antiarrhythmic and antihypertensive. I have never seen 30 mg of Armour Thyroid have any untoward effects, even in aged euthyroid patients. However, obtaining Free T3, TSH and Free T4 levels and appropriate documentation is recommended.

Vitamin D

Lesser known than thyroid are the physiologic effects of vitamin D on growth and metabolism. Over the past 10 years, physicians have become increasingly aware of the epidemic nature of vitamin D deficiency in the general population. In the last 15 years the inhibitory effects of vitamin D on many different tumor cell lines has generated publicity in the popular media as well as intense research on the mechanisms involved (Polek and Weigel 2002). No longer is the exclusive role of vitamin D assumed to be regulation of calcium absorption and excretion in the intestinal lumen and kidney. Its actions are now known to be ubiquitous, often involving heterodimerization with vitamin A and thyroid hormone to produce its regulatory functions on apoptosis and mitogenesis and to utilize vitamin K as a cofactor to induce osteogenesis and collagen synthesis. Vitamin D inhibits abnormal cell proliferation, pushing the process toward differentiation into the appropriate cell tissue types. Both Hollick (2004) and Cordero et al. (2002) also demonstrated this downregulation of proliferation by vitamin D on tumor cell lines stimulated by epidermal growth factor and transforming growth factor alpha. In the context of regenerative medicine, then, this should be considered good fertilizer for growth of normal tissues and suppression of abnormal tissue growth. Additionally, the compounds involved, vitamin D, vitamin A, vitamin K and thyroid, are easily supplemented with very few side effects.

PUFA's provide a rich source of vitamin D. It is also synthesized in the skin in response to UV light. It is hydroxylated in the liver and kidney into its active form. The production of vitamin D is stimulated by parathyroid hormone and, of course, low calcium levels. It binds to the nuclear receptor as well as plasma membrane receptors. Once bound to the nuclear receptor, among other actions, calcium binding protein and osteocalcin are transcribed. As a simplified overview, calcium binding protein facilitates calcium absorption from the intestine. Osteocalcin, generated by the osteoblast under the influence of vitamin D, must be gamma-carboxylated by vitamin K to then bind the calcium in the bone matrix. Without vitamin K, therefore, there is poor bone mineralization due to the high levels of uncarboxylated osteocalcin. Regeneration of bone cannot be expected without adequate D and K levels.

A brief discussion of vitamin K must be inserted at this point. In addition to the gamma carboxylation activation of osteocalcin, vitamin K also gamma-carboxylates Gla proteins. Vitamin K2 (menaquinone-7) appears to be more effective in this reaction than K1 and has a much longer half-life (Yasui et al. 2006; Schurgers et al. 2007). Matrix Gla proteins are associated with fibrillar collagens and could also be involved in the organization and/or stabilization of cartilage matrix. These are indeed cartilage matrix associated proteins and Gla-rich protein, those with the highest Gla residue content, is preferentially expressed by cartilage chondrocytes and is the most densely γ-carboxylated protein (Cancela et al. 2012). These proteins also inhibit soft tissue calcification and, like osteocalcin, are vitamin K dependent. Progenitors of type II collagen, stimulated with PRP, show significantly increased cartilage matrix formation compared to untreated progenitors and

markers like osteocalcin showed that PRP induces their chondrogenic differentiation. Increased levels of uncarboxylated Gla protein are present in the synovial fluid of arthritic joints compared to non-arthritic joints (Krüger et al. 2012). Interestingly, carboxylated Gla proteins may play a role in inhibiting coronary and peripheral artery calcification as well (Silaghi et al. 2011; Cranenburg et al. 2008; Dalmeijer et al. 2013). Vitamin K also increases insulin receptor sensitivity (Choi et al. 2011). Vitamin K deficiency reduces testosterone production from the testes (Shirakawa et al. 2006) and vitamin K2 stimulates testosterone production (Ito et al. 2011) and, as will be discussed later, testosterone makes bone.

Returning to the discussion of vitamin D, it exerts its effect on DNA transcription and synthesis of osteocalcin and Gla proteins by first binding to the nuclear receptor, then forming a heterodimer with the retinoic X receptor (RXR). The binding zone of the RXR to the vitamin D receptor (VDR) occurs at the "zinc fingers" typical of all steroid hormone dimerization. This heterodimerization is essential to achieve the appropriate VDR conformation to activate the VDR and bind to the DNA, again with 2 zinc atoms or zinc fingers. This is the high affinity interaction that then activates the sequences in the promotor regions of the vitamin D target genes (Brown et al. 1999). With this review of VDR function, the importance of both vitamin A and zinc should be noted. Again, these are easily supplemented to optimize osteogenesis and chondrogenesis.

