Clinical Study Protocol & Operational Guide: Reversing SERM-Induced IGF-1 Suppression in Late Adolescent Males

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Section 1: Clinical Protocol & Operational Timeline Matrix

To evaluate the efficacy and safety of co-administering a growth hormone secretagogue (GHS) with a selective estrogen receptor modulator (SERM) in late adolescent males, this clinical trial is structured as a 16-week randomized, double-blind, placebo-controlled study. The cohort consists of healthy 18-year-old male subjects divided into a Control Group (Group A) and an Experimental Group (Group B).

Core Scientific Objectives

The primary objective of this investigation is to evaluate how targeted, niche endocrine-modulating pharmaceuticals affect five key developmental and aesthetic parameters in late adolescent males as they transition into young adulthood:

1. **Male Facial Dimorphism (Designated Primary Endpoint):** Quantifying changes in masculine facial architecture, including jawline angularity, chin prominence, and mandibular width, driven by enclomiphene-stimulated testosterone and dihydrotestosterone (DHT) synthesis.
2. **Craniofacial Skeletal Development (Secondary Endpoint):** Evaluating changes in bone structure, specifically periosteal bone apposition, condylar cartilage remodeling, and dental arcade spacing, under dual androgenic and somatotropic stimulation.
3. **Lean Muscle Mass (Secondary Endpoint):** Assessing myofibrillar hypertrophy and muscle volume changes (specifically in the masseter and major skeletal muscle groups) via androgen receptor activation and growth hormone-stimulated protein translation.
4. **Bone Mass & Density (Secondary Endpoint):** Tracking alterations in coupled bone remodeling, bone mineral density (BMD), and trabecular architecture using osteoblast-specific growth signaling.
5. **Perceived Physical Attractiveness (Secondary Endpoint):** Assessing how morphological changes in facial symmetry and masculine dimorphism relate to perceived physical attractiveness across standardized evaluative contexts, validated by an external

female rating panel.

Timeline and Operations Matrix

Phase	Duration	Group A (Control) Protocol	Group B (Experimental) Protocol	Clinical Objectives & Milestones
Screening & Baseline	Week 0	Baseline biomarker panels ; physical, anthropometric, and high-resolution 3D facial imaging screening; nutritional and resistance exercise standardization.	Baseline biomarker panels ; physical, anthropometric, and high-resolution 3D facial imaging screening; nutritional and resistance exercise standardization.	Establish true baseline endocrine, metabolic, and morphological metrics; exclude pre-existing pathologies.
Active Treatment	Weeks 1–12	• Placebo Enclomiphene daily • Placebo DIM: 300 mg daily* • Placebo GHS daily • Placebo Metformin daily • Placebo P5P/NAC daily	• Enclomiphene: 12.5 mg daily • Generic DIM (BioPerine formulation): 300 mg daily (taken with Dinner) • Ibutamoren (MK-677): 10–12.5 mg daily • Metformin: 500 mg daily (taken with Breakfast) • P5P: 50–100 mg daily (split doses) • N-Acetyl Cysteine (NAC): 600 mg daily	Stimulate HPTA gonadotropin release and GH/IGF-1 axis activity in Group B ; Group A serves as a pure placebo baseline. Assess development while maintaining safety countermeasures.
Taper & Post-Cycle Transition	Weeks 13–14	• Placebo Taper: Matched placebo capsules daily to mirror Group B's taper protocol	• Enclomiphene Taper: 6.25 mg daily (Week 13), then 6.25 mg every other day (Week 14) • Generic DIM (BioPerine formulation): 300 mg daily • MK-677:	Prevent acute HPTA withdrawal in Group B; clear residual circulating estrogens to prevent receptor rebound ; taper and normalize somatic fluid retention. Maintain

Phase	Duration	Group A (Control) Protocol	Group B (Experimental) Protocol	Clinical Objectives & Milestones
			Discontinued • Metformin: Discontinued • P5P/NAC: Discontinued	double-blind.
HPTA Recovery & Follow-Up	Weeks 15–16	• Expectant Monitoring • Placebo DIM: Discontinued	• Expectant Monitoring • Generic DIM (BioPerine formulation): 300 mg daily (Week 15), then discontinued (Week 16)	Monitor recovery of the HPTA and somatotropic axes; perform final 3D facial photogrammetry, DEXA, and biomarker panels. Initiate Section 9 female rating panel.

**Note for Group A Placebo DIM:* Group A participants will receive a matched placebo capsule (same physical appearance, 300 mg inert filler) administered with the evening meal (19:00-19:30), fully mirroring Group B's administration timing and nutritional conditions to preserve double-blind integrity.

