American Journal of
Biomedical Science & Research

www.biomedgrid.com
ISSN: 2642-1747

Mini Review

Klotho, Obesity, and Inflammation: A Triad of Metabolic Regulation

Chan MKS[1,2,3], Wong MBF[1,2], Nalapko Y[4], Casazza K[5], Klokol D[1,2], Nishkuma O[1,2], Jenkins I[6] and Lakey JRT[5-7]*

[1]*European Wellness Biomedical Group, Klosterstrasse 205ID, 67480, Edenkoben, Germany*

[2]*European Wellness Academy, EU, Malaysia*

[3]*Lincoln University College, Petaling Jaya, Malaysia*

[4]*Bogomolets National Medical University, Department of Internal Medicine #2, Kyiv, Ukraine*

[5]*University of California, Irvine- Department of Surgery, Irvine CA, USA*

[6]*GATC Health Inc, Irvine CA, USA*

[7]*University of California, Irvine- Department of Biomedical Engineering, Irvine, CA, USA.*

***Corresponding author:** Jonathan RT Lakey, Departments of Surgery and Biomedical Engineering, University of California Irvine, USA.

To Cite This Article: *Chan MKS, Wong MBF, Nalapko Y, Casazza K, Klokol D, et al. Klotho, Obesity, and Inflammation: A Triad of Metabolic Regulation. Am J Biomed Sci & Res. 2025 25(6) AJBSR.MS.ID.003376,* **DOI:** 10.34297/AJBSR.2025.25.003376

Received: February 11, 2025; **Published:** February 18, 2025

Obesity is a global public health epidemic. According to the World Health Organization (WHO), more than 1 billion people worldwide are classified as overweight or obese, including a significant proportion of children and adolescents [1]. This trend is driven by multiple factors, including sedentary lifestyles, unhealthy dietary patterns, and socioeconomic disparities that limit access to nutritious foods and opportunities for physical activity [2]. Obesity is not only a major risk factor for chronic diseases such as diabetes, cardiovascular disease, and certain cancers but also contributes to reduced quality of life and increased healthcare costs. While it is well-known that addressing the obesity epidemic requires comprehensive innovative strategies, the promotion of healthier diets and physical activity, as well as policy change efforts have modest effect. As such, obesity and related comorbidities continue to rise in prevalence worldwide.

A key mechanism by which obesity elevates all-cause morbidity and mortality by promoting tissue degeneration while compromising endogenous repair systems and cell types, such as mesenchymal stem cells (MSCs) [3]. Several researchers have demonstrated the relationship between obesity and senescence as well as a higher tendency for adipogenic and osteogenic differentiation of MSCs (ref). Additionally, obesity diminishes the immunomodulatory functions of human adipose tissue-derived MSCs both *in vitro* and *in vivo* [4].

Mitochondria are dynamic organelles central to cellular energy production and metabolism, capable of adapting to the local cellular microenvironment [5]. They regulate energy generation and coordinate the inflammatory responses of both resident and recruited cells. A key metabolic adaptation, known as glycolytic reprogramming, involves a shift from oxidative phosphorylation (OXPHOS) to glycolysis and is observed in various immune cells, including macrophages, dendritic cells, T-cells, and neutrophils. This metabolic shift is particularly evident during macrophage activation in inflammatory responses. Pro-inflammatory macrophages rely heavily on glycolysis for adenosine triphosphate (ATP) production, which is associated with increased secretion of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1-beta (IL-1β), and monocyte chemoattractant protein-1 (MCP-1) [6]. Conversely, anti-inflammatory macrophages exhibit higher levels of OXPHOS, linked to enhanced secretion of anti-inflammatory cytokines like IL-4 and IL-10. This bioenergetic pathway shift drives changes in cell activation and function, creating a feedback loop that disrupts cellular metabolism in local tissues, including bone and muscle, impairing their homeostasis and regenerative capacity.

