Klotho and Follistatin 2026 Studies: Report of Preliminary Findings

Highlights

- Klotho improved processing efficiency and analytical/spatial reasoning, but its impact on other domains of fluid intelligence (like working memory) is unclear.
- Klotho showed early signs of efficacy (decreased parathyroid hormone) and kidney health improvement (potential decreases in cystatin C), but a stronger signal across more blood markers was expected.
- Follistatin improved upper but not lower body performance.
- Follistatin may have increased IL-10, which is a meta-regulator of other cytokines; however, the signal was weak and only trended toward significance.
- Background health blood panels stayed stable in both studies. This means our gene therapy didn't throw off bodily systems that we weren't targeting.

Executive Summary

Objectives

- Characterize the safety and efficacy of two gene therapies that use a nonviral plasmid vector.
- Investigate the cognitive and systemic health effects of klotho, which at the time of this summary has not yet been given to humans in an experimental setting.
- Understand effects of experimentally delivered follistatin on immune function while capturing muscle function/structure improvements.

Method

- We conducted two pilot studies. Both were open-label (participants knew what they were receiving) and neither study had a placebo group (in line with these being pilot studies).
- **Klotho study:** We gave klotho gene therapy to 14 healthy adults (ages 23-80).
- **Klotho + follistatin study:** Using a combined approach, we gave two gene therapies (klotho and follistatin) to 14 healthy adults (ages 50-80).

Key Findings

- **Klotho improved processing efficiency** (how quickly and fluently you process new information) **and analytical/spatial reasoning**, but its impact on other domains of fluid intelligence (like working memory) is less clear.
- **Follistatin improved upper body performance**, but not lower body performance. This is likely due to how we measured strength. Next time, instead of bodyweight exercises, we will use weights to test people's maximum exertion. Follistatin is more likely to affect maximum force output than endurance.

- **A few blood biomarkers moved in a favorable direction:**
    - **Klotho gene therapy led to decreased parathyroid hormone and trended towards decreasing cystatin C.** The decrease in parathyroid hormone is consistent with klotho's regulatory effects in the FGF-23-klotho axis, where klotho enables FGF-23 to suppress parathyroid hormone. Cystatin C estimates kidney function (eGFR) more accurately than other biomarkers like creatinine, and high levels of it can indicate risk of heart disease.
    - **Follistatin trended towards increasing IL-10**. IL-10 is a meta-regulator and can suppress other immune markers, including IL-1β, IL-6, TNF-α, and IFN-γ.
- **Background health blood panels stayed within physiological norms across both studies**.
    - This means our gene therapy didn't throw off bodily systems that we weren't targeting.

Main Takeaways

- **Any positive effects that occurred did so in the context of broader physiological stability.**
- **Klotho improved several domains of fluid cognition on RIAS-2 and NIH Toolbox.**
    - That is, klotho improved processing speed as well as analytical and spatial reasoning.
- **Klotho showed early signs of efficacy** (parathyroid hormone) **and kidney health improvement** (cystatin C), but a stronger signal across more blood markers was expected.
- **Follistatin signals were found but were weaker than we expected**.
    - Follistatin improved upper but not lower body performance.
    - Follistatin trended towards increasing IL-10 (a meta-regulator of other cytokines) but not other cytokines.
    - A higher dose may be needed to see the immune and strength effects we expected.

Remaining questions:

- *Does klotho affect all cognitive processes globally (and processing speed is just the first needle to move), or is there a mechanism by which klotho could affect individual subdomains of cognition in a vacuum without crossing the blood-brain barrier?*
- *Do we need to increase the follistatin dose, or will switching muscle measurements to maximum force output (weight lifting instead of bodyweight) be enough to see the changes we expect?*
- *Why didn't klotho substantially affect other klotho-mineral axis markers beyond parathyroid hormone?*
- *IL-10 is a master regulator, so it makes sense that this is the first signal we saw – but is the lack of other cytokine changes because we need a stronger follistatin dose, or is something else obscuring the signal of other cytokines?*

Detailed Summary of Preliminary Findings: Klotho and XPrize 2026 Studies

Current aging interventions focus largely on symptom management and do not directly target the underlying biological mechanisms contributing to age-related functional decline. Follistatin is a glycoprotein that counters muscle wasting and chronic low-grade inflammation. It blocks myostatin and activin to promote muscle growth and may be able to treat degenerative muscle diseases. Klotho is a protein that has multiple anti-aging properties, including enhancing cognition, shielding the kidneys from injury, and regulating mineral balance and metabolic function. For example, administration of klotho in mice leads to neural resilience and cognitive enhancement, whether they are young, aging, or transgenic α-synuclein (i.e., genetically modified to model neurodegenerative disease) (Leon et al., 2017). Research on klotho in humans has so far been observational and not experimental, so the preliminary data discussed here are an important step in klotho research.

