Chapter 3

Evaluation of the Relationship between Exercise, Osteocalcin, Insulin, and Interleukin-6

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Abstract

The evaluation of the relationship between exercise, osteocalcin, insulin, and interleukin-6 is a complex topic that involves understanding how these factors interact in the context of human physiology and health. Exercise refers to physical activity that involves bodily movement and is typically structured to improve or maintain physical fitness. Regular exercise, especially weight-bearing and high-impact activities, can stimulate the production of osteocalcin by osteoblasts. Osteocalcin may contribute to improved bone density and quality, and it may also have metabolic effects that enhance insulin sensitivity. Physical activity, particularly aerobic exercise and resistance training, can enhance insulin sensitivity. This means that exercise helps cells respond better to insulin, leading to better blood sugar control. Exercise can lead to a temporary increase in IL-6, which can have anti-inflammatory effects in the short term. This is part of the body's response to the physical stress of exercise. However, excessive or chronic inflammation, including sustained IL-6 elevation, can be detrimental to health. Consequently, exercise can have a positive impact on both osteocalcin and insulin, potentially improving bone health and metabolic function. The relationship between these factors and interleukin-6 is complex, with exercise having both short-term anti-inflammatory effects and the potential for long-term influences on inflammation. Further research is needed to fully elucidate these relationships and their clinical implications.

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1. Introduction

Osteocalcin, also called Bone Gamma-Carboxyglutamate (Protein BGLAP), is defined as a bone gamma-carboxyglutamic acid containing protein. It belongs to the non-collagenous protein group. Its synthesis starts with Vitamin D binding to osteoblasts' specific receptor (VDR). Proosteocalcin, which undergoes proteolysis, undergoes carboxylation or decarboxylation; if Vitamin K is present, osteocalcin is carboxylated, that is, inactive osteocalcin (Gla-OCN) is formed and binds to calcium and hydroxyapatite crystals in the bone matrix. Osteocalcin that does not undergo the process, i.e. remains decarboxylated, becomes active osteocalcin (Glu-OCN), and enters the bloodstream. It stimulates insulin release in the pancreas and testosterone synthesis in the testis and creates insulin sensitivity in muscle, liver, and adipose tissues. The anticoagulant warfarin, as a Vitamin K antagonist, inhibits carboxylation and increases the level of active osteocalcin (Tüylek, 2018).

Insulin is a polypeptide hormone released from beta cells in the islets of Langerhans of the pancreas and is involved, together with glucagon, in the regulation of carbohydrate metabolism in the body. In the context of the effects of insulin on carbohydrates, insulin accelerates the facilitated diffusion of glucose into cells in almost all tissues (except erythrocytes and the brain) and produces an effect to reduce blood glucose levels. In other words, insulin is involved in the regulation of blood glucose levels. Aside from its primary role in stabilizing carbohydrate metabolism, insulin also exerts significant influences on fat and protein metabolism, which are interconnected with carbohydrate metabolism (Ünal, 2012).

Exercise capacity is a crucial physiological function, and exercise offers a plethora of general health benefits. Some cytokines have demonstrated their ability to bolster muscle function during exercise, thereby enhancing exercise capacity (Bente K. Pedersen & Febbraio, 2012). Interleukin 6 (IL-6) is one such cytokine, and its levels in circulation have been observed to rise during exercise (Lang Lehrskov et al., 2018; Ostrowski et al., 1998; B. K. Pedersen et al., 2003; Steensberg et al., 2000; Wedell-Neergaard et al., 2019). Due to IL-6's expression by various cell types, the question arises as to which cell type(s) is accountable for this surge in circulating levels during exercise. It has been proposed that IL-6, once released into the general circulation, augments exercise capacity by stimulating gluconeogenesis and lipolysis, the production of two crucial nutrients for muscle fibers (Catoire & Kersten, 2015; Febbraio et al., 2004; Hawley et al., 2014; Lang Lehrskov et al., 2018; Ostrowski et al., 1998; B. K. Pedersen et al., 2003; Bente K.

