Tetratide Labs

PEPTIDE SOLUTIONS FOR HUMANS, EQUINES AND ANIMALS

TETRATIDE LABS / SCIENTIFIC SERIES

THYMOSIN β4 & TB-500

Scientific handbook on molecular biology, signalling pathways, tissue-repair research and cross-species evidence

HUMANS • EQUINES • DOGS • CATS

TETRATIDE LABS
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HANDBOOK GUIDE

Contents

Purpose and scope

This handbook presents the supplied scientific overview of thymosin β4 and TB-500, including molecular structure, biological pathways, research history, cross-species literature and current regulatory context. It is an educational scientific reference and does not provide diagnosis, prescribing, administration or dosing guidance.

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QUICK REFERENCE

Terminology and molecular profile

TERM	SCIENTIFIC MEANING
Thymosin β4 (Tβ4)	A naturally occurring 43-amino-acid peptide and major actin-sequestering molecule in mammalian cells.
TB-500	A name commonly used for the synthetic N-acetylated heptapeptide Ac-LKKTETQ, corresponding to amino acids 17-23 of Tβ4; the label is also used inconsistently for full-length Tβ4.
G-actin sequestration	Binding of unpolymerised actin monomers, influencing the availability of actin for cytoskeletal assembly and cell movement.
Angiogenesis	Formation of new blood vessels from existing vasculature, a process studied in tissue repair and injury models.
Preclinical evidence	Evidence generated in cell systems, tissue models or animals before established clinical use.

FULL-LENGTH Tβ4	TB-500 FRAGMENT
43 amino acids	7 amino acids
Endogenous, highly conserved mammalian peptide	Ac-LKKTETQ; residues 17-23 of Tβ4
Major G-actin-sequestering molecule	CAS 885340-08-9
Multiple functional regions across the sequence	$C_{39}H_{67}N_9O_{13}$; 877.99 g/mol

CHAPTER 1

Introduction to Thymosin β4 and TB-500

1.1 Discovery of Thymosin β4

The story of thymosin β4 (Tβ4) begins in the laboratories of the Albert Einstein College of Medicine in the late 1960s and early 1970s, where researchers led by Allan Goldstein isolated a mixture of small proteins from calf thymus gland tissue. This mixture was designated "thymosin fraction 5" and attracted considerable scientific interest due to its apparent ability to restore immune function in animals that had undergone thymus removal. Within this complex fraction existed a 43-residue peptide that would later be identified and named thymosin beta-4.

The structural foundation for all subsequent Tβ4 research was established in 1981, when Tung-Leung Low, Su-Kuei Hu, and Allan Goldstein published the complete amino acid sequence of bovine thymosin beta-4 in the Proceedings of the National Academy of Sciences. This landmark paper (PMID 6940133) revealed that the peptide possessed an acetylated N-terminus and demonstrated the ability to induce terminal deoxynucleotidyl transferase activity in mouse thymocytes - a biochemical marker indicative of early lymphocyte development. This discovery positioned Tβ4 initially as an immunologically active thymic hormone, a classification that would dominate research focus for approximately the first decade following its sequencing. **[1]**

The thymosin family was subsequently categorised into three distinct groups - α, β, and γ thymosins - based on differences in their isoelectric points, with thymosin β exhibiting an isoelectric point range of 5.0-7.0. To date, 15 distinct types of β-thymosin have been identified, of which three main forms (Tβ4, Tβ10, and Tβ15) are found in the human body, with Tβ4 being the most abundant, accounting for approximately 70%-80% of all β-thymosins. Tβ4 is distributed widely throughout various tissues, with particularly high concentrations found in the thymus, spleen, and peritoneal macrophages, as well as elevated expression in the brain, liver, kidney, testis, myocardium, platelets, and leukocytes.