We recently conducted two human clinical trials testing plasmid gene therapy. The first study examined klotho gene therapy in healthy adults (n = 14, ages 23-80), with the goal of improving cognitive function. The second study tested a combined approach of two gene therapies (klotho and follistatin) in healthy adults (n = 14, ages 50-80), with the goal of improving cognitive, immune, and muscle function. Preliminary results from 3-month (klotho study) and 1-month (klotho + follistatin study) post-intervention follow-ups are discussed here. **All reported p-values are FDR-corrected (Benjamini-Hochberg procedure) unless otherwise noted.**

Cognition

Klotho study: **Processing speed improved significantly**, with a mean increase of 14 points on the NIH Toolbox Pattern Comparison Processing Speed task (*SE* = 2.6, $p < .001$). Additionally, RIAS-2 Composite Intelligence Index scores, which combine verbal and nonverbal intelligence indices, increased by a mean of 5 points (*SE* = 1.2, $p = .007$). Speeded Processing Index scores increased by 8 points (*SE* = 3.0, $p = .019$). The other NIH Toolbox outcomes measured (Dimensional Change Card Sort, Flanker, and Picture Vocabulary) only trended toward significant improvement (each $p = .108$), and no change was found in the RIAS-2 Composite Memory Index performance. The lack of change in other NIH measures may either point to klotho as better at enhancing processing efficiency than other cognitive domains, or it may be due to low statistical power. In either case, future studies with more participants will be useful to understand klotho's effects on human cognition.

Klotho + follistatin study*:* We conducted this study as part of XPrize Competition, which outlines specific requirements for outcome variables that resulted in collection of NIH Toolbox but not RIAS-2 measures. **Performance improvement was found in the Pattern Comparison Processing Speed task** (+9 points, *SE* = 2.6, $p = .041$). It is interesting to note that **processing speed was also the strongest NIH Toolbox signal in the klotho cohort**, adding to the evidence here of a potential link between klotho gene therapy and processing speed. Other NIH Toolbox (Dimensional Change Card Sort, Flanker, and Picture Vocabulary) test scores did not change significantly. From the current data, it is currently unclear if these measures of task switching (Dimensional Change Card Sort) and inhibition (Flanker Test) did not change

due to klotho affecting only certain cognitive domains, or if a true effect was hidden due to a relatively small sample. While it would be ideal to be able to combine measures across the two studies to increase statistical power, direct comparisons between these cohorts should be treated with caution: klotho and follistatin are not expected to affect one another when combined as they act on separate pathways, but combinatorial effects have not yet been characterized in humans due to experimental human gene therapy being a young field.

Brain activity

Klotho study: We also collected brain activity data using Kernel Flow, which is a headset that uses a combination of functional near-infrared spectroscopy (fNIRS) and electroencephalography (EEG). Kernel's BrainAge score, which represents resting-state functional connectivity (relaxed and not doing a task), did not show significant change (Δ = -0.27 percentile units, SE = 1.47, p = .856).

Kernel's Composite Cognition score represents active task performance; specifically, a combination of performance on a set of tasks and simultaneously-captured brain activity. Their proprietary calculation rewards performance for how well it matches task performance and distributions of brain activity observed in an age-matched normative reference population; in other words, **how typical the brain activity looks relative to peers.** This means that a higher score reflects a combination of better task performance and more similarity in brain activation to the population average (which does not necessarily mean superior brain activation). Results showed that the composite cognition score increased after the klotho intervention: Baseline composite score: 60th percentile, SE = 7.6; Month 3 composite score: 75th percentile, SE = 5.4; p = .0072. The increase in score is in line with our other findings that klotho gene therapy led to increases in several domains of fluid intelligence. With that said, brain activity that is more "typical" is not always more efficient. Two individuals could achieve the same score on a task, but if one was more efficient and relied on already-automatized neural pathways that use less HbO and the other was working much harder, the score would only reward whichever activation pattern is more common to the reference dataset. While these findings are interesting, they should be interpreted with caution.