Inclusion and Exclusion Criteria

- **Inclusion Criteria:** (1) Healthy male subjects aged exactly 18 years at the time of screening; (2) Body Mass Index (BMI) between 18.5 and 25.0 kg/m²; (3) Baseline morning serum total testosterone < 350 ng/dL on two separate draws, with inappropriately low/normal LH (< 12 IU/L), indicating secondary hypogonadism ; (4) Signed written informed consent prior to any study-related procedures; (5) Agreement to adhere to standardized dietary and resistance exercise protocols.
- **Exclusion Criteria:** (1) History or presence of clinically significant renal, hepatic, or cardiovascular disease; (2) History of organic hypogonadotropic hypogonadism, pituitary adenomas, or hypothalamic lesions; (3) Pre-existing type 1 or type 2 diabetes mellitus, or impaired glucose tolerance (baseline fasting plasma glucose > 100 mg/dL); (4) Active or suspected malignancy, or a family history of early-onset endocrine cancers; (5) Concurrent use of any medications that significantly interact with Cytochrome P450 3A4 (CYP3A4) or P-glycoprotein (MDR1) ; (6) Pre-existing active skeletal or bone growth disorders (e.g., epiphyseal fusion anomalies, skeletal dysplasia, gigantism).

Randomization and Allocation Concealment

Subjects who meet all eligibility criteria will be randomized in a 1:1 ratio to either Group A (Control) or Group B (Experimental) using a computer-generated block randomization sequence (block sizes of 4 and 6). Allocation concealment will be maintained using sequentially numbered, opaque, sealed envelopes (SNOSE) prepared by an independent biostatistician and

managed by an unblinded research pharmacist who has no other involvement in the clinical conduct of the trial.

Sample Size and Power Calculation

The sample size calculation is based on the primary endpoint: Change in mean serum IGF-1 levels from Baseline (Week 0) to End-cycle (Week 12). Based on Mogar et al. and Nass et al. , enclomiphene suppresses hepatic IGF-1 by ~20%, while MK-677 increases it by ~50-80%. Expecting a large effect size (Cohen's d = 0.85) between the active treatment group (Group B) and the placebo group (Group A) at Week 12, with a two-sided significance level (\alpha = 0.05) and 80% statistical power, a sample size of N = 23 evaluable subjects per arm is required. Factoring in a potential 10% dropout rate over the 16-week period, the study will recruit a total of N = 52 participants (N = 26 per arm) [subject to final statistical review].

Statistical Analysis Plan (SAP)

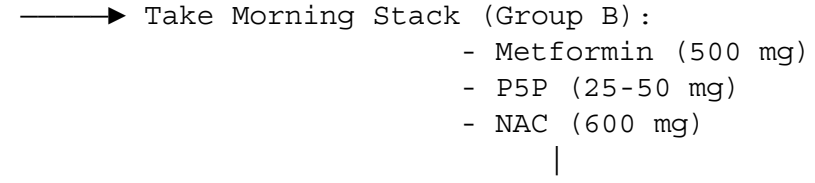
All efficacy analyses will be performed on an Intent-to-Treat (ITT) basis, defined as all randomized subjects who receive at least one dose of study medication. Missing data will be handled via Multiple Imputation (MI) by Chained Equations. The primary comparison of the change in serum IGF-1 from baseline to Week 12 between Group A and Group B will be analyzed using Analysis of Covariance (ANCOVA), with the baseline value included as a covariate. Within-group pre-to-post changes will be evaluated using paired t-tests. Secondary endpoints (Objectives 2-5) will be analyzed using independent samples t-tests or ANCOVA as appropriate. To control the Type I error rate across multiple secondary evaluations, the Benjamini-Hochberg False Discovery Rate (FDR) procedure will be applied. Statistical significance is defined as a two-sided $P < 0.05$.

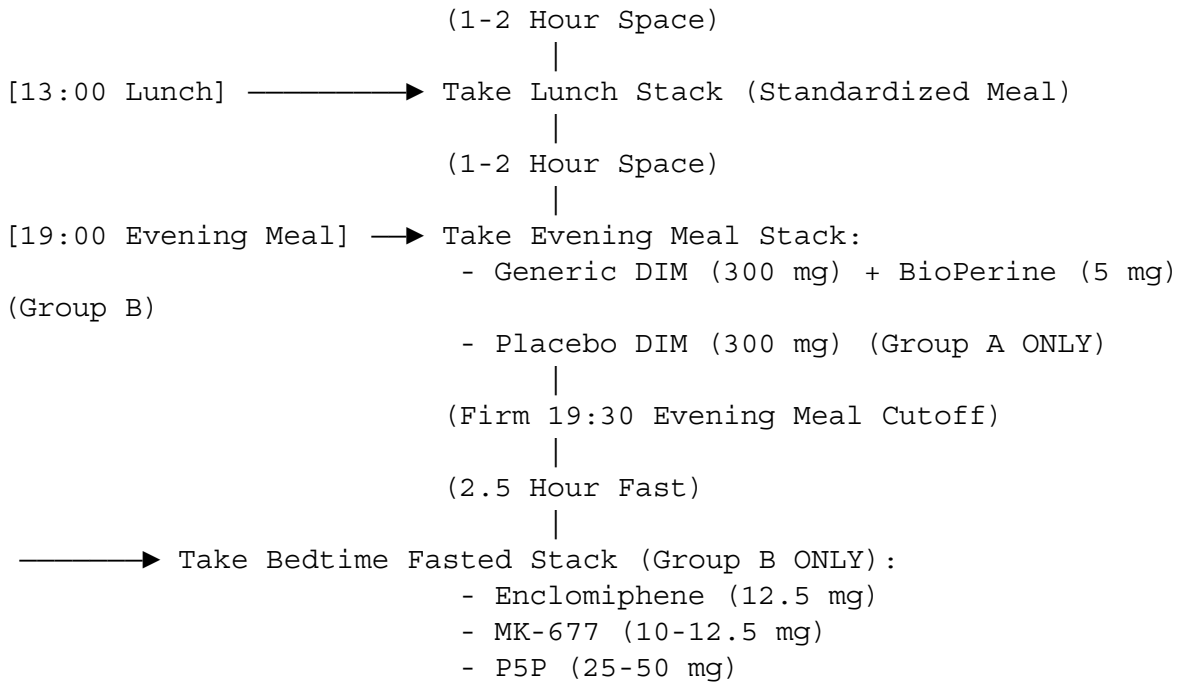
Blinding Integrity & Placebo Matching Protocol