1.2 Molecular Structure and Biology

Thymosin β4 comprises 43 amino acids, and its biological activity is determined by specific encoded gene fragments within this sequence. The peptide's structure-function relationship has been extensively characterised, revealing that different regions of the molecule mediate distinct biological activities. The first four amino acids of Tβ4 regulate anti-inflammatory and antifibrotic effects, while amino acids 1-15 inhibit apoptosis and reduce toxicity-induced cellular damage. The active fragment encoded by amino acids 17-23 (the heptapeptide Ac-LKKTETQ, CAS 885340-08-9) triggers angiogenesis and hair follicle growth. **[5]**

The primary physiological role of thymosin beta-4 was redefined in the late 1990s when researchers discovered that its fundamental function was G-actin sequestration. The peptide binds to unpolymerised actin monomers and regulates the actin cytoskeleton in virtually every mammalian cell type. This actin-binding function positioned Tβ4 as a tissue-repair agent and explained its pleiotropic effects across multiple physiological systems. The molecular formula for the TB-500 fragment (Ac-LKKTETQ) is C39H67N9O13, with a molecular weight of 877.99 g/mol. **[2]**

Tβ4 is a highly conserved and ubiquitous peptide among mammals, playing a critical role in cytoskeleton organisation. Its distribution and conservation across species have made it a subject of interest for both basic research and potential therapeutic applications in veterinary and human medicine.

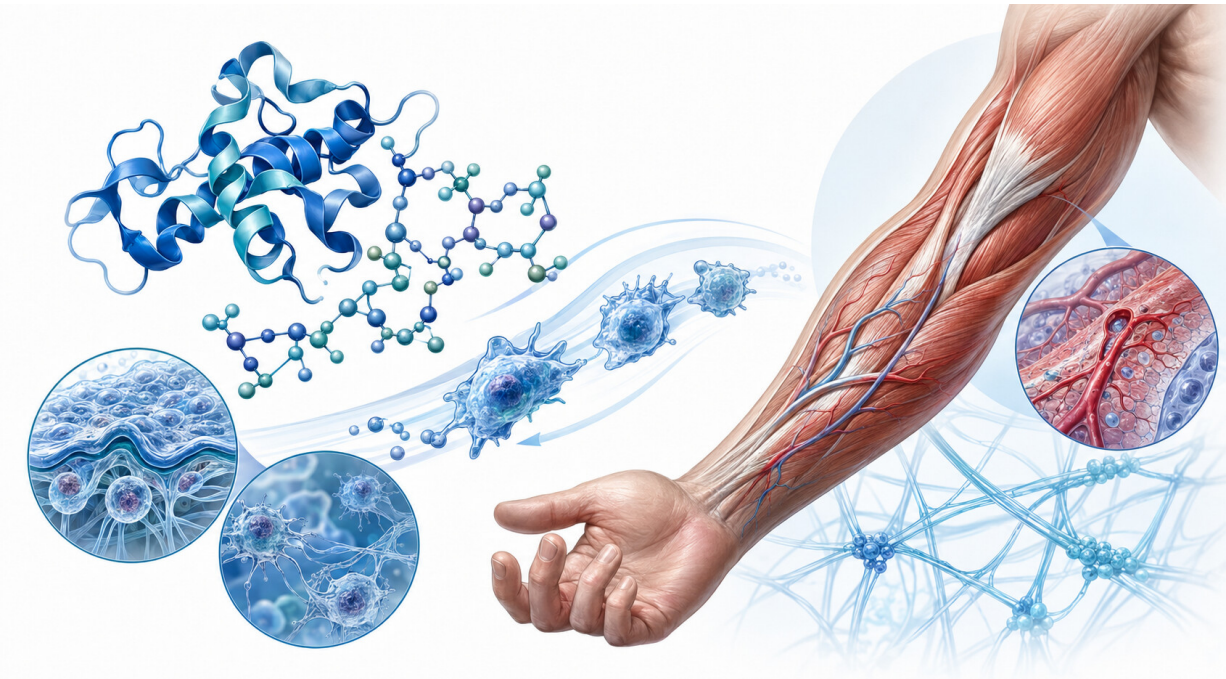
1.3 Why Researchers Developed TB-500

The designation "TB-500" emerged during the 2010s within online fitness and self-experimentation communities as a grey-market label for synthetic versions of thymosin beta-4 or its active fragment. The name technically refers to the heptapeptide Ac-LKKTETQ (amino acids 17 to 23 of thymosin beta-4), but vendors and users frequently apply it loosely to either the short fragment or the full 43-residue peptide. This distinction is scientifically important because the two compounds possess different structures and different published research profiles, yet are often sold interchangeably in grey-market contexts. **[11]**