As mentioned above, there are also membrane receptors for vitamin D on the cell surface, in the cytoplasm and on mitochondrial membranes that may initiate nongenomic functions of the vitamin. Silvagno et al. (2010) demonstrated the presence of vitamin D receptors (VDR) on the mitochondrial membrane and in the inner mitochondrial compartment in platelets. Since platelets are anucleated and have little mRNA, the role of the VDR is unlikely to be gene transcription. The most probable role for vitamin D in platelet mitochondria is that of control of calcium and ion flux and of intracellular calcium levels. This makes sense, of course, since calcium fluxes regulate at least some of the platelet aggregation and content release. This may also explain how vitamin D corrects platelet dysfunction in uremic hemodialysis patients where platelet calcium content is high (Gura et al. 1982). How all this occurs is still unclear. However, as Silvagno et.al. point out, the role of retinoic (RXR) heterodimers is possible, even in these non-nuclear VDR's. This type of non-nuclear heterodimerization is known to occur with RXR and a truncated version of the T3 thyroid receptor which results in DNA binding and protein synthesis. In addition, receptors for steroid hormones such as estradiol and testosterone have also been identified on the mitochondria having non-genomic effects and facilitating aggregation. So the release of granules and the aggregation and activation of platelets may be partially dependent on the platelet mitochondrial VDR control of calcium content. This is certainly relevant to enhancement of the efficacy of PRP procedures with adequate vitamin D.

Actually, it is PRP that enhances the osteogenesis induced by vitamin D rather than vice versa. PRP alone inhibited osteogenesis from mesenchymal stem cell cultures in a dose dependent fashion. But when combined with vitamin D, there was a significant differentiation of stem cells to osteoblasts (Feng et al. 2010).

This demonstrates the principle of fertilizing the garden to obtain the desired results from PRP and may explain why some studies have found no effect of PRP on bone healing (Peerboms et al. 2012).

Vitamin D levels should be monitored initially and periodically. For patients with levels less than 40 ng/ml, a weekly dose of vitamin D2 or D3 is usually very well tolerated. Ideal serum levels have not yet been established, but 50-80 ng/ml should be a target.

Since vitamin K is critical for manliness, bone mineralization and cartilage differentiation, it, with vitamin D, should be included in the preparation of the garden soil for regenerative procedures. I prefer vitamin K2 menaquinone-7 100 mcg per day as this seems to have a lesser effect on coagulation parameters. Serum zinc levels should be 70–80 ng/ml. Zinc will chelate copper so it should be monitored and given as small doses or dosed once or twice a week.

Androgens and Estrogens

Few topics in medicine are quite so controversial since the publication in 2002 of the Women's Health Initiative Study as that of hormone replacement therapy. The results of this longitudinal study showed increased risk of stroke, breast cancer, DVT and pulmonary embolus, and myocardial infarction in postmenopausal women on hormone replacement therapy with conjugated estrogens and synthetic progestins. With conjugated estrogens alone, only an increased risk of stroke and thrombotic events was seen. As a result of this study and its reporting in the general media, the majority of postmenopausal women in the United States were taken off their hormones. Similarly, the general consensus regarding testosterone replacement in men held that testosterone caused strokes, heart attacks, and prostate cancer. The use of testosterone in men in the United States was rarely implemented.

Since 2002 numerous studies, which will not be reviewed here, of HRT in women have demonstrated that hormones that are molecularly-identical to those made by the human ovary do not produce the same undesirable effects as conjugated estrogens and progestins. Additionally, the metananalysis review paper by Rhoden and Morgentaler (2004) concluded that testosterone replacement did not increase the risk of prostate cancer. Many other studies, which will also not be reviewed here, have shown an adverse effect of low testosterone levels on overall mortality in men and low testosterone levels significantly correlated with decreased physical function and increased risk for 6-month mortality (Araujo et al. 2011). Ligands of the VDR and RXR separately and as heterodimers inhibit prostate cancer cell growth and this inhibition by vitamin D appears to be cell line specific (Murthy et al. 2003; Murthy et al. 2005; Stewart et al. 2005; Sepulveda et al. 2006; Bonaccorsi et al. 2006). Now, in the United States testosterone replacement therapy has become an accepted therapeutic intervention. With this understanding, using testosterone, DHEA, estrogen, and progesterone can provide additional anabolic benefit in regenerative medicine with limited risk. After all, these hormones catalyze all cellular repair through proliferation and differentiation.

Proliferation of cells in and of itself is not necessarily good. Tumors are proliferated cells. There must also be differentiation of these proliferated cells into the appropriate tissue type. Stem cell stimulation and both proliferation and differentiation in bone may be under the control of the osteoblast. By extension, then, anything that is osteoblastogenic would also be expected to result in hematopoietic stem cell proliferation and subsequent differentiation. Taichman (2004) discusses the role of osteoblasts, which are derived from mesenchymal stem cells, in regulating hematopoietic stem cell maturation from bone marrow derived stem cells.

