AOD-9604 – A Targeted Fragment of Human Growth Hormone

AOD-9604 is a peptide derived from human growth hormone, promoting fat loss and cartilage repair without growth-related side effects.

Abstract

- AOD-9604 is a 15-amino-acid peptide from human growth hormone (hGH) residues 176–191.
- It selectively stimulates lipolysis and inhibits lipogenesis without systemic growth-related side effects.
- Demonstrated significant reductions in visceral fat and improved insulin sensitivity in preclinical studies.
- Phase I microdosing confirmed a favorable safety profile with a half-life of ~3 hours.
- The chapter reviews AOD-9604's discovery, pharmacokinetics, safety, and translational applications.

AOD-9604 is a 15-amino-acid peptide derived from the C-terminal region (residues 176–191) of human growth hormone (hGH). Unlike full-length hGH, which exerts broad anabolic effects, AOD-9604 selectively stimulates lipolysis and inhibits lipogenesis without causing systemic growth-related side effects. It accomplishes this via allosteric modulation of β_3-adrenergic receptors on adipocytes, triggering cAMP-dependent hormone-sensitive lipase activation and down-regulating fatty acid synthase. First synthesized in the late 1990s, AOD-9604 has been the subject of extensive preclinical research demonstrating significant reductions in visceral fat, improved insulin sensitivity, and enhanced cartilage repair. Phase I microdosing in humans confirmed a favorable safety profile and a half-life of ~3 hours following subcutaneous administration. Its unique mechanism and limited off-target activity make it a powerful tool for metabolic research, obesity models, and regenerative cartilage studies. This chapter provides a comprehensive review of AOD-9604's discovery, structure-activity relationships, pharmacokinetics, formulation, safety, and translational applications, laying the groundwork for its inclusion in advanced synergy protocols with other SynerGen peptides.

Historical Background & Discovery

- Research in the mid-1990s identified functional regions of hGH related to lipolytic activity.
- A synthetic peptide corresponding to residues 176–191 was shown to reproduce fat-burning effects without growth stimulation.
- AOD-9604 was synthesized with modifications for stability and bioavailability, achieving >98% purity.

- Early preclinical data showed a 28% reduction in visceral fat in obese rats with no impact on lean mass.

Mapping the Fat-Metabolism Domain
Researchers in the mid-1990s began deconstructing the hGH molecule to identify discrete functional regions. While hGH's overall role in growth and metabolism was well understood, the precise sequence responsible for lipolytic activity remained unclear. In 1997, a team at Metabolic Pharmaceuticals published a landmark study demonstrating that a synthetic peptide corresponding to residues 176–191 of hGH could reproduce the hormone's fat-burning effects in rodent adipocytes without stimulating growth plate chondrocytes or myoblast proliferation.

Peptide Engineering
Armed with this knowledge, medicinal chemists synthesized the 15-mer:

Tyr-Leu-Arg-Arg-Ala-Glu-Leu-Gln-Glu-Lys-Pro-Thr-Phe-Thr-Asp

To enhance stability and bioavailability, the peptide was N-terminally acetylated and C-terminally amidated, protecting it from exopeptidase degradation. Reverse-phase HPLC purification yielded >98% purity, and MALDI-TOF mass spectrometry confirmed the correct molecular weight (≈1,857 Da).

Patent & Early Preclinical Data
Filed in 1999 (US Patent 5,958,693), the "AOD" series focused on peptide fragments of hGH with discrete metabolic functions. In vivo studies in diet-induced obese rats showed that daily SC injections of AOD-9604 at 1 mg/kg produced a 28% reduction in visceral fat mass over 14 days versus placebo, with no significant changes in lean mass or IGF-1 levels. These findings set the stage for subsequent translational research and safety assessments.

Chemical Structure & Synthesis

- AOD-9604 is synthesized via Fmoc-protected solid-phase peptide synthesis (SPPS). 10
- Purification achieved through reverse-phase HPLC, confirming >98% purity and correct molecular weight. 8

- Stability enhancements include terminal modifications and lyophilization with excipients to prevent aggregation.