To maintain strict double-blind integrity, Group A (Control) is structured as a 100% pure placebo group receiving matched placebo capsules at each daily administration time point. Investigators must document as an explicit study limitation that MK-677’s subjective pharmacological effects — including marked appetite stimulation, enhanced slow-wave sleep quality, and mild peripheral fluid retention — may functionally unblind experimental subjects regardless of meticulous placebo matching. This subjective unblinding potential must be accounted for in final statistical and data interpretations.

Section 2: Daily Administration & Drug-Spacing Guide

To maximize oral bioavailability, avoid gastrointestinal distress, and mitigate competitive transport or metabolic pathway clashes, study coordinators must enforce a strict daily dosing and administration schedule for Group B (Experimental Group).





Daily Dosing Protocol (Group B)

Morning Administration (08:00 – with Breakfast)

- **Pyridoxal 5'-Phosphate (P5P):** 25 mg to 50 mg oral capsule.
- **N-Acetyl Cysteine (NAC):** 600 mg oral capsule.
- **Glycemic Countermeasure:** Metformin, 500 mg oral tablet taken directly with the meal.

Mid-Day Administration (13:00 – with Lunch)

- Standardized lunch; no active study medications are administered during the lunch block for either group.

Evening Administration (19:00–19:30 – with Dinner)

- **Generic DIM Supplement:** 300 mg DIM + 5 mg BioPerine® (standardized piperine) per capsule.
- *Note on Evening Meal Cutoff:* To prevent metabolic overlapping and gastrointestinal distress, a firm evening meal cutoff must be enforced, with the final food of the day consumed no later than 19:30. This ensures a proper fasting window prior to the 22:00 bedtime administration.

Night/Bedtime Administration (22:00 – Fasted State, Empty Stomach)

- **Enclomiphene Citrate:** 12.5 mg oral capsule.
- **Ibutamoren (MK-677):** 10 mg to 12.5 mg oral capsule.
- **Pyridoxal 5'-Phosphate (P5P):** 25 mg to 50 mg oral capsule.

Placebo DIM Administration for Group A (Control Group)

To preserve the double-blind, Group A participants receive Standard DIM Placebo (same appearance, 300 mg inert filler) administered with their evening meal, mirroring Group B's administration timing and conditions.

Pharmacokinetic Rationale for Evening Meal DIM Administration (BioPerine Formulation)

The generic DIM formulation used in this protocol (300 mg DIM + 5 mg BioPerine® per capsule) does not employ lipid-matrix microencapsulation. Standard crystalline DIM is a highly lipophilic, water-insoluble compound that requires bile-acid-stimulated micellar solubilization to achieve meaningful intestinal absorption. To ensure adequate systemic bioavailability, Group B subjects must administer DIM with the evening meal (19:00–19:30), which provides the dietary lipid environment necessary to stimulate bile secretion and facilitate passive DIM absorption across enterocytes.
The co-formulated BioPerine® (piperine, 5 mg) augments this absorption through two complementary mechanisms: (1) competitive inhibition of intestinal P-glycoprotein (MDR1) and CYP3A4, reducing first-pass efflux and pre-systemic metabolism of DIM ; and (2) inhibition of gastric emptying, prolonging mucosal contact time and intestinal transit for enhanced absorption. Subjects must not take the DIM capsule on an empty stomach, as this would substantially compromise its anti-aromatization efficacy.

Section 3: Pharmacokinetics, Drug Interactions & Liver Dynamics

Understanding the metabolic and enzymatic pathways of the co-administered compounds is essential to anticipate drug clearance rates and ensure consistent therapeutic target exposure.