Muscle function

Klotho + follistatin study: Given that klotho is not expected to directly impact muscle function, this variable was only examined in the klotho + follistatin cohort. We examined muscle via functional tasks and DEXA scans. Muscle function was measured via grip strength, number of repetitions per minute of pushups, situps, and squats, and leg lift time. At the 1-month mark, participants showed statistically significant **improvement in change from their baseline left and right grip strength** (M = +5.54 kg, p = .043; M = +4.66 kg, p = .043) **and push up performance** (M = +9 reps/min, p = .043). To put grip strength into context, a meta-analysis of 42 RCTs in 3,278 older adults with sarcopenia found that resistance intervention led to an increase in grip strength of 2.69 kg over approximately 12 weeks (Shen

et al., 2023). **The present sample's one-month gain of approximately 5 kg exceeded this value substantially**.

Situp performance increased by an average of 8 repetitions per minute (*SE* = 4.7), but this change did not reach statistical significance (unadjusted *p* = .137). Leg lift time and squat repetitions per minute did not change significantly. The lack of changes in lower limb function may be due to how muscle function was operationalized. Follistatin is better characterized as affecting muscle mass and direct force output, rather than repetition or endurance-based tasks (Haidet et al., 2008). Given that the present exercises were bodyweight-based, improvements were likely seen in pushup but not squat performance because the smaller upper-body muscles must move a relatively larger proportion of total body mass, meaning that under bodyweight they are operating closer to their force-producing capacity than larger lower body muscles.

DEXA scans did not show significant change in upper or lower body mass at the 1 month mark. It is likely that more time needed to elapse for structural muscle changes to show up on DEXA. It is also possible that the follistatin dose needed to be higher. Future studies will eventually test ideal follistatin dosages.

Subjective data

Klotho study: Quality of life is a predictor of tangible life outcomes, including employment, wealth, education success, mental health, and morbidity. We administered the brief version of the WHO quality of life questionnaire (WHOQOL-BREF), which is a 26-item self-report questionnaire covering four domains: physical health, psychological health, social relationships, and environment (score range for each domain: 4-20 points). Psychological health increased by 1 point (SE = 0.5), but this change only trended toward significance (*p* = .067). Other domains did not show statistically significant change.

Biological markers

Klotho study: Biological markers were taken via blood draw and included a klotho mineral axis panel, as well as renal, liver, and cardiometabolic panels. These panels stayed generally stable, which indicates that any benefits from klotho occurred without disrupting broader physiological stability. Moreover, **parathyroid hormone levels decreased** by 17 pg/mL (*SE* = 3.9, *p* = .007) and **cystatin C trended towards a decrease** of 0.05 mg/dL (*SE* = 0.016, uncorrected *p* = .006, *p-BH* = .059). The observed **parathyroid hormone decline is consistent with klotho exerting its canonical regulatory effect on the FGF-23-klotho axis** in which it suppresses parathyroid hormone at the parathyroid gland. **Low cystatin C is generally thought to indicate high kidney filtration capacity**, and this observed decrease, if it were to persist in a larger study, would be consistent with klotho's protective effects on the kidneys.

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) showed qualitative increases of 10 IU/L (SE = 6.5) and 16 IU/L (SE = 16.3), which, although not statistically significant, neared the expected normal thresholds and could indicate liver stress. However, both of these markers changed

only in the klotho study and not the klotho + follistatin study, so we are currently looking into whether any outliers are driving this change. This is especially likely given the high standard error of ALT (that is, the change and the standard error were both approximately 16 IU/L). A few other liver markers (total bilirubin, globulin, and protein) had indications of potentially shifting based on unadjusted significance thresholds, but all three measures stayed well within physiological norms.

In both studies, ADMA showed qualitative decreases of approximately 14 ng/mL (klotho study) and 13 ng/mL (klotho + follistatin study), but failed to reach statistical significance. Meanwhile, SDMA SDMA remained relatively stable. Given that ADMA is an indicator of cardiovascular risk, the decreases seen here could be advantageous if they were to persist in a larger study. Decreases in ADMA would be in line with klotho's protective effects on the endothelium. Elevated SDMA levels are considered an early indicator of kidney dysfunction and heart disease, so klotho gene therapy could lead to improvements here as well.

Klotho + follistatin study: Biological markers included the same panels as above, plus an immune panel. Panels were again generally stable. Most immune markers did not show change, although **IL-10 trended towards improvement** (+6.4 pg/mL, IQR: 2.5-7.4, p = .125). IL-10 is categorically different from other immune markers in that it acts as a meta-regulator and can suppress others like IL-1β, IL-6, TNF-α, and IFN-γ, so its increase shown here would be worth fleshing out in a larger study.