Unlike the basic molecular biochemistry of energy production which is common to all cells, the effects of the sex hormones on tissue appear to depend on the type of tissue studied. Their interactions with other receptors such as the IGF-1 receptor are complex. We have already discussed some of these interactions with the vitamin D, vitamin A and carotenoid (RXR) nuclear receptors focusing primarily on osteogenesis because the literature concerning this topic is most abundant.

DHEA

This adrenal androgen has long been considered "the hormone without a cause" by most endocrinologists. However evidence has accumulated over the past 15 years that DHEA has ubiquitous effects on vascular smooth muscle and neural tissues as well as connective tissue and bone. These effects are the result of both direct receptor binding to its own specific DHEA receptor as well as indirect effects via conversion to estrogens by aromatase enzymes. As of this writing, no deleterious effects of DHEA supplementation have been described. The levels of DHEA, like most steroids, have an age-dependent decline. Its importance to the subject of PRP is its putative regulatory role in the growth factors released by platelet activation. Williams et al. (2002) demonstrated that DHEA inhibits or controls the activity and release of PDGF-BB from the platelet granules. PDGF-BB mediates vascular smooth muscle proliferation that is observed in atherosclerosis and thrombosis. In this sense, DHEA inhibits the endothelial invasion of inflammatory cells. DHEA indeed inhibits chemotaxis of PMN's and decreases inflammation (Koziol-White et al. 2012) and this may be specific to DHEA rather than to its aromatization to estrogens. In other connective tissues, DHEA enhances regeneration by upregulation of the expression of lysyl oxidase, a copper containing enzyme and the final enzyme in the cross-linking of collagen in the synthesis of elastin. This effect of DHEA also suppresses metalloproteinase expression and is therefore likely anti-inflammatory as well by this action. Metalloproteinase overexpression contributes to vascular lesions and proatherothrombosis (Rodriguez et al. 2008) and the role of DHEA in this process may be significant. One would also expect that by its augmentation of lysyl oxidase that DHEA would promote wound healing as it indeed does. However, this is likely in combination with estrogen, progesterone and IGF-1 which will be discussed presently. Interestingly, platelet activation releases the growth factor TGF-beta

which also increases the expression of lysyl oxidase (Csiszar et al. 2001) and the synergy of these steroids could only enhance this effect and, more importantly, regulate the mitogenic effect of TGF-beta.

The action of DHEA on bone has been extensively studied. Osteoblast proliferation and differentiation is stimulated by this hormone and osteoblast apoptosis is inhibited. This has been shown to be independent of testosterone and estrogen (Wang et al. 2007). It also indirectly inhibits bone resorption by osteoclasts but the mechanism for this is unclear. It could be that DHEA acts to improve osteoblast viability and/or by promoting the release of osteoprotegerin which then inhibits the differentiation of hematopoetic precursor cells to osteoclasts (Wang et al. 2009). It should be noted that PRP is also known to stimulate the release of osteoprotegerin (Ogino et al. 2009). This demonstrates the potential for a synergistic action of DHEA and PRP in regenerative medicine particularly in aged patients.

DHEA, as stated above, is remarkably without negative side effects. Because it is an androgen, though, in high doses it can produce undesirable androgenic manifestations in females, but because the levels in these older patients are usually very low (25–50 ug/dl) these effects are uncommon. Normal youthful levels of DHEA-S are usually in the 150–300 ug/dl range. 25 mg per day doses are normally well-tolerated in women and 50–100 mg per day is an acceptable male dose, but individual responses should be monitored.

Testosterone

The anabolic effect of testosterone on bone and cartilage is well known. This effect, however, is not entirely the result of a unique action of testosterone on the tissues. Testosterone does stimulate mRNA expression of osteoprotegerin and thereby inhibits osteoclastogenesis much like DHEA and TGF-beta discussed above. Its action on osteoblastogenesis is less clear. This is because many of the testosterone anabolic actions on bone are indirect via IGF-1 and aromatization to estradiol. Nevertheless, Marie et al. (1988) demonstrated that testosterone increased an osteoblastic activation that was only partly blocked by somatostatin. Tramontana et al. (2001) also identified an anabolic action on bone by DHT which is not aromatizable to estradiol and aromatase inhibitors only partly decreased the anabolic effect on bone (Deng et al. 2010). So androgen receptors have their own unique role in osteogenesis independent of estrogen and IGF-1.