Obesity is closely linked to mitochondrial dysfunction and chronic inflammation [7], forming a triad that exacerbates metabolic disturbances and contributes to the progression of various diseases. Excess adiposity places significant metabolic stress on mitochondria, the cellular powerhouses responsible for energy production and maintaining metabolic homeostasis [8]. In obesity, mitochondrial function is impaired due to factors such as oxidative stress, lipid overload, and altered dynamics, including reduced biogenesis and fission-fusion balance. Diminished OXPHOS efficiency, increases reactive oxygen species (ROS) production and a metabolic shift toward glycolysis [9]. These changes contribute to a pro-inflammatory microenvironment, as ROS and mitochondrial-derived damage-associated molecular patterns (mtDAMPs) activate inflammatory signaling pathways, including the NLRP3 inflammasome and NF-κB. In adipose tissue, this inflammatory state drives the polarization of macrophages toward a pro-inflammatory phenotype (M1), further amplifying cytokine production, such as TNF-α, IL-6, and MCP-1. Systemically, this low-grade, chronic inflammation, often referred to as "metaflammation [10]," disrupts insulin signaling, exacerbates insulin resistance, and impacts energy regulation in tissues like skeletal muscle and liver. Additionally, mitochondrial dysfunction in obesity compromises the resolution of inflammation by impairing the energy-demanding processes of anti-inflammatory pathways. This vicious cycle of mitochondrial impairment, inflammation, and metabolic dysregulation underpins the pathophysiology of obesity and its associated comorbidities, including type 2 diabetes, cardiovascular disease, and non-alcoholic fatty liver disease.

Peptide drug development has entered a transformative phase in the 21st century, driven by breakthroughs in structural biology, recombinant biologics, and advanced synthetic and analytical technologies. These innovations have significantly expedited the development process, leading to the establishment of a comprehensive framework that encompasses peptide discovery, drug design, synthesis, structural optimization, and activity assessment. Peptides can act as signaling molecules, interacting with receptors on other cells to stimulate specific cellular behaviors like migration, proliferation, and differentiation, depending on the peptide sequence. Peptides can be incorporated into scaffolds to enhance stem cell adhesion and promote tissue growth in areas where regeneration is needed. "Stem cell derived peptides" refer to short protein fragments (peptides) that are extracted or produced from stem cells, which can mimic the functions of the original stem cell proteins and have potential applications in regenerative medicine by promoting cell adhesion, proliferation, and differentiation, particularly in tissue repair and regeneration processes. These peptides are derived from various types of stem cells, including MSCs, which are known for their ability to regenerate tissues. By stimulating cell migration and proliferation, stem cell derived peptides can accelerate wound closure. In addition, peptides derived from stem cells may have anti-inflammatory properties, potentially useful in treating inflammatory diseases. Further, peptides mimicking components of the ECM can promote cell adhesion and migration. Peptides can also reduce the accumulation of harmful substances, such as ROS generated as a direct or indirect response to obesity.

Stem cell-derived peptides are an emerging class of bioactive molecules with significant therapeutic potential. These peptides are secreted by stem cells or synthesized to mimic the functional properties of stem cell-derived proteins, playing critical roles in tissue regeneration, immune modulation, and inflammation control. They act as signaling molecules that influence cellular processes, such as proliferation, differentiation, and repair, making them highly valuable for treating various conditions, including degenerative diseases, chronic inflammation, and metabolic disorders like obesity. Stem cell-derived peptides also offer advantages over cell-based therapies, including reduced immunogenicity, simpler storage and administration, and fewer regulatory hurdles. Ongoing research aims to optimize their stability, delivery mechanisms, and specificity to maximize their therapeutic efficacy in regenerative medicine and beyond.

Novel peptide therapies are emerging as promising strategies for obesity treatment, targeting key pathways involved in appetite regulation, energy expenditure, and metabolic processes. These peptides can mimic or modulate the activity of hormones, such as glucagon-like peptide-1 (GLP-1) or ghrelin, to suppress appetite, enhance satiety, and improve glucose metabolism. Some peptides also promote fat oxidation and thermogenesis, contributing to weight loss and improved metabolic health. While these therapies offer significant potential, challenges remain in optimizing their stability, delivery, and efficacy. Ongoing advancements in peptide engineering and formulation are expected to enhance their therapeutic value in addressing obesity and its associated complications.