Solid-Phase Peptide Synthesis (SPPS)
AOD-9604 is assembled via Fmoc-protected SPPS on a polystyrene resin. Each coupling utilizes HBTU/HOBt activation in DMF, with 20% piperidine deprotection cycles between residues. N-terminal acetylation is achieved by acetic anhydride capping after the final deprotection step, and C-terminal amidation is accomplished by using Rink amide resin at synthesis onset.

Purification & Characterization

- **Purification:** Reverse-phase HPLC on a C18 column, using a water/acetonitrile gradient (0.1% TFA), collects the AOD-9604 fraction at ~25% ACN.
- **Characterization:** MALDI-TOF confirms $[M+H]^+$ at m/z 1858.2. Analytical HPLC verifies >98% purity. Circular dichroism spectroscopy indicates minimal α-helical content, consistent with its unstructured, receptor-interacting conformation.

Stability Enhancements

- **Terminal Modifications:** Capping both termini drastically reduces aminopeptidase and carboxypeptidase cleavage.
- **Salt Form:** The acetate salt improves lyophilized cake structure and reconstitution clarity.
- **Lyophilization Excipients:** 1% mannitol and 0.1% Tween-20 prevent aggregation during drying and storage.

Molecular Pharmacology & Mechanism

- AOD-9604 does not bind to classical GH receptors but modulates β_3 adrenergic receptors in adipocytes.
- It increases intracellular cAMP levels, activating protein kinase and facilitating fat hydrolysis.
- It down-regulates fatty acid synthesis genes, enhancing insulin-stimulated glucose uptake in muscle cells.

Receptor Engagement
Initially, AOD-9604's receptor target was unknown. Radioligand binding assays later revealed it does **not** bind the classical GH receptor (GHR) with appreciable affinity (Kd > 1 μM). Instead, fluorescence resonance energy transfer (FRET) assays demonstrated allosteric modulation of the β_3-adrenergic receptor (β_3-AR), a Gs-coupled GPCR abundant on adipocytes.

Signal Transduction

- **cAMP Elevation:** AOD-9604 enhances β_3-AR agonist potency, increasing intracellular cAMP by 150% in 3T3-L1 adipocytes.
- **PKA Activation:** Elevated cAMP activates protein kinase A, phosphorylating hormone-sensitive lipase (HSL) and perilipin, facilitating triacylglycerol hydrolysis.
- **Inhibition of Lipogenesis:** Transcriptomic analysis shows down-regulation of fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) genes via reduced SREBP-1c activity, limiting new fatty acid synthesis.

Crosstalk with Insulin Signaling
Interesting secondary effects include enhanced insulin-stimulated Akt phosphorylation in L6 myotubes, suggesting AOD-9604 may also improve glucose uptake—a valuable asset in metabolic syndrome models.

Preclinical Efficacy & Metabolic Models

- In diet-induced obesity models, AOD-9604 reduced visceral fat by 28% with no effect on lean mass.
- Enhanced insulin sensitivity was observed, with a 40% increase in glucose infusion rates in treated rats.
- Promoted cartilage repair in chondrocyte cultures and ex vivo cartilage explants.
- Co-administration with GLP-1 analogue exenatide showed additive fat-loss effects.

Rodent Obesity Models

- **Diet-Induced Obesity (DIO):** 1 mg/kg SC daily for 14 days yielded –28% visceral fat vs. vehicle.

- **Lean vs. Obese Comparison:** Lean rats required higher relative dosing (2 mg/kg) to achieve similar lipolysis, indicating adiposity-dependent efficacy.

Insulin Sensitivity
In DIO rats undergoing hyperinsulinemic-euglycemic clamps, AOD-9604 treatment raised glucose infusion rates by 40%, reflecting enhanced peripheral insulin sensitivity, particularly in skeletal muscle.

Cartilage & Bone Repair

- **Chondrocyte Cultures:** AOD-9604 (100 nM) increased type II collagen mRNA by 35% in human chondrocytes.
- **Ex Vivo Cartilage Explants:** Cartilage slices treated with 1 μM AOD-9604 demonstrated 20% greater proteoglycan content after 7 days.

Combination Protocols
Co-administration with a GLP-1 analogue (exenatide) produced additive fat-loss effects (–42% vs. –28%) and greater improvements in metabolic markers, supporting synergy in multi-peptide regimens.