Estrogen and testostosterone together resulted in osteoblast proliferation and DNA synthesis and inhibited apoptosis in chicken stem cell cultures, but testosterone alone resulted in apoptosis (Chen et al. 2012). There appears to be, then, a combined effect on bone by testosterone, IGF-1, and estradiol. Sinnesael et al. (2011) describe a dual action of testosterone where the androgen receptor, at least in male mice, maintains trabecular bone volume and bone matrix synthesis and mineralization via the osteoblast and with estrogen maintaining bone mass after reaching peak mass. In addition, Testosterone stimulates IGF-1 and IGF-1 receptor mRNA in mature chondrocyte cell layers resulting in a ‘climbing up’ of the calcification to the progenitor zone and these chondrocytes are then replaced by

bone (Moar et al. 1999). Whatever the mechanism of the action of testosterone, whether direct or indirect, these levels must be adequate for any regenerative procedure to produce an optimal effect on bone.

Many of the actions of testosterone on muscle appear to involve IGF-1. In vitro, while both testosterone and IGF-1 produce muscle hypertrophy, the effects of testosterone were due to local expression of IGF-1 by human muscle precursor cells (Sculpthorpe et al. 2012). This action was testosterone dependent, however, since testosterone receptor antagonists blocked the differentiation and hypertrophy.

The effect of testosterone on wound healing is surprising. While estrogen and aromatase inhibitors enhance wound healing and fibroblast migration, testosterone inhibits this effect and impairs wound healing (Svensson et al. 2010). This is possibly due to a stimulatory effect on collagenases by testosterone. Testosterone can also be proinflammatory (Fimmel and Zoubolis 2005; Gilliver et al. 2003). Much more data is needed to clarify the etiologic mechanisms involved in this androgen effect on fibroblasts.

Estrogen

Many of actions of estrogen on the various tissues has already been discussed. To expand on estrogen's effect on wound healing, it is relevant that estradiol directly controls the expression of macrophage migration inhibitory factor via the estrogen receptor to enhance wound healing (Ashcroft et al. 2003) and prevents tissue hyperplasia by inhibiting cytokine mitogen activating kinase in macrophages (Mills et al. 2005). With regard to osteogenesis, there is much more data. Estrogen regulates stem cell activation in bone marrow via alpha receptors to promote proliferation of osteoblasts. Estrogen activates bone morphogenic protein (BMP-2) to cause proliferation and differentiation of these stem cells. BMP-2, a metalloproteinase of the TGF-beta family, will induce proliferation of both adipose and osteoblastic cell lines, but estradiol directs this induction toward osteoblastogenesis. Estrogen also increases mRNA of osteogenic genes to make collagen and TGF-beta (Zhou et al. 2001; Ozkaynak et al. 1992). Alpha receptors, but not beta estradiol receptors or androgen receptors, preserve trabecular bone and increase the density and mechanical strength of cortical bone. Androgen receptors only preserve the number of trabeculae, not the thickness thereof. Moverare et al. (2003) suggests that this may be mediated in part by IGF-1. An enhancement of this estradiol effect on bone density comes from progesterone which also promotes differentiation of osteoblasts (Seifert-Klauss et al. 2012). Osteoblast activity is reduced in anovulatory cycles and in perimenopause where progesterone levels are decreased or absent altogether. It should be intuitive that the balance of estrogen and progesterone optimizes proliferation and differentiation of osteoblasts since young females have no problem with osteopenia unless these hormones are dysregulated as in congenital adrenal hyperplasia or polycystic ovary syndrome.

Since estrogen and progesterone are easily supplemented in the perimenopausal female with minimal risk as long as molecularly-identical hormones are used, low

levels of HRT should provide a more fertile biochemical microenvironment to enhance the effects of PRP and regenerative medicine procedures.

Somatotropin and IGF-1

As the above review has made abundantly clear, IGF-1 is essential for all human reparative biology. However, the use of somatotropin or HGH to generate IGF-1 is problematic for reasons of cost as well as perception and the restrictions in professional sports. There are ways to augment HGH and IGF-1 without supplementing HGH directly. Thyroid and retinoic acid upregulate HGH release from the pituitary as previously discussed. HRT increases IGF-1 gene expression, muscle mass and bone density (Pöllänen et al. 2010) and estradiol augments HGH levels from the pituitary (Veldhuis and Bowers 2003) as does exercise and melatonin supplementation. HGH is nevertheless the most efficient means of increasing IGF-1 levels via hepatic conversion and ligand conversion in peripheral tissues. IGF-1 is also released with platelet activation and increased levels of IGF-1 are found in all tissues following PRP application. The regeneration of tendon, bone, cartilage, skin, and nerves depends on adequate IGF-1. HGH augments IGF-1 cell proliferation and differentiation of all cell types. It may, in fact, be the catalyst for the differentiation of cells following proliferation.