Pedersen & Febbraio, 2012; Steensberg et al., 2000; Van Hall et al., 2003). However, this mechanism has not yet been verified in vivo, leaving room for the possibility that IL-6 may regulate exercise capacity through other yet undiscovered effects.

2. General Information

2.1. Osteocalcin

Osteocalcin, also called Bone Gamma-Carboxyglutamate (Protein BGLAP), is defined as a bone gamma-carboxyglutamic acid-containing protein. It belongs to the non-collagenous protein group. Its synthesis starts with Vitamin D binding to the specific receptor (VDR) in osteoblasts. Proosteocalcin, which undergoes proteolysis, undergoes carboxylation or decarboxylation; if Vitamin K is present, osteocalcin is carboxylated, that is, inactive osteocalcin (Gla-OCN) is formed and binds to calcium and hydroxyapatite crystals in the bone matrix. Osteocalcin that does not undergo the process, i.e. remains decarboxylated, becomes active osteocalcin (Glu-OCN), and enters the bloodstream. It stimulates insulin release in the pancreas and testosterone synthesis in the testis and creates insulin sensitivity in muscle, liver, and adipose tissues. The anticoagulant warfarin, as a Vitamin K antagonist, inhibits carboxylation and increases the level of active osteocalcin (Tüylek, 2018).

Discovered in 1972, osteocalcin is also known as bone Gla (γ carboxyglutamate) protein (Levinger et al., 2013). It is a protein synthesized by osteoblasts. Found in tooth and bone tissue, osteocalcin is a protein that gains affinity for hydroxyapatite by carboxylating three of the 4 glutamates it contains after translation (carboxylated osteocalcin cOC). Osteocalcin has a molecular mass of approximately 5700 daltons and is the most abundant non-collagenous protein in bone. It contains 49 amino acids and 3 gamma-glutamic acid (gamma-Gla) residues. Serum levels of osteocalcin are considered a marker of bone formation. Of the osteocalcin produced, 70-80% is found in bone and 20-30% in circulation. In case of an increase in the cycle of bone formation and destruction, an increase in circulating osteocalcin levels is also observed (Hauschka et al., 1989). In childhood and adolescence, when growth is more common, an increase in osteocalcin levels is observed due to an increase in bone formation (Gundberg et al., 1998). In postmenopausal women, although bone formation is less than bone destruction, a slight increase in osteocalcin levels is observed because osteocalcin increase is observed in the entire cycle (A. J. Lee et al., 2000). Changes in serum osteocalcin levels are not only related to bone formation

and bone resorption but may also be affected by variables such as gender, age, renal function, nutrition, exercise, menstrual cycle, pregnancy, and diurnal rhythm (Hauschka et al., 1989). When the relationship with diurnal rhythm is considered, the period in which osteocalcin levels are highest is 04. 00, while the lowest time is in the afternoon (Szulc & Delmas, 2008). Considering the relationship with nutrition, intake of vitamin D as well as vitamin K increases the osteocalcin level in serum as it positively affects bone mineralization (O'connor et al., 2007). In pregnancy, osteocalcin level decreases in the first trimester of pregnancy and even decreases by 50% compared to non-pregnant women, and tends to increase as the delivery approaches (Cole et al., 1987). In hyperparathyroidism, a condition in which bone resorption increases, osteocalcin levels increase 2-4 times (Rico et al., 1986). Serum osteocalcin levels are 50% higher in men who regularly perform muscle-building exercises compared to those who do not (Bell et al., 1988). Although osteocalcin has recently been shown to have effects on energy and glucose metabolism, its main biological function is thought to be bone formation and maturation. Nevertheless, its function in metabolism is not fully known (Gundberg et al., 2012; N. Lee et al., 2007).