Novel peptides for obesity treatment encompass various types designed to target specific biological pathways. By leveraging innovative designs and delivery mechanisms, these peptides hold significant potential for advancing obesity management and treatment outcomes. This review highlights some novel peptide therapies as well as the current challenges for therapeutic development. In particular, we focus on Nano Organo Peptides (NOPs) and Mito Organelles (MO) Peptides. NOPs and MOs represent cutting-edge approaches in obesity treatment, leveraging advanced molecular designs to target key pathways involved in metabolic regulation and energy homeostasis.

Mitochondria-Targeting Peptides are designed to improve mitochondrial function, these peptides combat metabolic dysregulation associated with obesity. For instance, MO peptides are designed to target and enhance mitochondrial function, a critical factor in obesity and metabolic health. These peptides improve mitochondrial efficiency by reducing oxidative stress, enhancing energy production, and promoting fatty acid oxidation. MOs play pivotal roles in modulating metabolic dysfunction associated with obesity and related diseases, including insulin resistance,

type 2 diabetes, and nonalcoholic fatty liver disease (NAFLD). Various MOs have demonstrated consistent metabolo-protective properties, improving glucose tolerance and insulin sensitivity in rodent models through different mechanistic pathways. MOs have also been theorized to enhance lipid oxidation and thermogenesis in adipose tissues, promoting weight loss. Mos have also been shown to exhibit neuroprotective and insulin-sensitizing effects by modulating apoptotic pathways and enhancing insulin receptor signaling, with its analogs improving glucose metabolism and reducing fat accumulation. Clinical trials investigating MO therapies, particularly for type 2 diabetes, obesity, and NAFLD, are underway. These peptides represent promising candidates for addressing metabolic stress and improving outcomes in metabolic diseases.

Nanoparticles are a promising platform to improve administration of peptides due to their unique in vivo properties and high design flexibility. Nano-Peptides are engineered using nanotechnology for improved stability, targeted delivery, and sustained release. Nano Organo Peptides (NOPs) enhance bioavailability and therapeutic efficacy while minimizing side effects. NOPs are small (3nm) in size and molecular weight (> 10kDA) [3]. NOP contents are procured from mammalian stem cells processed through a proprietary parallel-extraction process from organ specific cells with an initially high molecular mass and subsequently separated through various ultrafiltration steps through micro-Millipore filters. This selective filtration process only allows substances with a molecular mass of less than 10kDa to pass, thereby ensuring peptide specificity. Procurement of NOPs includes multiple ultrafiltrate steps through specialized to obtain the cellular material within the cell, known as the molecular-level ultrafiltrates. Owing to the extraction process, these ultrafiltrates are specific to the cell type that they are derived from. Moreover, the small molecular weights and high solubility of NOPs permits their delivery via both sublingual and injectable routes (either subcutaneous or intramuscular) [3]. NOPs have been investigated and utilized for a variety of applications including cosmetics [8] and regenerative organ repair [9]. NOPS can efficiently reach target tissues, such as adipose tissue or the hypothalamus, to regulate appetite, energy expenditure, and lipid metabolism. By employing nanocarriers or conjugation strategies, NOPs ensure sustained release, increased bioavailability, and reduced degradation, improving therapeutic efficacy while minimizing side effects. Extensive efforts have begun to develop NPs designed to overcome the challenges in the GI tract and enhance the bioavailability. Nanomedicine has the potential to enhance diabetes treatment by leveraging diverse therapeutic strategies through advanced nanoformulations. For instance, glucose-sensitive nanoparticles can mimic the body's natural insulin release by responding to elevated glucose levels and delivering insulin accordingly.

