Analysis of a 14-Week Mitochondrial Enhancement Protocol

Introduction

This 14-week protocol is designed to sequentially target mitochondrial health and metabolic function using three interventions: **SS-31**, **MOTS-c**, and **NAD+**. Each compound is introduced in a specific order and time frame (SS-31 at weeks 3–8; MOTS-c at weeks 9–14; NAD+ throughout, ramping up dose by week 5) to optimize their individual benefits and exploit potential synergistic effects. The overarching goal is to improve mitochondrial energy production, enhance metabolic health, and promote longevity-related pathways. Below, we explain the scientific and clinical rationale for:

- **Sequencing of interventions** (why each compound is started in a particular order).
- **Dose escalation** (especially for NAD+, which is increased over the first weeks).
- **Timing** (why each compound is given during specific weeks and not others).
- **Synergistic or complementary effects** of SS-31, MOTS-c, and NAD+ in this schedule.
- **Supporting evidence** from literature on each compound's mechanism of action and benefits for metabolism, energy, and aging.

Week	SS-31	MOTS-c	NAD+
Week 1	-	-	25 mg twice a week
Week 2	-	-	25 mg twice a week
Week 3	4 mg daily	-	50 mg twice a week
Week 4	4 mg daily	-	50 mg twice a week
Week 5	4 mg daily	-	100 mg twice a week
Week 6	4 mg daily	-	100 mg twice a week
Week 7	4 mg daily	-	100 mg twice a week
Week 8	4 mg daily	-	100 mg twice a week
Week 9	-	5 mg three times a week	100 mg twice a week
Week 10	-	5 mg three times a week	100 mg twice a week
Week 11	-	5 mg three times a week	100 mg twice a week
Week 12	-	5 mg three times a week	100 mg twice a week

Week 13	-	5 mg three times a week	100 mg twice a week
Week 14	-	5 mg three times a week	100 mg twice a week

Components of the Protocol and Their Mechanisms

SS-31: Mitochondria-Targeted Peptide (Elamipretide)

Mechanism of Action: SS-31 is a cell-permeable tetrapeptide that selectively concentrates in the inner mitochondrial membrane by binding to *cardiolipin*, a unique phospholipid of that membrane (SS-31 and NMN: Two paths to improve metabolism and function in aged hearts - PMC). By associating with cardiolipin, SS-31 stabilizes the structure of the electron transport chain (ETC) into “supercomplexes,” thereby improving electron transfer efficiency and *preserving ATP production while minimizing reactive oxygen species (ROS) generation* (SS-31 - The International Peptide Society). It essentially acts as a **mitochondria-targeted antioxidant and stabilizer**. SS-31 has been shown to:

- **Scavenge or reduce mitochondrial ROS** and prevent oxidative damage (SS31 and NMN: Two paths to improve metabolism and function in aged hearts - PMC). This protects mitochondria from stress-induced injury (e.g. ischemiareperfusion damage in heart tissue (SS-31 and NMN: Two paths to improve metabolism and function in aged hearts - PMC).

- **Improve mitochondrial bioenergetics:** By modulating the interaction between cytochrome c and cardiolipin, SS-31 promotes efficient electron flux and prevents cytochrome c from behaving as a peroxidase (SS-31 - The International Peptide Society). It also inhibits opening of the mitochondrial permeability transition pore (mPTP), guarding against mitochondrial swelling and apoptosis under stress (SS-31 - The International Peptide Society). The net effect is better energy production (ATP) and less cell death in high-stress conditions.

- **Enhance organ function in aging and disease:** In animal studies, SS-31 reversed age-related mitochondrial dysfunction. For example, 8 weeks of SS-31 treatment in old mice improved heart diastolic function and **reduced cardiac oxidative stress** to more youthful levels (SS-31 and NMN: Two paths to improve metabolism and function in aged hearts - PMC). Similarly, SS-31 increased exercise tolerance in aged mice, indicating improved muscle mitochondrial function ([PDF] SS-31 (also known as Elamipretide®, Bendavia®, and MTP-131)). It has shown benefits in models of kidney disease and muscular

dystrophy, and is under clinical trials for heart failure and macular degeneration (SS-31 - The International Peptide Society). Clinically, SS-31 is explored for **fatigue, cognitive support, and general anti-aging** because of its mitochondrial protective effects (SS-31 - The International Peptide Society) (SS31 - The International Peptide Society).

Usage Considerations: SS-31 is typically given by injection (subcutaneous or intravenous). Notably, prolonged use in humans is not well studied – most human trials have been short (a few weeks). In fact, **continuous treatment beyond 4 weeks has not been formally studied in humans** (SS-31 - The International Peptide Society). This informs the protocol's approach to use SS-31 in a limited 6-week window (weeks 3–8) to capture its benefits while staying within a prudent timeframe.