2.1.1. Senteces of Osteocalcin

The gene responsible for osteocalcin synthesis is the bone Gla protein (bone Gla protein-BGLAP) gene (Hauschka et al., 1989). Vitamin D in the BGLAP gene directly stimulates osteocalcin transcription through the vitamin D responsive element (VDRE), meaning that vitamin D has a direct effect on osteocalcin synthesis. After pre-proosteocalcin transcription, proosteocalcin is hydrolyzed to proosteocalcin and carboxylated by vitamin K and remains in the bone matrix or is transferred to the circulation (Patti et al., 2013). Many substances affect the synthesis of osteocalcin in osteoblasts; insulin-like growth factor (IGF-1), parathormone (PTH), leptin, glucocorticoids, estrogen, progesterone, triiodothyronine, and retinoic acid are hormones that regulate BGLAP gene expression. The increase of BGLAP gene expression in cells is regulated by parathormone, and other hormones, except leptin, regulate the rate of transcription by interacting with factors in the activation or transcription of the BGLAP gene (Villafán-Bernal J. R., 2011). Leptin causes suppression of osteocalcin expression (Ducy et al., 2000). In addition, IGF-1 suppresses osteocalcin expression in the mineralization process (Staal et al., 1998). Pre-proosteocalcin is the first molecule formed in the synthesis of osteocalcin. With the enzyme signal peptidase, the hydrophobic part with 23 residues is cleaved and 3 proosteocalcins are formed. These proosteocalcins contain Gla residues.

Proosteocalcin undergoes post-translational modification (carboxylation) by the vitamin K-dependent enzyme γ-carboxylase. The resulting osteocalcin contains carboxy gamma-Gla residues and is also called bone Gla protein (BGP). Most of the carboxylated osteocalcin that binds to calcium in hydroxyapatite is stored in the bone matrix (Hauschka et al., 1989). The affinity of carboxylated osteocalcin to calcium is low, so it is stored less in the bone matrix and most of it is found in serum. Both carboxylated and ancarboxylated osteocalcin are found in bone and serum (Cairns & Price, 1994).

Osteokalsin, when it enters the bloodstream, primarily affects glucose metabolism in two main ways. Firstly, osteocalcin binds to the GPRC6A receptor, directly impacting β-cell function by increasing their replication capacity and enhancing insulin synthesis and secretion. Furthermore, osteocalcin exerts control over insulin sensitivity and energy expenditure through a variety of mechanisms. It promotes energy expenditure by enhancing mitochondrial biogenesis in muscles and modulating the expression of genes associated with energy expenditure in brown adipose tissue and skeletal muscle. Osteocalcin also potentially influences insulin sensitivity by potentially elevating adiponectin expression in white fat and mitigating lipid accumulation and inflammation in a fatty liver. However, it should be noted that the direct role of osteocalcin as an insulin sensitizer is speculative and requires further investigation (Ferron & Lacombe, 2014).

2.2. Insulin

Insulin is a polypeptide hormone released from beta cells in the islets of Langerhans of the pancreas and is involved, together with glucagon, in the regulation of carbohydrate metabolism in the body. In the context of the effects of insulin on carbohydrates, insulin accelerates the facilitated diffusion of glucose into cells in almost all tissues (except erythrocytes and the brain Insulin exerts an influence to lower blood glucose levels, essentially participating in the control of blood glucose regulation. Beyond its primary role in stabilizing carbohydrate metabolism, insulin also wields significant effects on both fat and protein metabolism, interconnected with carbohydrate metabolism (Ünal, 2012).

2.2.1. The Discovery of Insulin

German scientists in the late 19th century reported that diabetes developed in animals whose pancreas had been removed. They believed that a substance from the pancreas was responsible for this condition. In subsequent years, research suggested that the destruction of the islets of

Langerhans was associated with diabetes. While Minkowski, Zuelzer, and Scott attempted to isolate the missing islet substance, in 1909, a Belgian researcher named de Meyer suggested the name "insulin," which was later proposed by British researcher Schaefer in 1916. The isolation, purification, and therapeutic use of insulin took until the year 1921. In 1921, under the supervision of McLeod, experiments began with dogs. Dogs were made diabetic by pancreatectomy, and they were intravenously administered with cooled saline extracts of the pancreas. As a result of this application, a decrease in blood sugar was observed. In December of the same year, the study was presented to the American Physiological Society, and biochemist Collip demonstrated that this extract also restored hepatic glycogen mobilization and ketone clearing capacity. By January 1922, the first human experiments were conducted on a 14-year-old diabetic child to correct biochemical abnormalities and clinical symptoms using pancreatic isolates. By May, this active substance was named "insulin" (Bliss, 1993).