Summary

To summarize, meaningful changes occurred in both studies within the context of physiological stability. Although these data are preliminary, improvements in cognition were seen in both studies as expected, and changes in upper body muscle performance were seen in the klotho + follistatin study. The follistatin dose would likely need to be increased to see further muscle gains in this study, as well as switching to measures of maximum force in place of endurance measures. Both experimental paradigms could benefit from larger test populations in order to better flesh out the effects of these gene therapies on humans.

References

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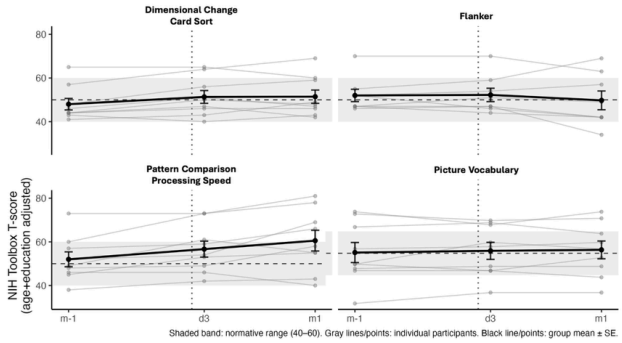
Leon J, Moreno AJ, Garay BI, Chalkley RJ, Burlingame AL, Wang D, et al. Peripheral elevation of a klotho fragment enhances brain function and resilience in young, aging, and α-synuclein transgenic mice. *Cell Rep.* 2017;20(6):1360-71. doi:10.1016/j.celrep.2017.07.024.

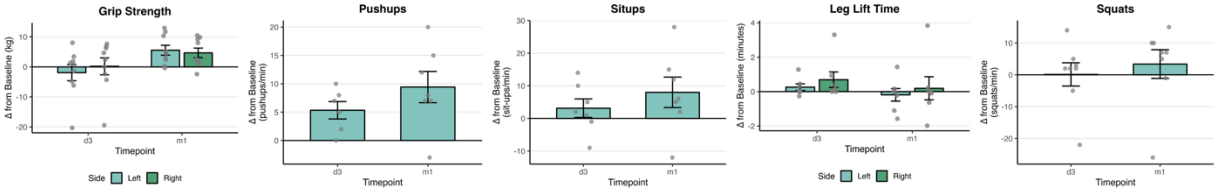
Shen Y, Shi Q, Nong K, Li S, Yue J, Huang J, et al. Exercise for sarcopenia in older people: a systematic review and network meta-analysis. J Cachexia Sarcopenia Muscle. 2023 Jun;14(3):1199-211. doi: 10.1002/jcsm.13225.