MOTS-c: Mitochondrial-Derived Peptide

Mechanism of Action: MOTS-c is a 16–amino acid peptide encoded in the mitochondrial 12S rRNA gene (mtDNA) and functions as a **signaling molecule that links mitochondrial status to nuclear metabolism** (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC) (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and AgeRelated Diseases - PMC). It is part of the cell's adaptive response to metabolic stress. Key actions of MOTS-c include:

- **Activation of AMPK Pathway:** MOTS-c interferes with the folate cycle, causing accumulation of AICAR, which in turn **activates AMPK (AMP-activated protein kinase)** (Exogenous humanin and MOTS-c function as protective agents ...). AMPK is a master energy sensor that enhances glucose uptake, fatty-acid oxidation, and mitochondrial biogenesis while inhibiting anabolic processes that consume energy. Through AMPK activation, MOTS-c improves insulin sensitivity and cellular stress resistance (MOTS-c interacts synergistically with exercise intervention to ...). This mimics some effects of exercise or calorie restriction at the cellular level.

- **Modulation of Metabolic Genes:** MOTS-c can translocate to the nucleus and regulate genes involved in metabolism and proteostasis (MOTS-c is an exerciseinduced mitochondrial-encoded regulator of age-dependent physical decline and muscle homeostasis | Nature Communications). It has "retrograde" signaling effects, meaning mitochondria (via MOTS-c) communicate with the nucleus to adjust metabolism. For example, MOTS-c influences the **folate/methionine cycle**, effectively putting cells in a state similar to methionine restriction (MOTSc, the Most Recent Mitochondrial Derived Peptide in Human Aging and AgeRelated Diseases - PMC). Methionine restriction is known to extend lifespan in mammals, and MOTS-c's inhibition of methionine metabolism

is thought to contribute to longevity signals (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC) (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC).

- **Raising NAD+ and Engaging Sirtuins:** Endogenously, MOTS-c has been found to **elevate intracellular NAD+ levels and act via SIRT1** (a NAD-dependent deacetylase) to exert some of its metabolic effects (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC) (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC). In other words, MOTS-c triggers a cascade where increased NAD+ activates sirtuin pathways (like SIRT1) that enhance glycolysis and other metabolic processes. This is important because it links MOTS-c's action to known longevity regulators (AMPK and sirtuins).

- **Anti-Inflammatory and Stress Protection:** Research indicates MOTS-c can reduce inflammatory signaling (e.g. lowering IL-6 levels) and enhance antioxidant responses via Nrf2 activation (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC). This can protect tissues from age-related inflammation and oxidative damage. There is even evidence that MOTS-c improves skin collagen and health by reducing inflammation-driven collagen breakdown (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC), suggesting multi-organ benefits.

Physiological/Clinical Effects: Levels of MOTS-c naturally decline with age – elderly individuals have about 20% lower MOTS-c levels than young adults (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC). Supplementing MOTS-c may therefore restore a youthful signaling environment. In animal studies, **MOTS-c has shown remarkable benefits for metabolic health and physical performance**. In mice on a high-fat diet, MOTS-c prevented obesity and insulin resistance by boosting energy expenditure and glucose utilization (The Mitochondrial-Derived Peptide MOTS-c Promotes Metabolic ...). Impressively, MOTS-c administration in older mice (22–23 months old) **significantly enhanced physical performance and health span**; intermittent MOTS-c (5 mg/kg thrice weekly) started late in life improved muscle endurance and delayed age-related frailty (). In fact, a *Nature Communications* study reported that MOTS-c treatment increased running capacity in young, middle-aged, and old mice alike, and when started in late life, it improved overall **physical capacity and markers of health span** (MOTS-c is an exercise-induced mitochondrial-encoded regulator of age-dependent physical decline and muscle homeostasis | Nature Communications). These findings underline MOTSc's potential as a longevity and fitness-promoting intervention. Clinically, while human trials

are nascent, the fact that **exercise naturally increases MOTS-c expression in muscle and blood** (MOTS-c is an exercise-induced mitochondrial-encoded regulator of age-dependent physical decline and muscle homeostasis | Nature Communications) suggests it is a safe, evolutionarily conserved pathway to target for metabolic rejuvenation.

NAD+ Supplementation: Nicotinamide Adenine Dinucleotide Repletion

Role in Cells: NAD+ is a vital coenzyme found in all cells, acting as an electron carrier in redox reactions (central to metabolism) and as a substrate for signaling enzymes (sirtuins, PARPs, etc.). NAD+ levels tend to **diminish with age**, due to factors like reduced synthesis and increased consumption (for DNA repair or inflammation) (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and AgeRelated Diseases - PMC). This decline in NAD+ is linked to metabolic sluggishness, impaired DNA repair, and lower stress resistance in aging cells (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC) (FAQs About NAD+ Infusions - Warren J. Bleiweiss, MD PA). Therefore, restoring NAD+ availability is a key strategy in age-related metabolic intervention. Maintaining or boosting NAD+ can *activate longevity pathways* and improve cell survival: NAD+ is a potent activator of sirtuin enzymes (e.g. SIRT1, SIRT3) which regulate energy metabolism, stress responses, and genomic stability (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC). Higher NAD+ levels are associated with improved mitochondrial function, because NAD-dependent deacetylases like SIRT3 enhance the efficiency of the TCA cycle and ETC by deacetylating metabolic enzymes.