2.2.2. Structure, Synthesis, and Functions of Insulin

Insulin is a protein molecule with anabolic effects, and it plays a crucial role in the activation and inactivation of membrane enzymes. This protein can also be involved in regulating the speed of synthesis or degradation of certain proteins and mRNA molecules. Insulin secretion increases in response to elevated blood glucose levels, helping to regulate the glucose concentration in the blood. This regulation balances the rate of glucose utilization within cells and the production of glucose in the liver. As a result, glucose is taken up from the bloodstream into muscle, blood cells, liver, and fat cells. Insulin is a polypeptide hormone consisting of 51 amino acids in its active functional form, with a molecular weight of 5808 Da (Amihaesei & Chelaru, 2014). It is synthesized in clusters within the exocrine part of the pancreas, specifically in the β cells of the Langerhans islets (G Wilcox, 2005). The insulin gene in humans is located on the short arm of chromosome 11. The precursor molecule of insulin, preproinsulin, is processed into proinsulin by microsomal enzymes. Proinsulin, in turn, is cleaved into insulin and C-peptide in the Golgi apparatus. The biological activity of proinsulin is only about 7-8% of insulin, but its half-life is approximately 3-4 times longer than that of insülin (Greenspan & Gardner, 2001).

2.3. Interleukin-6

The cytokine now known as Interleukin-6 (IL-6) was cloned approximately 40 years ago as a cDNA encoding B-cell stimulatory factor 2 (Hirano et al., 1986). Today, IL-6 is considered one of the key immunomodulatory

cytokines that regulate health and disease (Rose-John et al., 2023). IL-6 is an interleukin produced in various cells and tissues, with diverse functions. It plays a role in many diseases, including inflammation, the immune system, hematopoiesis, osteoporosis, and cancer. IL-6 can function both as a pro-inflammatory cytokine and an anti-inflammatory myokine. It is encoded by the IL-6 gene in humans and affects various tissues and organs. IL-6 is a protein consisting of 216 amino acids. It activates intracellular signaling pathways by binding to receptors on cell surfaces. IL-6 stimulates the production of pro-inflammatory cytokines such as IL-1 and TNF-α, leading to inflammation and pain. It also promotes the growth and differentiation of T cells and B cells, aiding the immune system in combating infections. Additionally, IL-6 stimulates the production of red blood cells, white blood cells, and platelets in the bone marrow. However, it also stimulates the growth and differentiation of cells known as osteoclasts, which promote bone resorption, potentially increasing the risk of osteoporosis (Tanaka et al., 2014).

2.4. Exercise

Exercise encompasses a regular and repetitive series of bodily movements, aimed at improving flexibility, muscle strength, endurance, and cardiorespiratory fitness. Activities like walking, running, swimming, cycling, and others fall into the categories of aerobic and endurance exercises (Baltacı & Düzgün, 2008). Exercise, or one's exercise capacity, is a crucial physiological function with numerous health benefits. Some cytokines have been found to support muscle function during exercise, consequently enhancing exercise capacity (Bente K. Pedersen & Febbraio, 2012). One such cytokine is interleukin 6 (IL-6), and it has been observed that circulating levels of IL-6 increase during exercise (Lang Lehrskov et al., 2018; Ostrowski et al., 1998; B. K. Pedersen et al., 2003; Steensberg et al., 2000; Wedell-Neergaard et al., 2019). Given that IL-6 is expressed by various cell types, questions have arisen regarding which cell type(s) is/are responsible for this increase in circulation during exercise (Catoire & Kersten, 2015; Febbraio et al., 2004; Hawley et al., 2014; Lang Lehrskov et al., 2018; Ostrowski et al., 1998; B. K. Pedersen et al., 2003; Bente K. Pedersen & Febbraio, 2012; Steensberg et al., 2000; Van Hall et al., 2003). It has been suggested that after IL-6 is released into the general circulation, it may promote the production of gluconeogenesis and lipolysis, potentially increasing exercise capacity by facilitating the production of essential nutrients for muscle fibers. However, this proposed mechanism has yet to be confirmed in live subjects, leaving open the possibility that IL-6 might regulate exercise capacity through other undiscovered means.