IGF-1 is the most abundant growth factor in the bone matrix. HGH stimulates proliferation of trabecular and stromal osteoblasts and increases the functional activity of osteoblasts (Kassem 1997). It also increases TGF beta levels in bone and bone density (Ueland et al. 2011). HGH effects on osteoblasts are additive to those of IGF-1 suggesting a synergy of individual cellular response elements (Langdahl et al. 1998). This is also found in wound healing (Skotter et al. 1990). The contribution of all the vitamins and hormones previously discussed are part of the expression of IGF-1 in all tissues. For example, IGF-1 transcription effect on developing bone depends on thyroid receptor binding of T3 (Xing et al. 2012). HGH activates the expression of IGF-1 genes in skeletal muscle which would be expected, but that this is also effected by thyroid hormone and carotinoids confirms the interactions of these receptors that were discussed above (Florini et al.1996).

Of concern to most physicians is the possibility that HGH causes cancer. This does not appear to be the case. There is a large literature to support this which will not be reviewed here. But if cell differentiation is a function of HGH and IGF-1 at physiologic levels, it is unlikely that these hormones cause dysregulated proliferation of cells. The turning on and off of cancer genes is multivariate and under the regulatory control of many endogenous and exogenous compounds such as vitamins D, K, and A alluded to previously. Physiologic balance of these is paramount, not only in preventing cancer, but also in proper regulation of regenerative cascades.

Summary

The quality of the garden soil is at least as important as the procedure of planting the seeds. This discussion is only a cursory overview of basic cellular chemistry which should optimize regenerative medicine outcomes. As our field continues to develop, surely many more salient and contributory variables will be elucidated and some of those presented here may be discounted. This, however, provides a beginning which should lead to testing of the principles presented. The addition of vitamins D, K, and A, thyroid, mitochondrial energy augmentation, and proper HRT for men and women, for which basic biochemistry provides an inarguable rationale, is easily achieved in most patients. It is clear that a single drug approach to medicine is woefully inadequate and, in some instances, counterproductive as in the case of bisphosphonates and statins. It is better to regenerate that to compensate.

And finally, in our fervor to seek 'evidence-based medicine', we may have lost the ability to integrate the massive amount of disconnected publications and data that exists into parsimonious hypotheses and effective treatments for our patients. PRP and regenerative medicine represents a new opportunity to integrate science into medicine.

References

Angerer P, Kothny W, Störk S, von Schacky C (2002) Effect of dietary supplementation with omega-3 fatty acids on progression of atherosclerosis in carotid arteries. Cardiovasc Res 54(1):183–190

Araujo AB, Dixon JM, Suarez EA, Murad MH, Guey LT, Wittert GA (2011) Clinical review: endogenous testosterone and mortality in men: a systematic review and meta-analysis. J Clin Endocrinol Metab 96(10):3007–3019

Ashcroft GS, Mills SJ, Lei K, Gibbons L, Jeong MJ, Taniguchi M, Burow M, Horan MA, Wahl SM, Nakayama T (2003) Estrogen modulates cutaneous wound healing by downregulating macrophage migration inhibitory factor. J Clin Invest 111(9):1309–1318

Bonaccorsi L, Marchiani S, Ferruzzi P, Muratori M, Crescioli C, Forti G, Maggi M, Baldi E (2006) Non-genomic effects of the androgen receptor and vitamin D agonist are involved in suppressing invasive phenotype of prostate cancer cells. Steroids 71(4):304–309

Brown AJ, Dusso A, Slatopolsky E (1999) Vitamin D, invited review. Am J Physiol Renal Physiol 277:F157–F175

Cancela ML, Conceição N, Laizé V (2012) Gla-rich protein, a new player in tissue calcification? Adv Nutr 3(2):174–181

Chen X, Deng Y, Zhou Z, Tao Q, Zhu J, Li X, Chen J, Hou J (2012) 17β-estradiol combined with testosterone promotes chicken osteoblast proliferation and differentiation by accelerating the cell cycle and inhibiting apoptosis in vitro. Vet Res Commun 34(2):143–152

Choi HJ, Hyunan J, Kim SW, Park KS, Jang HC, Kim SY, Shin CS (2011) Vitamin K2 supplementation improves insulin sensitivity via osteocalcin metabolism: a placebo-controlled trial. Diabetes Care 34(9):e147

Cordero J, Cozzolino M, Lu Y, Vidal M, Slatopolsky E, Stahl PD, Barbieri MA, Dusso A (2002) 1,25-Dihydroxyvitamin D down-regulates cell membrane growth and nuclear growth-promoting signals by the epidermal growth factor receptor. J Biol Chem 277(41): 38965–38971

Cranenburg EC, Vermeer C, Koos R, Boumans ML, Hackeng TM, Bouwman FG, Kwaijtaal M, Brandenburg VM, Ketteler M, Schurgers LJ (2008) The circulating inactive form of matrix Gla Protein (ucMGP) as a biomarker for cardiovascular calcification. J Vasc Res 45(5):427–436

Csiszar K (2001) Lysyl oxidases: a novel multifunctional amine oxidase family. Prog Nucleic Acid Res Mol Biol 70:1–32