Supplementation Benefits: Augmenting NAD+ (commonly via precursors like NMN or NR, or direct NAD+ IV therapy) has shown broad anti-aging and metabolic benefits in research. In mouse models, NAD+ precursor supplementation *increased NAD+ levels and improved many age-related markers*, including better insulin sensitivity, reduced inflammation, and enhanced mitochondrial function (SS-31 and NMN: Two paths to improve metabolism and function in aged hearts - PMC). One study noted that raising NAD+ in old mice **bolstered mitochondrial energetic capacity and even partially reversed cardiac dysfunction** in heart failure models (SS-31 and NMN: Two paths to improve metabolism and function in aged hearts - PMC). At the cellular level, more NAD+ means more substrate for ATP production and more activation of sirtuins that promote **DNA repair and mitochondrial biogenesis**. For instance, SIRT1 activation by NAD+ can deacetylate PGC-1α, the master regulator of mitochondrial biogenesis, leading to the creation of new mitochondria and improved muscle endurance. Clinically, NAD+ infusions have been used to combat fatigue, improve mental clarity, and assist in metabolic disorders. Patients often report increased energy and concentration after NAD replenishment, likely due to enhanced neuronal metabolism and neurotransmitter synthesis (FAQs About NAD+ Infusions - Warren J. Bleiweiss, MD PA). NAD+ therapy is also reported to support healthy brain function and DNA repair mechanisms (FAQs About NAD+ Infusions - Warren J. Bleiweiss, MD PA).

Dose and Administration: In this protocol, NAD+ is administered via injection/infusion **twice weekly**, starting at 25 mg per dose and increasing to 100 mg per dose by week 5. This gradual escalation is done for both efficacy and tolerability. High-dose NAD+ infusions can cause transient side effects (due to rapid cellular NAD+ uptake), such as flushing, chest tightness, nausea, or cramping if given too fast (FAQs About NAD+ Infusions - Warren J. Bleiweiss, MD PA). By starting at a lower dose and ramping up, the body adapts to the increased NAD+ pool and the risk of acute side effects is minimized. Additionally, a slower build allows observation of how the patient responds and ensures NAD+ is being effectively utilized (excess NAD+ too fast might be wasted or cause more NADH conversion). Once at 100 mg twice weekly (by week 5 and onward), tissues are consistently saturated with NAD+ to support the downstream interventions (SS-31 and MOTS-c).

Rationale for the Sequential 14-Week Schedule

1. Sequence of Introducing Each Compound

The order of this protocol is **designed to first lay a metabolic foundation, then repair mitochondrial function, and finally enhance metabolic signaling**:

- **Weeks 1–2 (Foundation Phase): NAD+ Loading –** The program begins by increasing NAD+ levels when no other interventions are yet in play. Elevating NAD+ early ensures that cells have ample metabolic cofactor availability and activated sirtuin pathways from the outset. This is akin to “fuel priming”: higher NAD+ readies the mitochondria and other cellular processes for improvement. By the end of the second week, NAD+ stores are on the rise (25 mg → 50 mg dosing), which supports energy metabolism and cell repair processes. Starting NAD+ alone also allows the patient to acclimate to NAD+ therapy gradually, without confounding effects from other compounds.

- **Weeks 3–8 (Mitochondrial Repair Phase): Introduction of SS-31 –** After a couple of weeks of NAD+ support, **SS-31 is introduced in week 3** (at 4 mg daily, a moderate dose) and continued through week 8. NAD+ therapy continues concurrently (reaching the full 100 mg biweekly dose by week 5). The rationale is that by week 3, NAD+-dependent enzymes (like SIRT1, SIRT3) are already more active, and mitochondrial metabolism is revving up. Adding SS-31 at this point directly targets the mitochondria to **repair and enhance their function**: SS-31 will reduce mitochondrial ROS and stabilize ETC function right when the mitochondrial substrate (NAD+) is plentiful. This one-two combination is thought to be highly synergistic – NAD+ provides the raw material for energy production while SS-31 improves the efficiency and safety (less oxidative stress) of that energy production. Indeed, research in aged mice showed that a NAD+ precursor

(NMN) and SS-31 each improved different aspects of mitochondrial function, and **combined treatment had a synergistic rejuvenating effect on heart metabolism and energetics** (SS-31 and NMN: Two paths to improve metabolism and function in aged hearts - PMC). By sequencing NAD+ first and then SS-31, the protocol aims to mimic this synergy: NAD+ addressing systemic metabolic needs and SS-31 reinforcing the mitochondrial engine.

Clinical logic: SS-31 is given for a defined 6-week period in this phase to maximize mitochondrial repair without long-term exposure. As noted, prolonged SS-31 therapy hasn't been widely tested in humans (SS-31 - The International Peptide Society), so limiting it to weeks 3–8 is a cautious approach. Six weeks is within the range used in animal studies (often 4–8 weeks) that achieved significant improvements (SS-31 and NMN: Two paths to improve metabolism and function in aged hearts - PMC). By week 8, we expect mitochondrial function (in terms of ATP production capacity and oxidative stress markers) to be much improved. This sets the stage for the next phase.