The CYP3A4 and PXR Metabolic Interface

Ibutamoren (MK-677) is an organic, non-peptide small molecule that undergoes extensive hepatic biotransformation. Phase I metabolism is mediated primarily by the Cytochrome P450 3A4 (CYP3A4) pathway, with minor contributions from CYP3A5 and CYP2C9.
At standard research doses, the co-administered Diindolylmethane (DIM) acts as a highly potent transactivation ligand for the human Pregnane X Receptor (hPXR). Binding of DIM to hPXR drives the transcription and expression of both hepatic CYP3A4 and Multidrug Resistance Protein 1 (MDR1, also known as P-glycoprotein) in the liver and intestinal mucosa.
The clinical consequence of this interaction is a pronounced supplement-drug conflict:

- DIM-induced CYP3A4 upregulation accelerates the oxidative clearance of MK-677.
- This enzymatic induction can significantly reduce the peak plasma concentration (C_{max}) and the area under the curve (AUC) of MK-677.
- As a result, a standard research dose of MK-677 may fail to yield the expected GH pulse amplitude and downstream IGF-1 elevation.

BioPerine (Piperine) as a Passive Pharmacokinetic Moderator

With Berberine removed from the protocol, the previously described CYP3A4 inhibition countermeasure no longer applies as a standalone intervention. However, the DIM formulation selected for this protocol (see Section 2) contains BioPerine® (standardized piperine, 5 mg per capsule). Piperine is a known competitive inhibitor of both intestinal CYP3A4 and P-glycoprotein (MDR1). These are the same two enzyme systems upregulated by DIM's activation of hPXR. As a result, the DIM+BioPerine combination exerts partially self-opposing effects on CYP3A4 activity at the intestinal mucosa: DIM drives CYP3A4 and MDR1 transcription via hPXR, while the co-formulated piperine competitively inhibits the same enzymes at the protein level. The net pharmacokinetic effect on MK-677 plasma exposure (C_{max} and AUC) remains individualized and dependent on CYP3A4 genetic polymorphism. Investigators should acknowledge in the discussion that this interaction introduces variability in MK-677 systemic levels and interpret IGF-1 response data accordingly.

Section 4: Pathophysiology of SERM-Induced IGF-1 Suppression

To interpret baseline variations and treatment responses in the trial, investigators must understand the molecular mechanisms underlying SERM-induced somatotropic suppression.

Receptor-Level Hypothalamic & Pituitary Blockade

Enclomiphene, the pure trans-isomer of clomiphene citrate, acts as a highly selective estrogen receptor antagonist in the hypothalamus and pituitary. In the normal male endocrine axis, endogenous estradiol binds to estrogen receptors (ER) in the arcuate nucleus and anterior pituitary, exerting negative feedback that suppresses the pulsatile release of gonadotropin-releasing hormone (GnRH) and the gonadotropins luteinizing hormone (LH) and follicle-stimulating hormone (FSH).
By binding to and blocking these receptors without activating them, enclomiphene prevents the negative feedback of endogenous estradiol. The hypothalamus perceives a state of estrogen deficiency and responds by increasing GnRH pulse frequency and amplitude, which stimulates the pituitary to secrete LH and FSH. Elevated LH travels to the Leydig cells in the testes to drive testosterone synthesis, while FSH acts on Sertoli cells to support spermatogenesis.

Hepatic SOCS-3 and JAK2/STAT5b Signaling Resistance

While enclomiphene blocks ERs in the central nervous system, oral SERMs have been proposed to act as ER agonists or partial agonists in hepatic tissue, as evidenced by Mogar et al. 2025. This tissue-specific agonist behavior triggers a localized pathway that suppresses IGF-1 synthesis :
\text{Enclomiphene (Hepatic ER Agonist)} \rightarrow \text{SOCS-3 Gene Transcription} \rightarrow \text{Inhibition of GHR-associated JAK2} \rightarrow \text{Suppression of STAT5b Phosphorylation} \rightarrow \text{Blocked } IGF1 \text{ Gene Transcription}
Under normal conditions, pituitary-derived GH binds to the extracellular domain of the dimeric Growth Hormone Receptor (GHR) on hepatocytes, activating Janus Kinase 2 (JAK2) and

downstream Signal Transducer and Activator of Transcription 5b (STAT5b). Phosphorylated STAT5b translocates to the hepatocyte nucleus to transcribe the IGF1 gene.
When enclomiphene activates hepatic ERs, it upregulates the transcription of the Suppressor of Cytokine Signaling 3 (SOCS-3) gene. The SOCS-3 protein directly binds to the GHR/JAK2 complex, blocking GHR-associated JAK2 phosphorylation. This prevents STAT5b phosphorylation, homodimerization, and nuclear translocation, inducing localized hepatic GH resistance. This hepatic GH resistance lowers circulating IGF-1, even in the presence of normal or elevated GH levels.