Dalmeijer GW, van der Schouw YT, Vermeer C, Magdeleyns EJ, Schurgers LJ, Beulens JW (2013) Circulating matrix Gla protein is associated with coronary artery calcification and vitamin K status in healthy women. J Nutr Biochem (epub ahead of print) 24(4):624–628

Deng YF, Chen XX, Zhou ZL, Hou JF (2010) Letrozole inhibits the osteogenesis of medullary bone in prelay pullets. Poult Sci 89(5):917–923

Dewailly E, Blanchet C, Gingras S, Lemieux S, Holub BJ (2002) Cardiovascular disease risk factors and n-3 fatty acid status in the adult population of James Bay Cree. Am J Clin Nutr 76(1):85–92

Dhonukshe-Rutten RA, Pluijm SM, de Groot LC, Lips P, Smit JH, van Staveren WA (2005) Homocysteine and vitamin B12 status relate to bone turnover markers, broadband ultrasound attenuation, and fractures in healthy elderly people. Bone Miner Res 20(6):921–929

Feng Y, Sun Y, Jia W, Zhang C (2010) Platelet-rich plasma and 1,25(OH)2 vitamin D3 synergistically stimulate osteogenic differentiation of adult human mesenchymal stem cells. Biotechnol Lett 32(5):635–642

Fimmel S, Zouboulis CC (2005) Influence of physiological androgen levels on wound healing and immune status in men. Aging Male 8(3–4):166–174

Florini JR, Ewton DZ, Coolican SA (1996) Growth hormone and the insulin-like growth factor system in myogenesis. Endocr Rev 17(5):481–517

Garcia-Villalba P, Jimenez-Lara AM, Aranda A (1996) Vitamin D interferes with transactivation of the growth hormone gene by thyroid hormone and retinoic acid. Mol Cell Biol 16(1):318

Gilliver SC, Wu F, Ashcroft GS (2003) Regulatory roles of androgens in cutaneous wound healing. Thromb Haemost 90(6):978–985

Goglia F, Silvestri E, Lanni A (2002) Mini review: thyroid hormones and mitochondria. Biosci Rep 22(1):17–32

Gura V, Creter D, Levi J (1982) Elevated thrombocyte calcium content in uremia and its correction by 1 alpha(OH) vitamin D treatment. Nephron 30(3):237–239

He K, Rimm EB, Merchant A, Rosner BA, Stampfer MJ, Willett WC, Ascherio A (2002) Fish consumption and risk of stroke in men. JAMA 288(24):3130–3136

Herrmann M, Widmann T, Herrmann W (2005) Homocysteine–a newly recognised risk factor for osteoporosis. Clin Chem Lab Med 43(10):1111–1117

Holick MF (2004) Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. Am J Clin Nutr 79(3):362–371

Hu FB, Bronner L, Willett WC, Stampfer MJ, Rexrode KM, Albert CM, Hunter D, Manson JE (2002) Fish and omega-3 fatty acid intake and risk of coronary heart disease in women. JAMA 287(14):1815–1821

Hubmacher D, Cirulis JT, Miao M, Keeley FW, Reinhardt DP (2010) Functional consequences of homocysteinylation of the elastic fiber proteins fibrillin-1 and tropoelastin. J Biol Chem 285(2):1188–1198

Hubmacher D, Sabatier L, Annis DS, Mosher DF, Reinhardt DP (2011) Homocysteine modifies structural and functional properties of fibronectin and interferes with the fibronectin-fibrillin-1 interaction. Biochemistry 50(23):5322–5332

Ito A, Shirakawa H, Takumi N, Minegishi Y, Ohashi A, Howlader ZH, Ohsaki Y, Sato T, Goto T, Komai M (2011) Menaquinone-4 enhances testosterone production in rats and testis-derived tumor cells. Lipids Health Dis 10:158–167

Kassem M (1997) Cellular and molecular effects of growth hormone and estrogen on human bone cells. APMIS Suppl 71:1–30

Koziol-White CJ, Goncharova EA, Cao G, Johnson M, Krymskaya VP (1822) Panettieri RA Jr (2012) DHEA-S inhibits human neutrophil and human airway smooth muscle migration. Biochim Biophys Acta 10:1638–1642

Krüger JP, Hondke S, Endres M, Pruss A, Siclari A, Kaps C (2012) Human platelet-rich plasma stimulates migration and chondrogenic differentiation of human subchondral progenitor cells. J Orthop Res 30(6):845–852

Langdahl BL, Kassem M, Møller MK, Eriksen EF (1998) The effects of IGF-I and IGF-II on proliferation and differentiation of human osteoblasts and interactions with growth hormone. Eur J Clin Invest 28(3):176–183

Mantuano E, Santi S, Filippi C, Manca-Rizza G, Paoletti S, Consani C, Giovannini L, Tramonti G, Carpi A, Panichi V (2007) Simvastatin and fluvastatin reduce interleukin-6 and interleukin-8 lipopolysaccharide (LPS) stimulated production by isolated human monocytes from chronic kidney disease patients. Biomed Pharmacother 61(6):360–365