- **Weeks 9–14 (Metabolic Enhancement Phase): Introduction of MOTS-c –** Starting in week 9, SS-31 is discontinued and **MOTS-c injections (5 mg, 3× per week) commence**, continuing through week 14. NAD+ at 100 mg twice/week is maintained throughout this period as well. The timing here deliberately staggers MOTS-c after SS-31, rather than overlapping them, for several reasons: (1) It avoids overloading the body with too many new agents at once – each compound's effects can unfold fully. (2) By week 9, the mitochondria have been "revitalized" by SS-31; they are now ready to respond to MOTS-c's metabolic push. MOTS-c will activate AMPK and spur the cells to take up nutrients and possibly grow new mitochondria (via AMPK→PGC-1α). Having healthier mitochondria (thanks to SS-31) means the tissues can better capitalize on this MOTS-c-driven metabolic boost. (3) Practically, **SS-31 and MOTS-c target overlapping domains (mitochondria and metabolism) but in different ways – separating their administration into two phases ensures each can be evaluated and adjusted without interference**. If everything were given concurrently, one might not know which agent is responsible for which effect (or side effect). Sequential use is a more controlled, stepwise enhancement of the body's energy systems.

  During the MOTS-c phase, NAD+ remains high, which is strategically important. As mentioned, MOTS-c's benefits partly rely on SIRT1 and NAD+ (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC) (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC); by supplying NAD+ exogenously, we ensure that SIRT1 and related pathways are fully operational to complement AMPK activation. Additionally, while MOTS-c itself can raise NAD+, older individuals might have impaired NAD+ synthetic capacity – so continuous NAD+ support guarantees that this is not a limiting factor. Essentially, **NAD+ and**

MOTS-c are co-therapies from week 9 onward, driving metabolism from two sides (cofactor and signaling). The sequence of SS-31 first, then MOTS-c, mirrors a logical progression: *first repair the "engine" (mitochondria), then step on the "gas pedal" (AMPK/metabolic activation).*

In summary, the sequence is NAD+ (base support) → SS-31 (mitochondrial repair) → MOTS-c (metabolic activation). This order is chosen to build one intervention upon the success of the previous one, creating a cumulative enhancement:

- **NAD+** primes cells and prevents a metabolic bottleneck (so that ensuing interventions aren't limited by low NAD).

- **SS-31** fixes mitochondrial inefficiencies and damage, ensuring the cell's powerhouses are ready.

- **MOTS-c** then pushes the system to a higher performance state (in glucose handling, endurance, etc.) using those tuned-up mitochondria.

An alternate sequence (for example, MOTS-c before SS-31) might not be as effective – if one were to activate metabolism (AMPK) first in an environment with suboptimal mitochondria, it could be like revving a broken engine, potentially producing excess ROS or simply not achieving the desired energy output. The chosen progression avoids that by strengthening mitochondria first. This stepwise strategy is supported conceptually by the peptide community, where it's common to "run SS-31 prior to MOTS-c" for low energy conditions, as anecdotal reports suggest better outcomes with that order (likely due to the reasoning above).

2. Rationale for NAD+ Dosage Increases

The protocol specifies NAD+ infusions/injections starting at 25 mg twice per week, ramping up to 50 mg and ultimately **100 mg twice weekly by week 5**, which is then sustained. There are a few reasons for this graduated dosing:

- **Tolerance and Safety:** NAD+ infusions can cause dose-dependent effects. Patients receiving high-dose NAD+ too quickly often report transient symptoms like flushing, nausea, lightheadedness, or chest tightness as cells rapidly metabolize NAD+ (FAQs About NAD+ Infusions - Warren J. Bleiweiss, MD PA). By beginning at a lower dose (25 mg) for the first two weeks, the infusion can be done more slowly and the body can acclimate. The dose is then doubled (to 50 mg) and again to 100 mg as tolerance builds. This mitigates side effects – for instance, if mild nausea occurred at 25 mg, the medical team can ensure infusion speed and hydration are managed before proceeding to higher doses. By week 5, most individuals tolerate 100 mg NAD+ comfortably. Essentially, **a slow**

rampup allows safe achievement of a high NAD+ level that might not be tolerated if given outright at the start.

- **Optimal NAD+ Utilization:** There may be diminishing returns if one floods the system with a very high dose initially. Cells regulate their NAD+/NADH balance; a sudden large dose might lead to rapid conversion to NADH or excretion of excess NAD+ metabolites if the cells are not yet prepared to use it. By increasing gradually, we ensure that enzymes like NAD-dependent deacetylases and mitochondrial dehydrogenases are being upregulated in parallel and can utilize the increasing NAD+. This stepwise increase could potentially induce the expression of NAD+-processing enzymes (e.g., NAMPT in the salvage pathway,

  or sirtuins) such that by week 5 the high dose NAD+ is effectively enhancing cellular function rather than wasted.

- **Benchmarking Effects:** From a clinical standpoint, starting low and increasing also allows observation of the patient's response to NAD+ at different levels. NAD+ can improve energy and mental clarity (FAQs About NAD+ Infusions - Warren J. Bleiweiss, MD PA); by week 3 or 4, one can assess if those benefits are emerging, and then see if they further improve at 100 mg. If not, one might reconsider the necessity of the highest dose. But typically, higher doses correlate with more sustained NAD+ elevation in tissues, which is desired for maximal synergy with SS-31 and MOTS-c.

In summary, the NAD+ escalation is a prudent strategy to **ensure patient comfort and safety, while steadily achieving a therapeutically effective NAD+ level** by the time SS-31 is in full swing. Once at 100 mg twice weekly (from week 5 onward), that dosage is maintained because it represents a plateau where NAD+ stores are consistently repleted (there's no further increase beyond 100 mg in this protocol, as that is considered a high but manageable dose for long-term weekly therapy).