Figures – Klotho + follistatin study

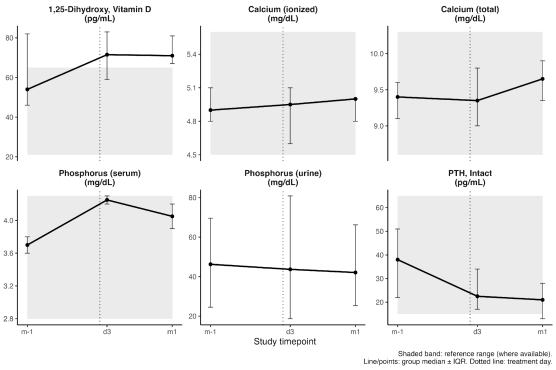
Cognition



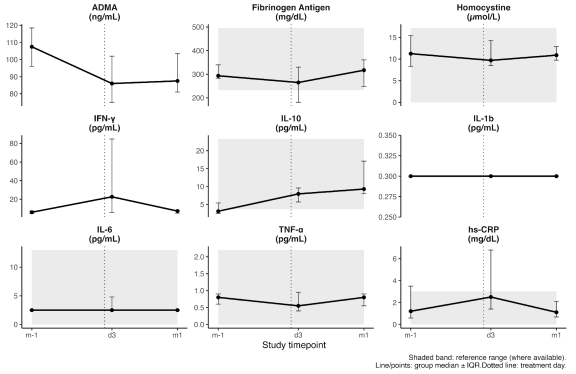
Muscle function



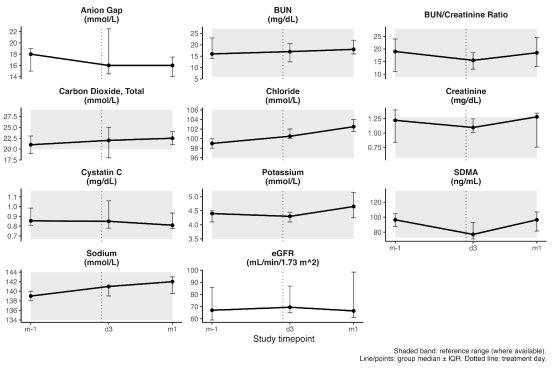
KL-FGF23 mineral axis



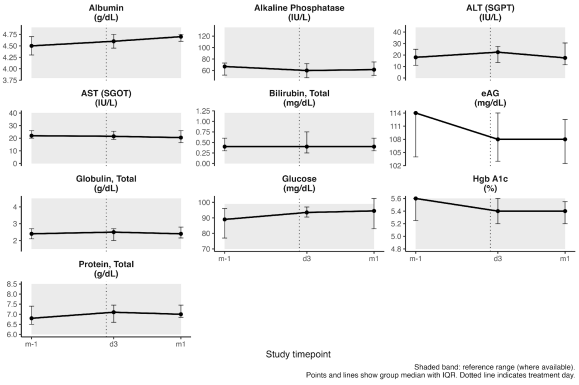
Immune markers



Renal markers

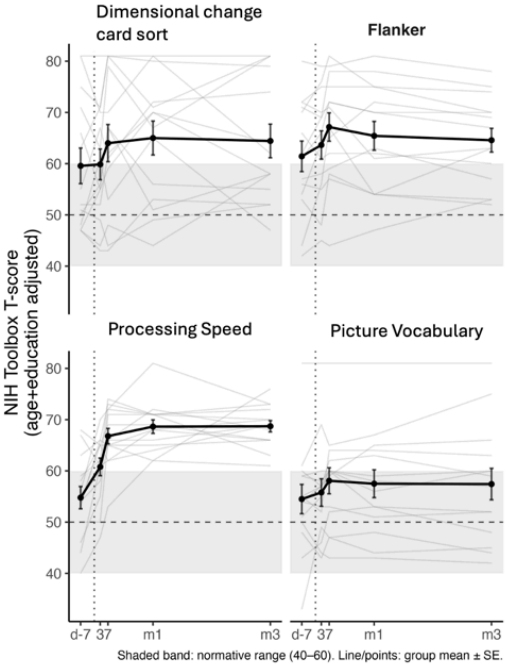


Liver and metabolic markers

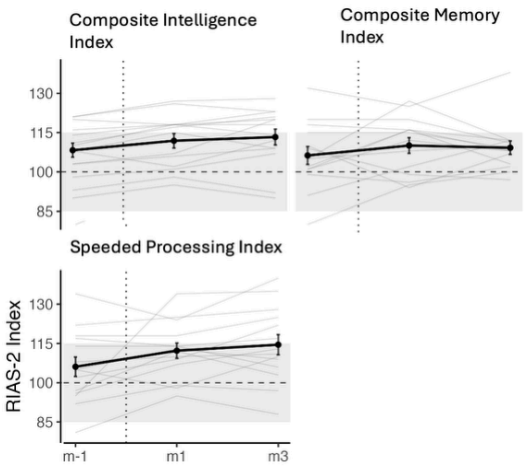


Figures – Klotho study

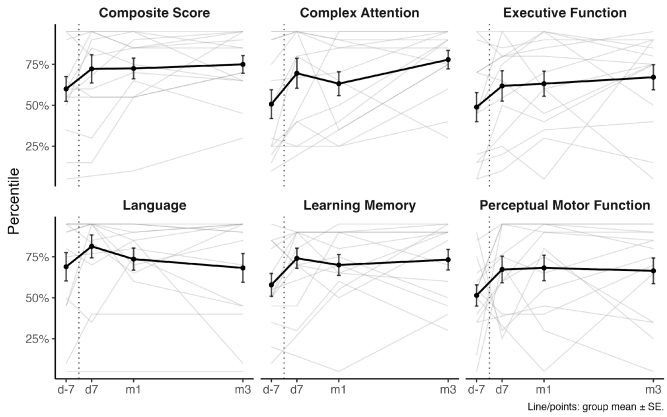