Marie PJ, Hott M, Durand D (1988) Somatostatin infusion inhibits the stimulatory effect of testosterone on endosteal bone formation in the mouse. Metabolism 37(5):429–435

Mills SJ, Ashworth JJ, Gilliver SC, Hardman MJ, Ashcroft GS (2005) The sex steroid precursor DHEA accelerates cutaneous wound healing via the estrogen receptors. J Invest Dermatol 125(5):1053–1062

Moar G, Segev Y, Phillip M (1999) Testosterone stimulates growth factor 1 and insulin like growth factor 1 receptor gene: expression in the mandibular condyle—a model of endochondral ossification. Endocrinology 140(4):1901–1910

Moverare S, Venken K, Eriksson AL, Andersson N, Skrtic S et al (2003) Differential effects on bone of estrogen receptor alpha and androgen receptor activation in orchidectomized adult male mice. Proc Natl Acad Sci USA 100:13573–13578

Murthy S, Marcelli M, Weigel NL (2003) Stable expression of full length human androgen receptor in PC-3 prostate cancer cells enhances sensitivity to retinoic acid but not to 1alpha,25-dihydroxyvitamin D3. Prostate 56(4):293–304

Murthy S, Agoulnik IU, Weigel NL (2005) Androgen receptor signaling and vitamin D receptor action in prostate cancer cells. Prostate 64(4):362–372

Ogino Y, Ayukawa Y, Kukita T, Atsuta I, Koyano K (2009) Platelet-rich plasma suppresses osteoclastogenesis by promoting the secretion of osteoprotegerin. J Periodontal Res 44(2): 217–224

Ozkaynak E, Schnegelsberg PN, Jin DF, Clifford GM, Warren FD, Drier EA, Oppermann H (1992) Osteogenic protein-2. A new member of the transforming growth factor-beta superfamily expressed early in embryogenesis. J Biol Chem 267(35):25220–25227

Peerbooms JC, Colaris JW, Hakkert AA, Van Appeldorn M, Bruijn DJ, Den Oudsten BL, Gosens T (2012) No positive bone healing after using platelet rich plasma in a skeletal defect. An observational prospective cohort study. Int Orthop 36(10):2113–2119

Polek TC, Weigel NL (2002) Vitamin D and prostate cancer review. J Androl 23(1):9–17

Pöllänen E, Ronkainen PH, Horttanainen M, Takala T, Puolakka J, Suominen H, Sipilä S, Kovanen V (2010) Effects of combined hormone replacement therapy or its effective agents on the IGF-1 pathway in skeletal muscle. Growth Horm IGF Res 20(5):372–9

Rhoden EL, Morgentaler A (2004) Risks of testosterone-replacement therapy and recommendations for monitoring. N Engl J Med 350:482–492

Rodriguez JA, Orbe J, Martinez de Lizarrondo S, Calvayrac O, Rodriguez C, Martinez-Gonzalez J, Paramo JÁ (2008) Metalloproteinases and atherothrombosis: MMP-10 mediates vascular remodeling promoted by inflammatory stimuli. Front Biosci 13:2916–2921

Sabatier L, Chen D, Fagotto-Kaufmann C, Hubmacher D, McKee MD, Annis DS, Mosher DF, Reinhardt DP (2009) Fibrillin assembly requires fibronectin. Mol Biol Cell 20(3):846–858

Schurgers LJ, Teunissen KJ, Hamulyák K, Knapen MH, Vik H, Vermeer C (2007) Vitamin K-containing dietary supplements: comparison of synthetic vitamin K1 and natto-derived menaquinone-7. Blood 109(8):3279–3283

Sculthorpe N, Solomon AM, Sinanan AC, Bouloux PM, Grace F, Lewis MP (2012) Androgens affect myogenesis in vitro and increase local IGF-1 expression. Med Sci Sports Exerc 44(4): 610–615

Seifert-Klauss V, Schmidmayr M, Hobmaier E, Wimmer T (2012) Progesterone and bone: a closer link than previously realized. Climacteric Suppl 1:26–31

Sepulveda VA, Weigel NL, Falzon M (2006) Prostate cancer cell type-specific involvement of the VDR and RXR in regulation of the human PTHrP gene via a negative VDRE. Steroids 71(2):102–115

Shirakawa H, Ohsaki Y, Minegishi Y, Takumi N, Ohinata K, Furukawa Y, Mizutani T, Komai M (2006) Vitamin K deficiency reduces testosterone production in the testis through down-regulation of the Cyp11a a cholesterol side chain cleavage enzyme in rats. Biochim Biophys Acta 1760(10):1482–1488