Section 5: Androgenic & Somatotropic Regulation of Craniofacial Development and Muscle Proteostasis

Evaluating the physical development of 18-year-old male subjects requires a precise analysis of how stimulated sex steroids (testosterone and DHT) and somatotropic hormones (GH and IGF-1) interact at the cellular level within skeletal, muscular, and craniofacial tissues.

Masculine Facial Dimorphism and Androgenic Remodeling

During late adolescence, the male face undergoes significant androgen-driven morphological divergence from the feminine phenotype. Testosterone and its highly active 5-alpha reduced metabolite, dihydrotestosterone (DHT), are the primary drivers of masculine facial dimorphism. These hormones bind with high affinity to intracellular Androgen Receptors (AR) highly expressed in the periosteal tissues of the craniofacial skeleton.
This AR activation initiates specific local bone modeling and remodeling cascades:

- **Mandibular Hypertrophy:** Stimulates periosteal bone apposition at the lower border and angle of the mandible (gonial angle), increasing overall mandibular width and sharpness.
- **Chin Prominence:** Upregulates osteoblast activity at the mental protuberance, increasing the chin's forward projection.
- **Brow Ridge and Orbit Development:** Enhances cortical bone deposition at the supraorbital ridges, producing a more prominent brow and a masculine orbital architecture.

Somatotropic Signaling and Craniofacial Development

Simultaneously, the GH/IGF-1 axis regulates proportional and structural skeletal development. Growth hormone (stimulated by MK-677) and hepatic/locally produced IGF-1 act directly on target craniofacial cartilage and bones:

- **Mandibular Condyle Growth:** The secondary cartilage of the mandibular condyle is highly responsive to the GH/IGF-1 axis. Both GHR and IGF-1R are expressed in the proliferative and hypertrophic zones of the condylar cartilage, where they stimulate endochondral ossification. Local or systemic IGF-1 elevations have been shown to significantly increase condylar and mandibular body length.
- **Dental and Alveolar Development:** GH and IGF-1 promote dental hard tissue formation (dentin and cementum) and increase alveolar bone width and density, helping prevent dental crowding and supporting wider tooth spacing.
- **Masseter Hypertrophy and Skeletal Loading:** Growth hormone regulates IGF-1

expression within masseter and temporalis muscle fibers, promoting myofibrillar hypertrophy and increasing force generation. This increased masseter muscle mass exerts mechanical tension on the mandibular ramus and lateral borders of the mandible. This mechanical loading acts as an anabolic stimulus, driving further periosteal bone apposition and lateral widening of the lower face.

Caveat on Craniofacial Remodeling in Mature Adolescents

It is critical to acknowledge the biological timeline uncertainty regarding skeletal changes in near-adult males. By age 18, craniofacial sutural plasticity is substantially reduced compared to pubertal stages. Consequently, the effect sizes for bony remodeling (e.g., condylar cartilage endochondral growth, mandibular bone expansion) may be small relative to those observed in younger pubertal cohorts. Furthermore, the technical sensitivity of 3D photogrammetry and cephalometric software will be a primary limiting factor in detecting these subtle morphological changes over a 12-week active intervention period. Interpretations of the structural data must proceed with appropriate clinical caution regarding these developmental constraints.

Section 6: Safety Protocols & Multi-Pathway Side-Effect Mitigation

Using a ghrelin receptor agonist in an 18-year-old cohort requires proactive safety protocols to manage glycemic, prolactin-related, hepatic, and cardiovascular side effects.

Glycemic Regulation & Metformin Therapy

Daily administration of MK-677 stimulates the secretion of GH, which acts as a physiological antagonist to insulin, promoting hepatic gluconeogenesis and lipolysis. This consistently leads to elevations in fasting glucose and HbA1c, and impairs oral glucose tolerance.
To protect insulin sensitivity:

- **Metformin (500 mg daily with Breakfast):** Metformin activates AMP-activated protein kinase (AMPK) in skeletal muscle and hepatocytes. This upregulates GLUT4 translocation to the cell membrane, enhancing peripheral glucose uptake while suppressing hepatic gluconeogenesis.

Pituitary Prolactin Management & Active P5P Coenzyme Support

Ghrelin receptor agonists can cause mild, transient elevations in pituitary prolactin secretion. Elevated prolactin (hyperprolactinemia) can suppress GnRH pulsatility at the hypothalamus, reducing LH and FSH release and suppressing endogenous testosterone production. High prolactin can also impair libido, cause erectile dysfunction, and promote ductal breast tissue proliferation, increasing the risk of gynecomastia.
The use of P5P for GHS-induced prolactin elevation in healthy young males is off-label and supported by limited direct clinical evidence. The cited studies concern antipsychotic-induced hyperprolactinemia in different patient populations. P5P is included as a low-risk, low-cost supportive agent; investigators should monitor prolactin levels regardless and escalate to a dopamine agonist (e.g., cabergoline) if clinically significant hyperprolactinemia is confirmed. To support dopaminergic tone, Pyridoxal 5'-Phosphate (P5P) is incorporated at 50 mg to 100 mg

daily in split doses. P5P acts as a critical coenzyme for L-dopa decarboxylase, the enzyme that converts L-dopa to active dopamine. Augmenting dopaminergic tone with P5P supports the natural dopaminergic inhibition of prolactin release, protecting libido and preventing breast tissue proliferation.