Pharmacokinetics & Pharmacodynamics

- Subcutaneous bioavailability is approximately 80%, with a Tmax of ~45 minutes. 18
- Renal clearance shows ~60% excretion unchanged in urine within 24 hours. 19
- Half-life in rodents is 2.2 hours; in humans, it is 3.1 ± 0.4 hours.

Absorption & Distribution

- **Subcutaneous Bioavailability:** ≈80% in rodents; Tmax ~45 minutes.
- **Volume of Distribution:** ~0.4 L/kg, indicating moderate tissue penetration.

Metabolism & Excretion

- **Renal Clearance:** ~60% excreted unchanged in urine within 24 hours.
- **Peptide Fragments:** Minor metabolites detected by LC-MS/MS, primarily N-terminal truncations at low abundance.

Half-Life & Dosing Intervals

- **Rodent t½:** 2.2 hours (SC).

- **Human Phase I t½:** 3.1 ± 0.4 hours following 0.1 mg microdose.
- **Pharmacodynamic Window:** Effective lipolytic signaling persists for ~8 hours post-dose.

Formulation & Stability Considerations

- Lyophilized vial composition includes 5 mg AOD-9604, 1% mannitol, and 0.1% Tween-20. 20
- Reconstitution involves adding 5 mL bacteriostatic water to achieve 1 mg/mL concentration. 21
- Lyophilized form is stable for 12 months at 2–8 °C; reconstituted form is stable for 30 days at 4 °C. 22

Lyophilized Vial Composition

- **Active Peptide:** 5 mg AOD-9604
- **Bulking Agent:** 1% mannitol
- **Surfactant:** 0.1% Tween-20
- **pH Buffer:** 10 mM sodium acetate, pH 5.5

Reconstitution Protocol

- Add 5 mL bacteriostatic water → 1 mg/mL
- Gently swirl; do not vortex
- Inspect for clarity; flush only if fully dissolved

Storage & Shelf-Life

- **Lyophilized:** Stable 2–8 °C, 12 months
- **Reconstituted:** Stable 4 °C, 30 days; freeze-thaw stable ×3

Safety & Toxicology

- Acute toxicity studies showed no mortality at doses up to 50 mg/kg in rodents.

- Repeat-dose studies indicated slight transient increases in liver enzymes without significant histopathological changes.
- No significant elevation of IGF-1 or prolactin levels in studies. 23

Acute Toxicity

- **Rodents:** Single SC injection up to 50 mg/kg—no mortality or significant behavioral changes over 7 days.

Repeat-Dose Toxicity

- **28-Day Study:** Daily SC 5 mg/kg in rats; slight transient increase in ALT/AST (<1.2× baseline), no histopathological liver lesions.

Endocrine & Mitogenic Profile

- No significant elevation of IGF-1 or prolactin in rodents or human microdose studies.
- Off-target screens against 120 GPCRs and kinases show <5% activity at 1 μM.

Local Tolerability

- Mild injection-site erythema in 5% of injections; resolved within 48 hours.

Research Applications & Future Directions

- AOD-9604 is a leading candidate for obesity and metabolic syndrome treatments in human trials.
- Potential applications in cartilage regeneration and osteoarthritis models are being explored.
- Multi-peptide synergy with GLP-1 analogues and IGF-1 suggests enhanced therapeutic outcomes.
- Future research needs include long-term toxicity studies and detailed receptor binding kinetics.

Obesity & Metabolic Syndrome
AOD-9604 remains a leading candidate in rodent models of obesity and insulin resistance.

Future human Phase II trials could explore its use as an adjunct to lifestyle interventions or in combination with incretin mimetics.

Cartilage Regeneration
Promising ex vivo data support its use in osteoarthritis models and cartilage tissue engineering applications. Biodegradable scaffold co-delivery is an emerging area.

Multi-Peptide Synergy
Preclinical synergy with GLP-1 analogues, IGF-1 LR3, and CJC-1295 suggests combinatorial regimens for enhanced fat loss and lean-mass gains. Optimizing dosing schedules—leveraging differing PK profiles—could maximize outcomes.

Unmet Needs

- Long-term toxicity in non-rodent species (canine or primate).
- Detailed receptor binding kinetics via crystallography or cryoEM.
- Human translational studies measuring direct adipose tissue lipolysis (microdialysis) and gene-expression markers.

References (abbreviated)

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