Researchers and developers pursued TB-500 and related Tβ4 preparations based on the extensive preclinical evidence accumulated from the 1990s through the 2000s, which documented wound-healing effects in dermal and corneal injury models and proposed potential applications following cardiac hypoxia. A 2005 review in Trends in Molecular Medicine by Goldstein, Hannappel, and Kleinman described how Tβ4's actin-binding function positioned it as a tissue-repair agent, with proposed mechanisms including down-regulation of inflammatory chemokines, promotion of cell migration, new blood-vessel formation, and stem-cell maturation. **[2]**

The development interest was further fuelled by parallel research in cardiac biology from around 2010, where the peptide received significant attention as a potential heart-repair agent following myocardial infarction. Reviews in journals including Expert Opinion on Biological Therapy and Current Medicinal Chemistry summarised preclinical data in heart-injury models, though none of this preclinical work ultimately produced an approved cardiac medicine. The 2010 review by Philp and Kleinman in the Annals of the New York Academy of Sciences catalogued evidence from dermal, corneal, and cardiac wound models, generating substantial interest in potential therapeutic applications. **[3]**

1.4 Mechanisms Under Investigation



Conceptual scientific illustration of peptide structure, cellular migration, microvasculature and musculoskeletal tissue - core themes discussed in Tβ4/TB-500 research.

PATHWAY	RESEARCH FOCUS DESCRIBED IN THE LITERATURE
PI3K/Akt/eNOS	Cell migration, survival, endothelial progenitor-cell activity and angiogenesis
Notch	Endothelial signalling, lumen formation and context-dependent fibrotic responses
TGFβ/Smad	Fibrotic signalling and hepatic stellate-cell activation
Wnt/β-catenin	Cell differentiation, hair-follicle morphogenesis and regenerative models
Apoptosis	Bcl-2/Bax balance, mitochondrial integrity and caspase activity

Tβ4 affects the secretion of multiple cytokines and regulates various signalling pathways, positioning it as a multifunctional regulatory peptide. The peptide alleviates inflammatory damage by regulating the NF-κB and Toll-like receptor pathways while reducing the release of cytokines such as TNF-α and IL-1 receptor-associated kinases. During tissue repair, Tβ4 regulates PI3K/Akt/eNOS, Notch, angiopoietin-1/Tie2, and other pathways, while also regulating the TGF-β pathway to attenuate fibrosis and the Wnt pathway to promote hair follicle generation. **[5]**

PI3K/Akt/eNOS Pathway

The PI3K/Akt pathway is an important signalling cascade associated with microangiogenesis and plays a pivotal role in cell migration, cell survival, and angiogenesis. PI3K/Akt serves as the upstream pathway of eNOS and affects its transcription and translation, with eNOS increasing the local mobilisation of endothelial progenitor cells (EPCs) and participating in angiogenesis. Exogenous Tβ4 stimulates EPC proliferation, migration, and adhesion via the PI3K/Akt/eNOS signal transduction pathway. After intraperitoneal injection of Tβ4 in rats with cerebral ischemia and reperfusion, the level of Akt phosphorylation and the expression of eNOS in the cerebral cortex increased, regeneration of blood vessels around the infarction occurred, and neurological function recovered. Furthermore, Tβ4 induces angiogenesis via the PI3K/AKT signalling pathway in ischemic limb diseases. Systemic injection of a Tβ4-specific C-terminal tetrapeptide enhanced early myocyte survival by activating Akt-mediated signalling, increased coronary vessel growth, and inhibited inflammation in mice and pigs.

Notch Pathway

The Notch signalling pathway comprises four Notch receptors (Notch-1, 2, 3, and 4) and five ligands, and is crucial in neuronal function, tumor cell proliferation, apoptosis, angiogenesis, arterial endothelial cell stability, and expansion of bone marrow hematopoietic stem cells. Tβ4 induces angiogenesis in human umbilical vein endothelial cells (HUVECs) via the Notch signalling pathway. In the presence of Tβ4, the expression of Notch1 and Notch4 increased in a dose- and time-dependent manner, and the speed of lumen formation was accelerated; when the Notch pathway was inhibited, the efficacy of Tβ4 decreased. Moreover, Tβ4 inhibited the proliferation and activation of hepatic stellate cells (HSCs), attenuated liver fibrosis by inhibiting Notch signalling, and markedly reduced expression levels of Notch2 and Notch3, which were increased in liver cells.