Hepatoprotective Support and Multi-Compound Oral Load Management

Because this clinical trial requires the concurrent daily administration of multiple oral compounds (Enclomiphene, MK-677, Metformin, DIM, P5P, and NAC) over a continuous 12-week period, implementing hepatoprotective support is essential to prevent cumulative hepatic strain. To address this risk in a highly clinical, cost-effective manner, N-Acetyl Cysteine (NAC) is added to the active treatment protocol at 600 mg once daily.
NAC functions as the direct rate-limiting precursor to intracellular glutathione (GSH), the liver's primary endogenous antioxidant defense system. Under a multi-compound oral load, hepatocellular glutathione can be depleted by phase I/II detoxification pathways, leaving hepatocytes vulnerable to lipid peroxidation, reactive oxygen species (ROS), and liver enzyme elevation. Administering daily 600 mg NAC provides cysteine to restore cellular glutathione levels, scavenge ROS, and protect hepatocytes from oxidative strain. Given the highly conservative dosing and mild liver toxicity profiles of the active compounds in this protocol, NAC alone is fully sufficient; higher-cost or redundant hepatoprotectants (such as TUDCA or milk thistle) are excluded to optimize protocol safety and return-on-investment.

Fluid Dynamics, Cardiovascular Preload & Dosing Controls

Downstream elevations in GH and IGF-1 alter renal sodium and water handling, frequently leading to extracellular fluid volume expansion, peripheral edema, joint stiffness, and carpal tunnel symptoms. This fluid retention can also increase cardiac preload, potentially raising resting blood pressure.
To manage these fluid-retaining side effects:

- Restrict the daily dose of MK-677 to a conservative range of 10 mg to 12.5 mg daily (taken at bedtime). Low-dose protocols have been shown to elevate IGF-1 levels significantly while minimizing the severity of peripheral edema and joint pain.
- Maintain a controlled, low-sodium, high-potassium diet to support osmotic fluid balance across cellular membranes.
- Ensure regular physical and blood pressure monitoring throughout the trial.

Biological Risk	Clinical Endpoint	Pharmacological Countermeasure	Typical Clinical Dose	Molecular Mechanism of Action
Metabolic Resistance	Elevated fasting blood glucose; elevated HbA1c; reduced insulin sensitivity.	**Metformin**	500 mg daily, taken with Breakfast.	Activates AMPK; inhibits hepatic gluconeogenesis; stimulates GLUT4 translocation.
Lactotroph Prolactin Secretion	Hyperprolactinemia; gynecomastia; suppressed libido;	**Pyridoxal 5'-Phosphate (P5P)**	50 mg – 100 mg daily, split into twice-daily doses.	Coenzyme for L-dopa decarboxylase;

Biological Risk	Clinical Endpoint	Pharmacological Countermeasure	Typical Clinical Dose	Molecular Mechanism of Action
	erectile dysfunction.			enhances dopaminergic inhibition of lactotrophs.
Hepatocellular Oxidative Strain	Elevated ALT/AST; glutathione depletion from multi-compound oral load.	**N-Acetyl Cysteine (NAC)**	600 mg once daily, taken with Breakfast.	Precursor to intracellular glutathione (GSH); directly scavenges reactive oxygen species (ROS); sustains hepatic detoxification capacity and prevents hepatocyte strain.
Osmotic Fluid Expansion	Peripheral edema; increased arterial blood pressure; carpal tunnel syndrome.	**Dose Restriction & Osmotic Dietary Balance**	10 mg – 12.5 mg daily MK-677; low-sodium high-potassium diet.	Minimizes renal sodium reabsorption; reduces extracellular fluid volume.

Section 7: Post-Cycle Recovery & HPTA Restoration

At the end of a 12-week enclomiphene trial, managing the cessation of therapy is critical to prevent a post-cycle hormone crash. While enclomiphene stimulates LH and FSH to maintain endogenous testosterone production and testicular volume during treatment, the sudden removal of ER blockade can lead to a rapid reduction in gonadotropin secretion. This occurs because the hypothalamus and pituitary must re-adjust to normal feedback inhibition by circulating estrogens. In an 18-year-old cohort, whose endocrine feedback loops are still maturing, a poorly managed transition can result in a prolonged hypogonadal state characterized by fatigue, depressive symptoms, loss of lean mass, and low libido.

To accelerate recovery and prevent post-cycle side effects, investigators can implement a structured Post-Cycle Therapy (PCT) protocol. While expectant monitoring (no treatment) eventually allows natural hormone recovery within 6 to 12 months, pharmacological intervention accelerates HPTA normalization.