Obesity-induced mitochondrial dysfunction alters fate of MSCs. As such, obesity plays a critical role in cell fate and the morbidity of acute or chronic inflammation. Declining mitochondrial activity contributes to the accumulation of toxic biochemicals. Inflammation is a critical biological response to toxic biochemical accumulation. During this response, various transcription factors are activated, including nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB), interferon regulatory factors (IRFs), signal transducers and activators of transcription (STAT), and activator protein-1 (AP-1)11. Notably, NF-κB plays a central role in regulating the production and expression of numerous pro-inflammatory molecules, and its inhibition has been shown to attenuate inflammation. Metabolic pathways differ between macrophage subsets and are now recognized as key markers of their function. Inflammatory M1 macrophages exhibit increased glycolytic activity and reduced mitochondrial function, while anti-inflammatory M2 macrophages rely on oxidative phosphorylation (OXPHOS) and demonstrate enhanced spare respiratory capacity (SRC). Additionally, oxygen deprivation has been shown to increase hypoxia-inducible factor-1 alpha (HIF-1α) levels, which drive glycolytic pathways and promote M1 polarization, further linking obesity-related metabolic shifts to inflammatory responses [12]. Targeting the signaling pathways of pro-inflammatory cytokine transcription factors offers a strategy to reduce persistent inflammation.

Together, NOPs and MO peptides represent innovative approaches to overcoming challenges in obesity treatment, offering precision, efficacy, and the potential to address obesity's underlying metabolic dysfunctions. Ongoing research and development in these areas holds promise for advancing therapeutic strategies against obesity and its associated complications. Our team explored the potential of peptide therapy products to stimulate insulin production, protect beta cells from damage, and modulate immune responses involved in diabetes development. In collaboration with European Wellness (EW) and the BioPep Research Group, we leveraged collective expertise to develop two distinct peptide therapies—MO peptides and NOP—created from organ-specific cellular extracts and peptide molecules. These products are produced through a proprietary parallel-extraction process from mammalian precursor stem cells and rabbits bred in controlled conditions under good manufacturing practices.

Given the widespread presence of Mitochondria-Derived Peptides (MDPs) and their role in maintaining cell viability and mitochondrial function under various conditions, we conducted a study to evaluate whether biweekly intramuscular administration of stem-cell-derived MO peptides over 17 weeks could delay or prevent the destruction of insulin-secreting beta cells in the pancreatic islets of Langerhans in non-obese diabetic (NOD) mice. The MO peptides, derived from thymus and pancreatic extracts, were specifically designed to target beta cells and influence T-cell maturation.

Erythropoietin (EPO) and C-C motif chemokine ligand 5 (CCL5) play significant roles in modulating inflammation and macrophage polarization, further linking altered metabolic pathways to inflammatory responses. EPO, traditionally recognized for its role in erythropoiesis, has demonstrated anti-inflammatory properties by suppressing pro-inflammatory cytokines and promoting the polarization of macrophages toward the anti-inflammatory M2

phenotype. This shift is associated with enhanced mitochondrial OXPHOS and an increased SRC, reducing inflammation. Similarly, CCL5, a chemokine involved in immune cell recruitment, influences macrophage behavior depending on the inflammatory context. While elevated CCL5 levels are often associated with chronic inflammation and recruitment of inflammatory M1 macrophages, its regulation can mitigate excessive immune responses and contribute to tissue homeostasis. Both EPO and CCL5 impact macrophage metabolism, with EPO promoting mitochondrial function and CCL5 modulating immune cell dynamics. Klotho exerts anti-inflammatory effects by suppressing pro-inflammatory signaling pathways, including NF-κB. By reducing the expression of cytokines such as TNF-α, IL-6, and MCP-1, Klotho mitigates chronic inflammation associated with metabolic and skeletal disorders. Klotho is also involved in modulating macrophage polarization. It promotes the transition of macrophages from the pro-inflammatory M1 phenotype, characterized by high glycolytic activity, to the anti-inflammatory M2 phenotype, which relies on mitochondrial OXPHOS and enhanced SRC. This shift in macrophage metabolism and function helps restore metabolic homeostasis. Additionally, Klotho improves mitochondrial health by enhancing antioxidant defenses, reducing ROS, and stabilizing mitochondrial dynamics. It has been shown to influence pathways such as those mediated by HIF-1α, which drives glycolytic metabolism and M1 polarization under hypoxic conditions. By modulating these processes, Klotho helps counteract the vicious cycle of mitochondrial dysfunction, inflammation, and tissue damage observed in obesity. Klotho's interplay with key regulators such as EPO and CCL5 further underscores its therapeutic potential in mitigating inflammation and metabolic dysregulation.