TGFβ/Smad Pathway

The TGFβ/Smad signalling pathway is crucially mediated by TGFβ, with TGFβ1 playing an important role in HSC activation in fibrosis models. TGFβ1 initiates intracellular signal transduction by binding to the TGFβ receptor type II (TGFβR II) and activates TGFβ receptor type I (TGFβR I) kinase, which subsequently activates the downstream proteins Smad2 and Smad3 via phosphorylation. Chen et al. reported that Tβ4 reduced the expression of TGF-β1, TGFβR II, Smad2, and Smad3 in the liver tissues of mice with bile duct ligation, and demonstrated that Tβ4 reduced TGFβR II expression levels in human hepatic stellate cells LX-2 in vitro, indicating that Tβ4 alleviated cholestatic liver fibrosis by inhibiting the TGFβ/Smad pathway. Zhang et al. reported that Tβ4 treatment markedly inhibited the TGFβ1/NF-κB signalling pathway, which affects neuroprotection and neurorestoration after traumatic brain injury.

Wnt Signalling Pathway

The Wnt signalling pathway is crucially associated with cell proliferation and differentiation and is functionally important for hair follicle morphogenesis. Gao et al. reported that Tβ4 stimulated Wnt ligands on the cytomembrane to transmit signals that accumulate unphosphorylated β-catenin, which plays a pivotal role in hair follicle growth. After Tβ4 treatment, the number of hair follicles in mice significantly increased, and Tβ4 accelerated hair growth via the Wnt signalling pathway by elevating the mRNA levels of β-catenin and Lef-1. Additionally, Tβ4 activates the Wnt/β-catenin signalling pathway in limb progenitor cells and promotes limb regeneration in a frog model.

Apoptosis Pathway

Tβ4 decreases apoptosis by increasing antiapoptotic proteins and reducing the Bax/BCL2 ratio. Sosne et al. demonstrated that Tβ4 treatment decreased deleterious mitochondrial alterations, significantly decreased cytochrome c release from mitochondria, and increased Bcl-2 expression in ethanol-exposed human corneal epithelial cells, wherein it inhibited caspase-2, -3, -8, and -9 activity, with caspase-8 exhibiting the highest inhibition. Furthermore, FasL-mediated activation of caspases-8 and -3, as well as H_2O_2-triggered stimulation of caspases-9 and -3 in human corneal epithelial cells, was abolished by preincubation with Tβ4.

1.5 Equine Research

PROPOSED EQUINE BENEFIT	SCIENTIFIC RATIONALE
Tendon and ligament support	Actin regulation and cell migration are fundamental to soft-tissue remodelling and repair.
Muscle recovery	Tβ4 has increased myoblast migration and experimental wound closure in skeletal-muscle injury models.
Wound and skin repair	Animal studies report accelerated closure, collagen deposition and re-epithelialisation.
Vascular support	Angiogenic activity may support formation of microvasculature around recovering tissue.
Inflammatory regulation	Research describes modulation of inflammatory chemokines, cytokines and cellular stress responses.
Mobility and rehabilitation	Proposed as a downstream benefit of improved muscle and soft-tissue recovery.

Thymosin β4 is a highly conserved mammalian peptide with biological activities directly relevant to the demands placed on equine muscle, tendon, ligament, skin and vascular tissue. Its regulation of actin dynamics, cell migration, angiogenesis, inflammatory signalling and cell survival has made it a prominent research target in recovery-focused models. These mechanisms underpin the proposed equine benefits associated with Tβ4 and TB-500. **[3, 4]**

Soft-tissue and muscle recovery are central areas of interest. In injured skeletal-muscle models, endogenous Tβ4 expression rises during the early regenerative phase, while Tβ4 and its sulfoxide form have accelerated experimental wound closure and increased the migration of myoblasts - the precursor cells involved in rebuilding muscle fibres. This supports research interest in post-exercise muscle repair, recovery from muscular strain and the restoration of normal tissue function. **[14]**

Dermal and connective-tissue research has reported accelerated wound closure, increased collagen deposition, enhanced cell migration and new blood-vessel formation in animal models. In equine-focused applications, these findings are commonly discussed in relation to skin repair, tendon and ligament remodelling, improved vascular support to recovering tissue and the maintenance of tissue flexibility during rehabilitation. **[4, 15, 16]**

Tβ4 has also been studied for its ability to regulate inflammatory chemokines and cytokines while supporting cell survival. Within an equine recovery framework, these actions are proposed to support a more controlled tissue response following strenuous work or injury. Collectively, the research themes explain the claimed benefits of faster recovery, improved mobility, reduced stiffness, soft-tissue support and more efficient return to normal training activity. **[3, 4]**

> **Competitive-sport status**
> Tβ4 derivatives, including TB-500, are prohibited in competitive equestrian and racing sport. Participants should consult the current FEI, IFHA and applicable national rules.