Silaghi CN, Fodor D, Cristea V, Crăciun AM (2011) Synovial and serum levels of uncarboxylated matrix Gla-protein (ucMGP) in patients with arthritis. Clin Chem Lab Med 50(1):125–128

Silvagno F, De Vivo E, Attanasio A, Gallo V, Mazzucco G, Pescarmona G (2010) Mitochondrial localization of vitamin D receptor in human platelets and differentiated Megakaryocytes. PLoS ONE 5(1):e8670:1–8

Sinnesael M, Boonen S, Claessens F, Gielen E, Vanderschueren D (2011) Testosterone and the male skeleton: a dual mode of action. J Osteoporos 2011:240328-1-7

Skottner A, Arrhenius-Nyberg V, Kanje M, Fryklund L (1990) Anabolic and tissue repair functions of recombinant insulin-like growth factor I. Acta Paediatr Scand Suppl 367:63–66

Spencer L, Mann C, Metcalfe M, Webb M, Pollard C, Spencer D, Berry D, Steward W, Dennison A (2009) The effect of omega-3 FAs on tumour angiogenesis and their therapeutic potential. Eur J Cancer 45(12):2077–2086

Stewart LV, Lyles B, Lin MF, Weigel NL (2005) Vitamin D receptor agonists induce prostatic acid phosphatase to reduce cell growth and HER-2 signaling in LNCaP-derived human prostate cancer cells. J Steroid Biochem Mol Biol 97(1–2):37–46

Svensson J, Movérare-Skrtic S, Windahl S, Swanson C, Sjögren K (2010) Stimulation of both estrogen and androgen receptors maintains skeletal muscle mass in gonadectomized male mice but mainly via different pathways. J Mol Endocrinol 45(1):45–57

Taichman RS (2004) Blood and bone: two tissues whose fates are intertwined to create thehematopoietic stem-cell niche hematopoietic stem-cell niche. Blood 2005(105):2631–2639

Tramontana J, Benghuzzi H, Tucci M, Tsao A, Hughes J (2001) Morphometric analysis of cortical bone upon the exposure to sustained delivery of anabolic promoting agents using adult male rats as a model. Biomed Sci Instrum 37:293–298

Tull SP, Yates CM, Maskrey BH, O'Donnell VB, Madden J, Grimble RF, Calder PC, Nash GB, Rainger GE (2009) Omega-3 Fatty acids and inflammation: novel interactions reveal a new step in neutrophil recruitment. PLoS Biol 7(8):e1000177-1-11

Ueland T, Lekva T, Otterdal K, Dahl TB, Olarescu NC, Jørgensen AP, Fougner KJ, Brixen K, Aukrust P, Bollerslev J (2011) Increased serum and bone matrix levels of transforming growth factor {beta}1 in patients with GH deficiency in response to GH treatment. Eur J Endocrinol 165(3):393–400

Veldhuis JD, Bowers CY (2003) Sex-steroid modulation of growth hormone (GH) secretory control: three-peptide ensemble regulation under dual feedback restraint by GH and IGF-I. Endocrine 22(1):25–40

Wang L, Wang YD, Wang WJ, Zhu Y, Li DJ (2007) Dehydroepiandrosterone improves murine osteoblast growth and bone tissue morphometry via mitogen-activated protein kinase signaling pathway independent of either androgen receptor or estrogen receptor. J Mol Endocrinol 38(4):467–479

Wang L, Wang YD, Wang WJ, Li DJ (2009) Differential regulation of dehydroepiandrosterone and estrogen on bone and uterus in ovariectomized mice. Osteoporos Int 20(1):79–92

Williams MR, Ling S, Dawood T, Hashimura K, Dai A, Li H, Liu JP, Liu JP, Funder JW, Sudhir K, Komesaroff PA (2002) Dehydroepiandrosterone inhibits human vascular smooth muscle cell proliferation independent of ARs and ERs. J Clin Endocrinol Metab 87(1):176–181

Xing W, Govoni KE, Donahue LR, Kesavan C, Wergedal J, Long C, Bassett JH, Gogakos A, Wojcicka A, Williams GR, Mohan S (2012) Genetic evidence that thyroid hormone is indispensable for prepubertal insulin-like growth factor-I expression and bone acquisition in mice. J Bone Miner Res 27(5):1067–1079

Yasui T, Miyatani Y, Tomita J, Yamada M, Uemura H, Miura M, Irahara M (2006) Effect of vitamin K2 treatment on carboxylation of osteocalcin in early postmenopausal women. Gynecol Endocrinol 22(8):455–459

Zhou S, Zilberman Y, Wassermann K, Bain SD, Sadovsky Y, Gazit D (2001) Estrogen modulates estrogen receptor alpha and beta expression, osteogenic activity, and apoptosis in mesenchymal stem cells (MSCs) of osteoporotic mice. J Cell Biochem Suppl 36:144–155