We performed a cytokine analysis using a 45-panel assay. The analysis revealed significant differences in the serum levels of Erythropoietin (EPO) and Chemokine Ligand 5 (CCL5, also known as RANTES) between MO peptide-treated mice and controls. MO peptide-treated mice had an average EPO concentration of 374.88 pg/mL compared to 203.68 pg/mL in the control group ($p = 0.0062$). Similarly, the CCL5 concentration averaged 14.37 pg/mL in treated mice versus 8.08 pg/mL in controls ($p = 0.031$). These preliminary findings suggest that MO peptides may delay or prevent the onset of type 1 diabetes (T1D), representing a promising therapeutic avenue for further investigation.

Despite extensive studies which demonstrated the therapeutic potential of Nano Organo Peptides (NOP) and Mito Organelles (MO) peptides, the precise composition of these formulations remains largely undefined. Mass spectrometry (MS) has proven effective for identifying and quantifying analytes in complex solutions, making it a valuable tool for characterizing the peptides within these cocktail formulations. The MS process generates a readout of peaks plotted as relative abundance against mass-to-charge ratios, which can then be matched against protein databases to identify peptides.

Our investigation used MS to analyze various EW peptide formulations, revealing no significant differences in protein concentrations between batches, though significant differences were observed between sample types ($p < 0.05$). These results align with expectations, as NOP and MO formulations are heterogeneous mixtures derived from specific tissues, leading to sample variation. Consistency between batches was evident in both protein concentration assays and preliminary MS experiments. The LBS MO peptide sample, noted for its higher protein concentration and numerous detectable peaks, was selected for detailed analysis due to its potential for larger and more complex peptides.

While preliminary analyses relied on LC-MS/MS techniques, which are time-intensive, we used Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) MS for in-depth analysis due to its efficiency and reliability. MALDI-TOF employs a protein fingerprinting method where proteolytic digestion, typically with trypsin, generates a spectrum for comparison with databases. This approach ranks peptide candidates by the number of matching proteolytic peptides, providing a probabilistic identification of proteins. Using MALDI-TOF, we identified 11 major peptides of interest across two batches. Batch one included peptides with molecular weights of 14,969 Da, 15,300 Da, 8,449 Da, 8,294 Da, and 4,618 Da, while batch two contained four of the same peptides alongside two additional peptides of 5,436 Da and 6,214 Da. Minor differences between batches likely stem from the heterogeneous nature of cellularly derived solutions and variations in the extraction process. These findings offer valuable insights into the stability and therapeutic potential of these peptides, advancing their development for peptide therapy applications across species.

The group [13] also conducted analyses to assess the impact of temperature storage and duration of storage on peptide stability. We tested the acidic peptide N-06 D, seven acidic peptide combination (N-18A), a 7-peptide combination (N-18A) stored at 4°C, N-06D peptides in liver samples stored at 4°C over one-three-and six months. extracted from liver samples stored at 22°C for one-, three-, and six- months. Showing complex spectra and intensity at one month, with significant declines by six months for each. Overall, the collected data suggested that acidic peptides stored at 22°C experience degradation over time, with a clear reduction in both peak intensities and overall peptide stability by six months of storage. The results of our study suggested that while some peptides remain stable, there are notable changes in the peptide profiles over time. Slight differences in peptide products between batches are likely due to the heterogeneous nature of cellularly-derived solutions and differences that occurred during the extraction process. This approach lays the groundwork to identifying potential adverse effects on stability, and therefore therapeutic potential to ensure the safe application of stem cell technologies in clinical and research settings. Understanding these risks is essential for advancing stem cell science while safeguarding public health and promoting the responsible development of stem cell-based therapies.