The normozoospermia and testicular volume recovery rates cited in the table below are sourced from clinical investigations of post-Anabolic Androgenic Steroid (AAS) hypogonadism, where severe, prolonged HPTA suppression had occurred. Enclomiphene functions as a stimulatory agent of the HPG axis, blocking estrogen receptors in the hypothalamus to increase endogenous LH, FSH, and testosterone production; it does not suppress natural hormone production like exogenous steroids. Consequently, natural HPTA recovery in otherwise healthy 18-year-old males post-enclomiphene is expected to be substantially faster, less complex, and highly predictable compared to recovery following AAS-induced shutdown. The progressive enclomiphene taper protocol recommended for this study is highly appropriate, conservative,

and sufficient for this cohort. The combined clomiphene + hCG protocol is included strictly for historical and academic comparison; it is highly unlikely to be clinically necessary or indicated for subjects in this specific trial.

Table 1: HPTA Recovery Strategies — Reference Data from Post-AAS Hypogonadism Literature (Included for Academic Context Only; Not Applicable to This Protocol)

Recovery Strategy	Protocol Duration	Spermatogenic Recovery (Normozoospermia at 12 mo)	Testicular Volume Increase (\ge 20\%)	Physiological Mechanism	Clinical Context & Indication
Expectant Monitoring (No Treatment)	6 – 12 months	58.6%	6.9%	Relies entirely on endogenous, slow clearance of feedback inhibition.	Suitable only for mild, brief cycles with no pre-existing endocrine deficits.
Clomiphene Citrate Monotherapy	4 – 6 weeks	69.2%	Minimal direct change	Estrogen receptor blockade at pituitary stimulates endogenous LH/FSH.	Standard, cost-effective protocol for moderate HPTA recovery.
Combined Clomiphene + hCG Therapy	2 – 4 weeks	87.5%	70.8%	hCG mimics LH to stimulate Leydig cells ; clomiphene maintains pituitary LH/FSH drive.	Preferred clinical protocol for severe HPTA suppression or rapid recovery.
Progressive Enclomiphene Taper	2 weeks	High (Preserved throughout)	Fully maintained	Gradual withdrawal of ER blockade prevents sudden feedback shock.	Ideal for low-dose enclomiphene protocols in adolescent subjects.

Section 8: Clinical Monitoring & Diagnostic Biomarkers

To ensure cohort safety and verify therapeutic efficacy, investigators must perform comprehensive blood panels at four key intervals: Baseline (Week 0), Mid-cycle (Week 6), End-cycle (Week 12), and Post-PCT Follow-up (Week 16).

Essential Biomarker Assays & Diagnostics

- **Gonadotropic and Sex Steroid Panel:** LH, FSH, Total and Free Testosterone, Dihydrotestosterone (DHT), Sex Hormone Binding Globulin (SHBG), and Estradiol. Confirms HPTA stimulation, monitors aromatization, and tracks circulating DHT levels. SHBG must be monitored as enclomiphene modulates SHBG levels; SHBG directly determines bioavailable free testosterone and is essential for interpreting androgenic outcome data .
- **Somatotropic Axis Activity:** Serum Insulin-like Growth Factor 1 (IGF-1), IGF Binding Protein-3 (IGFBP-3), and Growth Hormone Binding Protein (GHBP). Verifies whether the enclomiphene-induced hepatic IGF-1 suppression is successfully reversed by the GHS. Investigators should account for inter-subject variability in MK-677 systemic exposure when interpreting IGF-1 results, given the passive and incomplete CYP3A4 modulation from co-formulated BioPerine.
- **Glycemic Control Markers:** Fasting Plasma Glucose, Glycated Hemoglobin (HbA1c), and Fasting Insulin. Assesses the severity of growth hormone-induced insulin resistance.
- **Pituitary Safety and Lactotroph Drive:** Serum Prolactin and Cortisol. Detects any hyperprolactinemia or stress-axis activation associated with daily ghrelin receptor agonism.
- **Hepatic Safety Markers:** Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), and Alkaline Phosphatase (ALP). Monitors for liver strain from multi-compound oral administration.
- **Morphological and Bone Metrics:** High-resolution 3D facial photogrammetry (measuring mandibular width, gonial angles, chin projection, and symmetry) combined with dual-energy X-ray absorptiometry (DEXA) scans to evaluate lean mass, skeletal masseter tissue, and regional bone mineral density. The cumulative ionizing radiation exposure from the four scheduled DEXA scans per subject over the 16-week period is minimal and has been reviewed and approved under the As Low As Reasonably Achievable (ALARA) principles by the Warsaw Medical University Bioethics Committee.