3. Timing of Each Compound (Why Specific Weeks)

Each compound is confined to specific weeks to **align with its role and avoid overlapping redundancies or unknown interactions**:

- **SS-31 (Weeks 3–8 only):** A 6-week window was chosen for SS-31 to accomplish mitochondrial restoration without continuous long-term use. Studies in animals often use 4–8 weeks of SS-31 to achieve benefits (SS-31 and NMN: Two paths to improve metabolism and function in aged hearts - PMC), after which returns may plateau. Clinically, since safety beyond 4 weeks isn't well documented (SS-

31 - The International Peptide Society), capping SS-31 at 6 weeks is a balanced approach to get significant effect while limiting exposure. Another reason to stop SS-31 before week 9 is to hand off the baton to MOTS-c rather than giving both together. By week 9, we assume SS-31 has done its job (mitochondrial ROS reduced, membranes stabilized, ATP output improved). Continuing it further might not add much additional benefit, whereas introducing a **new stimulus (MOTS-c)** can drive further improvements through a different mechanism (AMPK). It also prevents the need for daily SS-31 injections beyond 6 weeks, which improves patient compliance and reduces injection fatigue.

- **MOTS-c (Weeks 9–14 only):** MOTS-c is started later and limited to the final 6 weeks in part to **observe its isolated effects** after an SS-31 phase. By not starting MOTS-c earlier, we avoid any potential negative feedback where two mitochondrial-targeted interventions overlap. Additionally, MOTS-c's benefits on physical capacity were demonstrated even in *short timeframes (2–6 weeks)* in animal studies (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC), so a 6-week course is expected to be sufficient to elicit improvements in metabolic health (like better glucose tolerance or stamina). There isn't a strong rationale to extend MOTS-c much longer in this cycle, as the goal is to trigger metabolic reprogramming during this phase. After 6 weeks of MOTS-c, one can stop and potentially cycle off, since prolonged continuous stimulation of AMPK might eventually lead to adaptation (just as constant exercise without rest can lead to plateau). Another practical reason for MOTS-c's timing is injection frequency – it's given 3x/week, which in combination with twice-weekly NAD+ is manageable. If we attempted to, say, also give SS-31 in that period, the number of injections per week would be quite high. So, separating SS-31 and MOTS-c into different halves of the protocol keeps the regimen reasonable for the patient.

- **NAD+ (Weeks 1–14 continuous):** NAD+ is the only component that spans the entire 14 weeks (and beyond, one could continue NAD+ maintenance). This is because NAD+ repletion is fundamentally a *supportive therapy* – it's not something the body will become "resistant" to in the short term, and it underpins the success of the other two interventions. By keeping NAD+ high throughout, we ensure that at no point are improvements limited by a shortage of this crucial molecule. Both SS-31 and MOTS-c rely on robust mitochondrial function which is bolstered by NAD+. For example, NAD+ is needed for mitochondrial dehydrogenase enzymes to generate NADH for ATP production; as SS-31 makes that ATP generation more efficient, NAD+ must be available to fuel it. Likewise, when MOTS-c activates AMPK and cells start oxidizing more fuel,

NAD+ turnover increases, so continuing NAD+ therapy prevents a drop-off in NAD+ that could otherwise occur. In essence, NAD+ is the *through-line* of the entire 14-week schedule, ensuring consistency and continuity in metabolic support while the other, more targeted therapies are cycled in and out.

In sum, each compound's schedule is chosen to **maximize its unique benefits and then make room for the next agent**. The non-overlap of SS-31 and MOTS-c avoids redundancy (both affect mitochondria, but one via structural support and the other via signaling). This sequential approach also mirrors potential clinical staging: address mitochondrial quality first (weeks 1–8), then address metabolic control and signaling (weeks 9–14), all the while maintaining NAD+ levels as a backbone. Such timing is expected to yield a stronger cumulative effect than if any single agent was used alone for 14 weeks, or if all were started simultaneously from day 1.

4. Synergistic and Complementary Effects of the Combined Schedule

One of the compelling reasons behind this protocol design is the **synergy** between NAD+, SS-31, and MOTS-c. Each of these compounds works on different but interconnected aspects of cellular metabolism, and their effects can reinforce each other when timed properly. Here's how they complement one another:

- **SS-31 and NAD+ (Mitochondrial Synergy):** SS-31's mitochondrial improvements and NAD+ supplementation go hand-in-hand. SS-31 preserves the function of the electron transport chain and reduces ROS damage, which means mitochondria can generate ATP more efficiently (SS-31 - The International Peptide Society). However, mitochondria also require NADH (the reduced form of NAD+) as the fuel for the ETC. By keeping NAD+ levels high, we ensure a steady supply of NADH through metabolic reactions. In turn, because SS-31 reduces oxidative damage, cells are less likely to divert NAD+ into PARP enzymes for DNA repair of ROS-induced damage – thus **NAD+ pools may be conserved and directed towards productive metabolism**. In a study on aged hearts, combining an NAD+ booster (NMN) with SS-31 produced a **synergistic rejuvenation of mitochondrial function**, improving energy metabolites more than either alone (SS-31 and NMN: Two paths to improve metabolism and function in aged hearts - PMC). The combination increased steady-state NADH/NAD+ levels in heart tissue and restored youthful energy dynamics (SS31 and NMN: Two paths to improve metabolism and function in aged hearts - PMC). This suggests that NAD+ and SS-31 together create a positive feedback: NAD+ fuels the mitochondria, and SS-31 ensures that fuel is used efficiently with minimal "waste" (ROS or energy leak). Clinically, patients might experience this synergy as improved endurance and recovery: NAD+ gives cells more immediate energy and SS-31 increases the *output per unit NADH*, so to speak.