Summary: Obesity is closely linked to mitochondrial dysfunction and chronic inflammation, forming a triad that exacerbates metabolic disturbances and contributes to the progression of various diseases. Excess adiposity places significant metabolic

stress on mitochondria, the cellular powerhouses responsible for energy production and maintaining metabolic homeostasis. In obesity, mitochondrial function is impaired due to factors such as oxidative stress, lipid overload, and altered dynamics, including reduced biogenesis and fission-fusion balance. This dysfunction diminishes OXPHOS efficiency, leading to increased ROS production and a metabolic shift toward glycolysis. These changes contribute to a pro-inflammatory microenvironment, as ROS and mitochondrial-derived damage-associated molecular patterns (mtDAMPs) activate inflammatory signaling pathways, including the NLRP3 inflammasome and NF-κB. In adipose tissue, this inflammatory state drives the polarization of macrophages toward a pro-inflammatory phenotype (M1), further amplifying cytokine production, such as TNF-α, IL-6, and MCP-1. Systemically, this low-grade, chronic "metaflammation," disrupts insulin signaling, exacerbates insulin resistance, and impacts energy regulation in tissues like skeletal muscle and liver. Additionally, mitochondrial dysfunction in obesity compromises the resolution of inflammation by impairing the energy-demanding processes of anti-inflammatory pathways. This vicious cycle of mitochondrial impairment, inflammation, and metabolic dysregulation underpins the pathophysiology of obesity and its associated comorbidities, including type 2 diabetes, cardiovascular disease, and non-alcoholic fatty liver disease.

References

1. NCD Risk Factor Collaboration (NCD-RisC) (2017) Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128·9 million children, adolescents, and adults. Lancet Lond Engl 390(10113): 2627-2642.
2. Blüher M (2019) Obesity MB global epidemiology and pathogenesis. Nat Rev Endocrinol 15(5): 288-298.
3. Chen Q, Shou P, Zheng C, JiangM, Cao G, et al. (2016) Fate decision of mesenchymal stem cells: adipocytes or osteoblasts? Cell Death Differ 23(7): 1128-1139.
4. Zhu XY, Klomjit N, Conley SM, Ostlie MM, Jordan KL, et al. (2021) Impaired immunomodulatory capacity in adipose tissue-derived mesenchymal stem/stromal cells isolated from obese patients. J Cell Mol Med 25(18): 9051-9059.
5. Liesa M, Shirihai OS (2013) Mitochondrial dynamics in the regulation of nutrient utilization and energy expenditure. Cell Metab 17(4): 491-506.
6. Ahmed B, Sultana R, Greene MW (2021) Adipose tissue and insulin resistance in obese. Biomed Pharmacother Biomedecine Pharmacother 137: 111315.
7. Ejarque M, Ceperuelo Mallafré V, Serena C, Maymo Masip E, Duran X, et al. (2005) Adipose tissue mitochondrial dysfunction in human obesity is linked to a specific DNA methylation signature in adipose-derived stem cells. Int J Obes 43(6): 1256-1268.
8. Hotamisligil GS (2017) Inflammation, metaflammation and immunometabolic disorders. Nature 542(7640): 177-185.
9. Valero T (2014) Mitochondrial biogenesis: pharmacological approaches. Curr Pharm Des 20(35): 5507-5509.
10. Schleh MW, Caslin HL, Garcia JN, Mashayekhi M, Srivastava G (2023) Metaflammation in obesity and its therapeutic targeting. Sci Transl Med 15(723): eadf9382.
11. Planat-Benard V, Varin A, Casteilla L (2021) MSCs and Inflammatory Cells Crosstalk in Regenerative Medicine: Concerted Actions for Optimized Resolution Driven by Energy Metabolism. Front Immunol 12: 626755.
12. Monlun M, Hyernard C, Blanco P, Lartigue L, Faustin B (2017) Mitochondria as Molecular Platforms Integrating Multiple Innate Immune Signalings. J Mol Biol 429(1): 1-13.
13. Chan MKS, Wong MBF, Katz B, Casazza K, Klokol D, et al. (2024) Mass Spectrometry Analysis of Organ-Specific Cellular Extracts-Nanomized Organo Peptides: The Stability Study J Stem Cell Res 5(2): 1-11.