Cognition – NIH Toolbox



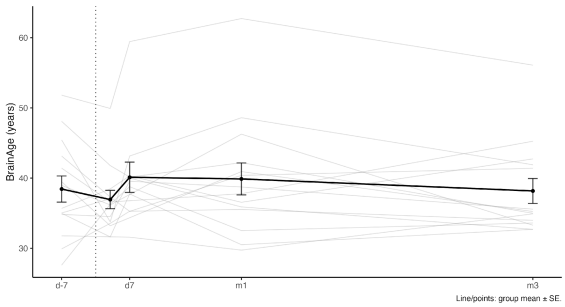
Cognition – RIAS-2



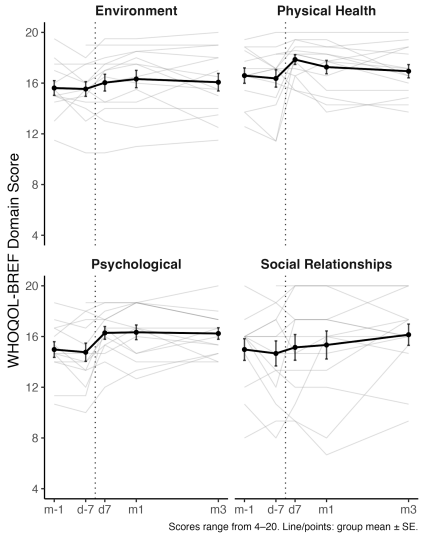
Kernel cognition/brain composites



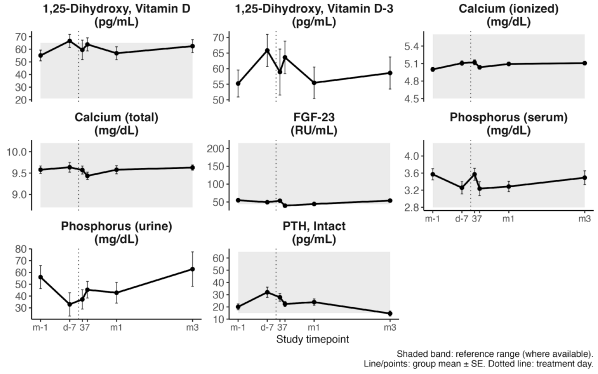
Brain Imaging – Kernel BrainAge



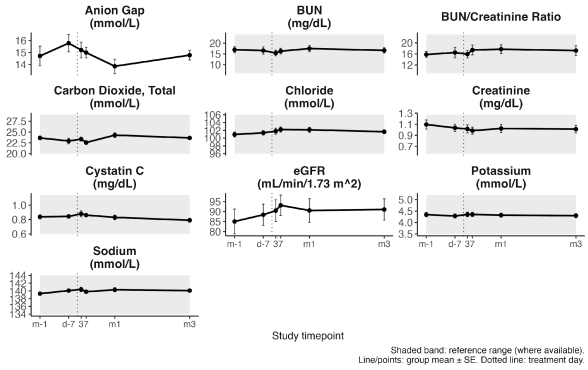
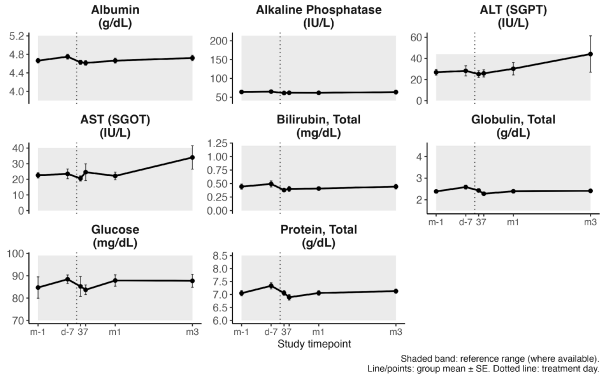
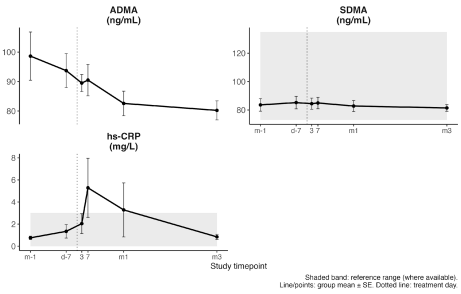
Quality of life survey (WHOQOL-BREF)



KL-FGF23 mineral axis



Renal markers



Metabolic and liver markers