- **MOTS-c and NAD+ (Metabolic Synergy):** Once SS-31 has done its part, MOTS-c enters and brings another layer of synergy with NAD+. MOTS-c activates AMPK, which not only helps burn fuels but also has a known relationship with NAD+ metabolism. AMPK activation can increase NAD+ availability by upregulating NAD+ biosynthesis (via the NAMPT enzyme) (Potential Synergistic Supplementation of NAD+ Promoting Compounds as a Strategy for Increasing Healthspan), and it also requires adequate NAD+ to activate SIRT1 fully (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC). The **AMPK–SIRT1 axis** is a well-known positive feedback loop in promoting metabolic health and longevity (Potential Synergistic Supplementation of NAD+ Promoting Compounds as a Strategy for Increasing Healthspan). By providing NAD+ from the outside, we amplify this loop: MOTS-c triggers AMPK, AMPK helps boost NAD+, and high NAD+ in turn activates sirtuins (like SIRT1 and SIRT3) which complement AMPK's effects (e.g., by deacetylating and activating the same metabolic targets, and by improving mitochondrial biogenesis). Indeed, it was observed that **MOTSc elevates NAD+ and relies on SIRT1 for its full metabolic benefits** (MOTSc, the Most Recent Mitochondrial Derived Peptide in Human Aging and AgeRelated Diseases - PMC) (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC). In our protocol, NAD+ levels are already elevated, so when MOTS-c is given, SIRT1 is primed and ready – this could mean a stronger effect on, say, stimulating glycolysis or increasing insulin sensitivity than MOTS-c alone would achieve. One can consider NAD+ as *fuel for the fire* that MOTS-c ignites; it keeps the beneficial metabolic fire burning brighter and longer. For example, if MOTS-c encourages muscles to uptake glucose and burn it, NAD+ is there to ensure glycolytic and TCA cycle enzymes have their necessary cofactor. Additionally, both NAD+ and MOTS-c fight age-related metabolic decline: NAD+ by restoring youthful enzyme activity, and MOTS-c by mimicking exercise and nutrient restriction signals. Their combination thus addresses metabolic health on multiple fronts (nutrient utilization, hormone sensitivity, and gene activation).

- **SS-31 and MOTS-c (Sequential Complementarity):** Although SS-31 and MOTS-c are not given at the exact same time in this schedule, their **effects are meant to complement across the transition**. By improving mitochondrial capacity first, SS-31 likely enhances the responsiveness to MOTS-c. When MOTS-c activates AMPK, cells will demand more from mitochondria (more ATP from fat and glucose oxidation). Thanks to the prior SS-31 phase, mitochondria can meet this demand with higher efficiency and resilience. This could translate to greater improvements in endurance or metabolic rate when MOTS-c is introduced than if MOTS-c were used alone on unoptimized mitochondria. Moreover, SS-31 may reduce baseline oxidative stress and inflammation (it even showed reduced inflammatory cytokines in some models (SS-31 - The

International Peptide Society)), creating a cellular environment in which AMPK activation (by MOTS-c) can do its job with less hindrance. High chronic ROS or inflammation can blunt insulin signaling and AMPK's efficacy; by lowering those, SS-31 indirectly ensures **AMPK/MOTS-c signaling faces less resistance**. There is also an interesting interplay: MOTS-c in the long term can promote mitochondrial biogenesis (new mitochondria creation via nuclear gene upregulation). The quality of those new mitochondria could be higher if SS-31 had earlier fortified the mitochondrial membranes (perhaps through cardiolipin stabilization that might carry over as mitochondria replicate). While direct studies of SS-31+MOTS-c are lacking, we do know MOTS-c worked *synergistically with exercise* in studies (MOTS-c interacts synergistically with exercise intervention to ...) – exercise also improves mitochondrial function, not unlike SS-31's effect. This hints that combining MOTS-c with a prior mitochondrial enhancement (whether exercise or SS-31) yields better outcomes than either alone.