1.6 Canine Research

Animal studies with thymosin beta-4 have covered a wide range of models across species, with research interest driven by the peptide's documented roles in wound healing, angiogenesis, and tissue repair. The broad distribution of Tβ4 in mammalian tissues and its high conservation across species suggest potential applicability to canine physiology, though specific peer-reviewed canine studies remain limited in the published literature. **[3]**

The biological mechanisms identified in other species - G-actin sequestration, regulation of inflammatory cytokines, promotion of cell migration, and angiogenesis through PI3K/Akt/eNOS, Notch, and VEGF pathways - are fundamentally conserved across mammals and would be expected to operate similarly in canine systems. Tβ4's effects on cell proliferation, apoptosis inhibition, and inflammation amelioration have been documented in multiple tissue types including cardiac, dermal, corneal, and hepatic models.

The 2010 review by Philp and Kleinman noted that animal studies across this period covered diverse models, with proposed mechanisms including down-regulation of inflammatory chemokines, promotion of cell migration, new blood-vessel formation, and stem-cell maturation. However, the translation of this preclinical evidence into veterinary therapeutic applications has been limited, and no approved veterinary medicines containing Tβ4 or TB-500 are currently available. **[3]**

1.7 Feline Research

Published research specifically addressing thymosin beta-4 in feline models is notably sparse in the peer-reviewed literature. The general principles of Tβ4 biology - ubiquitous distribution in mammalian tissues, high conservation across species, and critical role in cytoskeleton organisation - would suggest fundamental biological relevance to feline physiology. However, species-specific research is necessary to establish dosing parameters, safety profiles, and therapeutic efficacy in cats.

The absence of feline-specific Tβ4 research reflects a broader pattern in the field, where four decades of preclinical work have not translated into approved therapeutic uses for the full-length thymosin beta-4 or the TB-500 fragment in any systemic indication across any species. The only Western-standard controlled trial data comes from ophthalmology, specifically a Phase III trial of RGN-259 (a 0.1% thymosin beta-4 ophthalmic solution) in patients with neurotrophic keratopathy, published in 2022. **[8]**

1.8 Current Evidence and Limitations

As of July 2026, thymosin beta-4 and TB-500 hold no marketing authorisation from the FDA, EMA, MHRA, or any other national regulatory agencies in major jurisdictions. In all covered jurisdictions, these compounds sit in the grey-market category: not approved medicines, not explicitly scheduled as controlled substances in most places, but also not lawfully sold for human use. The FDA's Pharmacy Compounding Advisory Committee was scheduled to review thymosin beta-4 preparations for the 503A bulk-drug-substances list in 2026, addressing whether US compounding pharmacies may legally prepare the peptide; however, this review is not a path to marketing authorisation and will not produce an approved medicine regardless of outcome. **[12]**

The most rigorous human trial data on any thymosin beta-4 preparation comes from ophthalmology. RegeneRx Biopharmaceuticals conducted a randomised, placebo-controlled, double-masked Phase III trial of RGN-259, a 0.1% thymosin beta-4 ophthalmic solution, in patients with neurotrophic keratopathy (stages 2 and 3). Published in the International Journal of Molecular Sciences in 2022 (PMID 36613994), the trial found that 60% of treated patients achieved complete healing within four weeks compared with 12.5% receiving placebo, with no significant adverse effects observed. This study used a topical ophthalmic preparation, a different route and formulation from the subcutaneous injections sold in grey-market contexts, and the product has not been approved by the FDA or EMA as of mid-2026. **[8]**

SOURCE NOTES

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Editorial note

Scientific and regulatory information can change. This edition reflects the supplied manuscript and cited source context available in July 2026. Readers should consult the linked primary or official source for the most current position.