Furthermore, hormonal signaling within myofibers may also contribute to the enhancement of muscle function during exercise. Osteocalcin, a hormone originating from bones, has demonstrated its necessity and sufficiency in augmenting muscle function in mice during exercise (Mera et al., 2016). Osteocalcin also triggers an increase in IL-6 expression within muscle tissue and elevates circulating IL-6 levels during exercise. Moreover, in experiments conducted with cell cultures, IL-6 has been observed to boost the expression of Rankl, a crucial gene responsible for osteoclast differentiation in osteoblasts, which is essential for the formation of carboxylated and bioactive osteoclasts (Ferron et al., 2010; Johnson et al., 2014; Mera et al., 2016). In summary, there are still numerous unanswered questions regarding the roles of IL-6 during exercise and the intricate interactions involving IL-6, osteocalcin, and muscle-derived IL-6 (mIL-6) in the mechanisms responsible for augmenting exercise capacity through IL-6. These questions include the extent to which the increase in circulating IL-6 contributes to muscle function during exercise, whether mIL-6 independently improves exercise capacity, if so, what mechanisms are involved, and whether the regulatory events described in rodents can be confirmed in humans. It is widely recognized that exercise mitigates the detrimental effects of proinflammatory adipokines through proteins secreted by skeletal muscles (Bente K. Pedersen & Febbraio, 2012). A recent study conducted by Pedersen and colleagues demonstrated the endocrine effects of cytokines or peptides derived from muscle fibers, produced and secreted during skeletal muscle contractions (B. K. Pedersen et al., 2003). These cytokines and peptides, categorized as myokines, have been shown in numerous studies to be exercise-dependent (Bortoluzzi et al., 2006; Henningsen et al., 2010; Yoon et al., 2009). One of the most well-known exercise-induced myokines is IL-6, which is the first myokine identified in the circulation in response to muscle contractions (Bente K. Pedersen & Febbraio, 2008). IL-6 is a peptide that inhibits tumor necrosis factor-alpha, playing an anti-inflammatory role. Additionally, it regulates glucose uptake by activating AMP-activated protein kinase (AMPK) (Carey et al., 2006; Kariuki et al., 2015; Keller et al., 2006). Intriguingly, circulating levels of IL-6 increase during exercise without any indications of muscle damage (Fischer, 2006).

3.Conclusion

The relationship between exercise, osteocalcin, insulin, and IL-6 is associated with intricate interactions within physiological processes. Exercise is a crucial factor for bone health. Weight-bearing exercise can

increase bone density and reduce the risk of osteoporosis. Osteocalcin is a protein hormone produced by bone cells, with positive effects on both bone health and metabolism. Moderate-intensity exercise contributes to osteocalcin production, aiding in the preservation of bone health. Additionally, osteocalcin plays a role in regulating blood sugar by enhancing insulin sensitivity. Insulin is a hormone that regulates the uptake of glucose into cells and energy production. Both exercise and osteocalcin support glucose metabolism by increasing insulin secretion, potentially reducing the risk of type 2 diabetes. Exercise increases muscle activity and can trigger inflammatory responses during this process. Interleukin-6, known as a pro-inflammatory cytokine, exhibits anti-inflammatory effects when released from tissues such as muscles and adipose tissue in response to exercise. This can help protect the body against infections and inflammation. However, elevated levels of circulating IL-6 may negatively impact insulin sensitivity. High levels of circulating IL-6 can increase insulin resistance and raise the risk of type 2 diabetes. Nevertheless, IL-6 associated with exercise may have anti-inflammatory effects, potentially enhancing insulin sensitivity.

In conclusion, exercise can enhance osteocalcin production, improve insulin sensitivity, and trigger low-level anti-inflammatory responses. These factors appear to play roles in various physiological processes, from bone health to glucose metabolism, with complex interactions among them. However, further research is needed to fully understand how these relationships work and determine their precise effects.

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