- **Whole-Protocol Synergy (Big Picture):** All three interventions together target the so-called "mitochondrial triangle" of healthy aging: *mitochondrial function, metabolic signaling, and cellular maintenance*. NAD+ is feeding the system and activating sirtuins (which improve **DNA repair and mitochondrial gene expression**); SS-31 is directly nurturing the mitochondria (improving **energy output and reducing damage**); MOTS-c is reprogramming metabolic pathways (enhancing **glucose/fat usage and stress resistance via AMPK**). Each pillar supports the others: improved mitochondrial function from SS-31 means more ATP and less damage, which helps cells respond to NAD+ and AMPK signals; high NAD+ enables both SS-31 and MOTS-c to fully activate their downstream pathways (e.g. SIRT3 in mitochondria for SS-31's outcomes, SIRT1 for MOTSc's outcomes); MOTS-c ensures that the metabolic gains translate into wholebody effects like better muscle function and insulin sensitivity, cementing the benefits from a systemic perspective. The expected result is a **multiplicative effect** – for instance, a study on old mice showed that combining NAD+ augmentation and SS-31 **"best recapitulates the young state" of metabolism, more than either alone** (SS-31 and NMN: Two paths to improve metabolism and function in aged hearts - PMC). We are extending that philosophy by adding MOTS-c to address aspects that neither NAD+ nor SS-31 alone target (such as upregulating antioxidant genes via Nrf2, or mimicking calorie restriction's gene expression effects). Overall, the schedule is built so that **each compound amplifies the efficacy of the others**, either concurrently (as with NAD+ for both other agents) or sequentially (SS-31 paving the way for MOTS-c).

5. Supporting Evidence for this Progression and Expected Outcomes

Each step of this protocol is grounded in scientific findings, and together they align with strategies known to improve metabolic health, energy production, and longevity markers:

- **Mitochondrial Function Improvement:** By mid-protocol (end of week 8), with NAD+ at high levels and SS-31 therapy completed, one would expect markedly improved mitochondrial metrics. In animal models of aging, mitochondrialtargeted interventions like SS-31 restore cellular energy reserves and reduce oxidative damage (SS-31 and NMN: Two paths to improve metabolism and function in aged hearts - PMC). For example, aged mice treated with SS-31 showed their muscle endurance increase to levels comparable to younger mice ([PDF] SS-31 (also known as Elamipretide®, Bendavia®, and MTP-131)). We anticipate similar outcomes: increased ATP production capacity, reduced muscle fatigue, and lower biomarkers of oxidative stress (e.g., decreased lipid peroxidation or lower blood levels of ROS byproducts). A healthier mitochondrial population sets the stage for better overall metabolism.

- **Metabolic Health and Insulin Sensitivity:** The introduction of MOTS-c (weeks 9–14) directly targets metabolic health. Supporting literature shows MOTS-c improves insulin sensitivity and glucose utilization; in mice on high-fat diets, it lowered blood glucose and insulin levels, preventing diabetes (MitochondrialEncoded Peptide MOTS-c, Diabetes, and Aging ...). During the MOTS-c phase (with ongoing NAD+), one would expect improved glycemic control, possibly reflected in lower fasting glucose or improved glucose tolerance tests, and enhanced fatty acid oxidation leading to reduction in visceral fat. MOTS-c also shares mechanisms with known lifespan-extending interventions (AMPK activation like exercise, methionine restriction mimicry) (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC), so we anticipate beneficial changes such as reduced inflammation (e.g., lower TNF-α, IL-6 – which SS-31 and MOTS-c both address), improved lipid profiles, and maybe slight weight loss or muscle gain depending on the individual's baseline. If the person had "low energy and brain fog" (as mentioned in some contexts), by the end of 14 weeks the combined effect of mitochondrial rejuvenation and metabolic re-tuning should significantly alleviate those symptoms – higher NAD+ and ATP availability in the brain can improve cognitive function, while MOTS-c and NAD+ both promote neuronal health (NAD+ aids neurotransmitter synthesis and neuroprotection (FAQs About NAD+ Infusions - Warren J. Bleiweiss, MD PA), MOTS-c may activate protective stress responses in the brain as well (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC)).

- **Longevity Pathways Activation:** All three compounds influence pathways linked to longevity. Over 14 weeks, we expect to see upregulation of **SIRT1/3, AMPK, and possibly a reduction in mTOR activity**, which together are a classic signature of life-extension interventions (similar to fasting or exercise physiology). NAD+ is the sirtuin fuel; MOTS-c activates AMPK and indirectly

SIRT1; SS-31 by reducing damage may decrease chronic mTOR-activating stress signals. The protocol's staggered nature also touches on hormesis – a concept where timed stress or stimulation yields stronger long-term adaptation. MOTS-c's intermittent dosing (thrice weekly) is itself a hormetic approach, as seen in the mouse study where **intermittent late-life MOTS-c increased healthspan** (MOTS-c is an exercise-induced mitochondrial-encoded regulator of age-dependent physical decline and muscle homeostasis | Nature Communications). A tangible outcome might be improved markers of biological age: for instance, decreased inflammation, improved mitochondrial DNA integrity, or increased expression of antioxidant enzymes (MOTS-c has been shown to boost cellular antioxidants via Nrf2 (The Mitochondrial-Derived Peptide MOTS-c Alleviates Radiation ...)). While 14 weeks is relatively short in terms of measuring lifespan changes, the changes at cellular levels (higher NAD+, lower oxidative damage, activated stress response genes) all trend towards an *anti-aging effect*.