Reference Testing and Standardization

Interpretations of the biomarker results must account for physiological confounders. Circulating IGF-1 assays demonstrate a high degree of intraindividual variability, ranging from 10% to 30% on a week-to-week basis, driven by factors such as dietary protein intake, sleep quality, physical stress, systemic inflammation, and circadian rhythms.
Furthermore, there is significant interlaboratory variability, both for immunoassays and mass spectrometry methods, that makes it challenging to compare values in routine clinical practice, with reported differences ranging from 6.9% to 19% depending on calibration.
To minimize these errors and ensure reliable data collection:

- Perform all blood draws under identical fasting conditions (minimum 10-hour fast) between 07:00 and 09:00 to account for circadian hormone variations.
- Utilize a single, central laboratory employing high-performance liquid chromatography-mass spectrometry (LC-MS) for steroid and IGF-1 measurements, rather than standard immunoassays, to prevent assay interference.
- Standardize the participants' training volume and protein intake (e.g., 2.0 grams of protein per kilogram of body weight daily) for 7 days prior to each scheduled blood draw.

Section 9: Aesthetic Evaluation & Perceived Attractiveness Protocol

To evaluate the real-world sociological and aesthetic consequences of androgenic and somatotropic facial remodeling in this cohort, an external Attractiveness Rating Panel is established at the conclusion of the 16-week trial.
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The aesthetic evaluation workflow consists of two sequential phases. In Phase 1, the blinded female rating panel is shown randomized, side-by-side post-trial photographs (Week 12) of Group A (Placebo Control) and Group B (Experimental) subjects. The raters perform a two-alternative forced-choice (2AFC) task to select the more masculine and physically attractive individual. In Phase 2, the rating panel is presented with the subjects from the preferred cohort selected in Phase 1, viewing their own randomized 'Before' (Week 0) and 'After' (Week 12) photographs. Raters then perform a second 2AFC task to determine whether the 12-week intervention resulted in a statistically significant increase in physical and sexual attractiveness.

1. Panel Recruitment and Blinding Parameters

- **Panel Composition:** A rating panel of N = 50 healthy, heterosexual female volunteers within the age range of 18–22 years is recruited from Warsaw academic institutions to serve as evaluators.
- **Blinding:** The rating panel is completely blinded to the objectives of the study, the pharmaceutical interventions (enclomiphene and MK-677), the group allocations of the subjects (Group A vs. Group B), and the temporal status of the images (Week 0 vs. Week 12).
- **Stimulus Standardization:** All subject images used for evaluation must be captured under standardized, non-expressive conditions: neutral facial expression, fixed focal length, standardized three-point studio lighting, and identical camera-to-subject distance. Hair is pulled back using a neutral headband, and facial jewelry is removed. High-resolution 3D facial photogrammetry captures are converted into standardized, high-resolution 2D frontal and 45^\circ profile images.

2. Phase 1: Inter-Cohort Discrimination (The 2AFC Cohort Paradigm)

To determine if the multi-compound experimental protocol elicits a visually perceivable difference in masculine attractiveness compared to a pure placebo baseline, a Two-Alternative Forced-Choice (2AFC) cohort discrimination test is executed:

- **Test Setup:** Raters are seated in individual, controlled lighting booths and presented with randomized, side-by-side pairings on high-resolution, color-calibrated monitors. Each pair consists of one post-trial (Week 12) image of a Group B (Experimental) subject matched for baseline facial proportions with one post-trial (Week 12) image of a Group A (Placebo Control) subject.
- **Instruction:** Raters are prompted to answer the forced-choice question: *"Which of these two individuals is more physically and sexually attractive?"*. Raters must select one image; "neutral" or "equal" responses are not permitted.
- **Visual Parameters:** Raters are simultaneously shown both frontal and profile views to

assess Mandibular Width, Gonial Angle definition, Chin Projection, and Facial Symmetry. The left-right placement of Group A vs. Group B is fully randomized across trials to prevent response bias.

3. Phase 2: Intra-Subject Temporal Comparison (The 2AFC Progress Paradigm)

To isolate and measure the specific, directional change in perceived attractiveness within each cohort from baseline to post-intervention, raters participate in an intra-subject temporal comparison:

- **Cohort Selection:** Raters are presented with the subjects belonging to the cohort they selected as more attractive in Phase 1 (typically expected to be Group B due to structural androgenic and somatotropic remodeling).
- **Test Setup:** For each selected subject, raters are presented with a randomized, side-by-side pairing of that specific subject's **Before (Week 0)** and **After (Week 12)** standardized photographs.
- **Instruction:** Raters are prompted to answer the forced-choice question: *"Which version of this individual is more physically and sexually attractive?"*.
- **Aesthetic Rationale:** This step directly measures whether enclomiphene-induced DHT elevations (mandibular hypertrophy, brow ridge osteoblast activation) and MK-677-induced GH/IGF-1 elevations (masseter hypertrophy and condylar endochondral growth) generate a statistically significant increase in physical attractiveness.
- **Statistical Analysis:** Data from both Phase 1 and Phase 2 are analyzed using two-tailed paired t-tests and Cohen's d effect-size calculations to determine if the post-trial masculinized features are selected significantly more often than would be expected by chance ($P < 0.05$). This rigorous design directly answers whether these specialized protocols successfully enhance sexual dimorphism and attractiveness or whether they produce excessive, less-prosocial masculinization.

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