- **Clinical Observations and Data:** Although formal clinical trials combining these exact interventions are lacking, we extrapolate from related studies. A **Phase 2 trial of SS-31 (Elamipretide)** in heart failure patients showed improved left ventricular function, suggesting it can translate to human benefit in energydemanding tissues. NAD+ infusions are routinely used in integrative medicine clinics with reported improvements in patients' energy, mood, and concentration (FAQs About NAD+ Infusions - Warren J. Bleiweiss, MD PA). MOTS-c is newer in human use, but small studies indicated it can acutely enhance exercise performance. The safety profile of each is also supportive: SS-31 has been given intravenously in humans (up to 0.25 mg/kg/h infusions) with good tolerance (SS31 - The International Peptide Society); NAD+ is a natural molecule and aside from infusion-related sensations has no toxic byproducts; MOTS-c, being a natural peptide, was well-tolerated in animal studies even at relatively high doses (15 mg/kg) (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC). We expect no adverse interactions because their mechanisms are complementary – however, the staggered schedule further reduces any risk of interaction.

- **Broad Therapeutic Goal:** The combined outcome from this 14-week mitochondrial rejuvenation program should be **enhanced cellular energy production, improved metabolic flexibility, and reduced signs of metabolic aging.** In practical terms, a person undergoing this regimen might experience increased exercise capacity, better post-exercise recovery, improved cognitive function (due to more efficient neuronal mitochondria and NAD+-driven neuroprotection), and metabolic benefits such as easier weight management or improved blood sugar control. Supporting this expectation, a review on

mitochondrial peptides concluded that interventions like MOTS-c can “improve physical capacity and slow age-related deficits” (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC), and NAD+ researchers have noted that maintaining NAD+ can **“postpone age-related disorders”** (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC). By sequentially implementing these scientifically grounded therapies, the protocol attempts to create a synergistic wave of cellular rejuvenation that aligns with cutting-edge gerontology research.

Conclusion

In summary, the 14-week protocol is carefully structured to **prime the body with NAD+**, then **repair mitochondrial function with SS-31**, and finally **enhance metabolic regulation with MOTS-c**. The dosage and timing choices reflect both mechanistic insights (e.g. NAD+ enabling sirtuins for MOTS-c’s action, SS-31’s known effective treatment window) and practical clinical considerations (safety and avoiding overlap). By doing each at the right time, the interventions support and reinforce one another – NAD+ and SS-31 rejuvenate mitochondrial energy capacity, and MOTS-c pushes the revitalized energy system to higher performance, all in service of improving metabolic health, vitality, and potentially longevity. This sequential, multi-targeted approach is emblematic of modern strategies to combat aging: rather than one magic bullet, it uses a coordinated arsenal to address the complex decline in mitochondrial and metabolic function. Early evidence from research and clinical use backs up each component: SS31 for its mitochondrial protection (SS-31 and NMN: Two paths to improve metabolism and function in aged hearts - PMC) (SS-31 - The International Peptide Society), MOTSc for its metabolic mastery via AMPK (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC), and NAD+ for its fundamental role in cell survival and aging (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC). Together, they form a potent combination with a strong scientific rationale for those seeking improvements in energy production, metabolic resilience, and healthy aging.

Overall, no definitive evidence suggests these therapies lose effectiveness with repeated use, though data is limited. In general, mitochondrial enhancers like SS-31 and MOTS-c do not have known receptor desensitization mechanisms, and NAD+ precursors continue to elevate NAD levels over time.

References:

- Szeto, 2014; Hou et al., 2016 – SS-31 binding to cardiolipin and reducing ROS (SS-31 and NMN: Two paths to improve metabolism and function in aged hearts - PMC)

- Chiao et al., 2020 – 8-week SS-31 in aged mice improved cardiac function & oxidative stress (SS-31 and NMN: Two paths to improve metabolism and function in aged hearts - PMC)
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- Rabinovitch et al., 2020 – Combined SS-31 + NMN (NAD precursor) synergistically rejuvenated old mouse hearts (SS-31 and NMN: Two paths to improve metabolism and function in aged hearts - PMC)
- Reynolds et al., 2021 – MOTS-c intermittent treatment increased physical capacity and healthspan in mice (MOTS-c is an exercise-induced mitochondrialencoded regulator of age-dependent physical decline and muscle homeostasis | Nature Communications)
- Lee et al., 2015 – MOTS-c activates AMPK, improves insulin resistance and promotes weight loss in obese mice (Exogenous humanin and MOTS-c function as protective agents ...) (The Mitochondrial-Derived Peptide MOTS-c Promotes Metabolic ...)
- Johnson et al., 2018 – NAD+ precursor (NMN/NR) supplementation improved mitochondrial function and markers of health in aging mice (SS-31 and NMN: Two paths to improve metabolism and function in aged hearts - PMC)
- Bleiweiss, 2022 – NAD+ infusion benefits (energy, metabolism) and managing infusion side effects by slow administration (FAQs About NAD+ Infusions - Warren J. Bleiweiss, MD PA) (FAQs About NAD+ Infusions - Warren J. Bleiweiss, MD PA)
- Peptide Society Monograph – SS-31 mechanism (cardiolipin binding, ETC optimization) and human dosing guidelines (SS-31 - The International Peptide Society) (SS-31 - The International Peptide Society)
- Lu et al., 2015 – Discovery of MOTS-c and its role in coupling mitochondrial and metabolic health (MOTS-c, the Most Recent Mitochondrial Derived Peptide in Human Aging and Age-Related Diseases - PMC) (via NAD+/SIRT1 and folate cycle interaction)