

STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

Hormonal Normalization Is Not Metabolic Recovery: Adipokine Dynamics After Levothyroxine Replacement in Newly Diagnosed Primary Hypothyroidism

Official Title: Hormonal Normalization Is Not Metabolic Recovery: Adipokine Dynamics After Levothyroxine Replacement in Newly Diagnosed Primary Hypothyroidism

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Sponsor / Institution: University of Health Sciences, Basaksehir Cam and Sakura City Hospital

Ethics Committee: Clinical Research Ethics Committee of Basaksehir Cam and Sakura City Hospital

Study Design: Single-center, prospective observational cohort study

This document contains no participant names or directly identifying participant information.

Protocol Synopsis

Official Title	Hormonal Normalization Is Not Metabolic Recovery: Adipokine Dynamics After Levothyroxine Replacement in Newly Diagnosed Primary Hypothyroidism
Study Type	Observational
Observational Model	Cohort
Time Perspective	Prospective
Enrollment	88 participants
Population	Premenopausal female patients aged 18 years or older with newly diagnosed primary hypothyroidism.
Groups / Cohorts	One cohort: newly diagnosed primary hypothyroidism cohort.
Exposure of Interest	Standard-of-care levothyroxine replacement therapy administered according to routine clinical practice.
Primary Outcome	Change in serum adipokine levels after levothyroxine replacement therapy.
Time Points	Baseline before treatment initiation and 8 weeks after levothyroxine replacement therapy.
Biospecimen Retention	Serum samples retained without DNA extraction, stored at -80°C for adipokine and metabolic biomarker analyses.

1. Background and Rationale

Primary hypothyroidism is associated with systemic cardiometabolic disturbances, including dyslipidemia, insulin resistance, altered energy homeostasis, adipose tissue dysfunction, hepatic metabolic burden, and chronic low-grade inflammation. Levothyroxine replacement therapy is the standard treatment for primary hypothyroidism and generally produces biochemical improvement in thyroid function tests within the early treatment period. However, biochemical recovery of the thyroid axis may not necessarily indicate synchronous recovery of adipose tissue function and metabolic homeostasis.

Adipose tissue is an active endocrine organ that secretes adipokines involved in glucose regulation, lipid metabolism, inflammation, vascular function, and cardiometabolic risk. Asprosin, adipolin, omentin-1, and visfatin represent biologically distinct adipokine pathways related to hepatic glucose production, insulin sensitivity, vascular-metabolic protection, NAD⁺ metabolism,

and inflammatory activation. Evidence regarding the dynamic behavior of these adipokines after levothyroxine replacement in newly diagnosed primary hypothyroidism remains limited.

This study is designed to evaluate whether early biochemical thyroid recovery after levothyroxine replacement is accompanied by parallel changes in serum adipokines and metabolic parameters. The protocol follows a within-patient comparison model to reduce inter-individual biological variability and to characterize early adipokine dynamics during the first 8 weeks of treatment.

2. Objectives

2.1 Primary Objective

The primary objective is to evaluate changes in serum adipokine levels, including asprosin, adipolin, omentin-1, and visfatin, from baseline to 8 weeks after standard-of-care levothyroxine replacement therapy in newly diagnosed primary hypothyroid patients.

2.2 Secondary Objectives

- To evaluate changes in thyroid function tests, including TSH, free T4, and free T3.
- To evaluate changes in lipid profile parameters, including total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and non-HDL cholesterol.
- To evaluate changes in glucose-insulin metabolism and insulin resistance indices, including fasting glucose, fasting insulin, HOMA-IR, TyG index, and METS-IR.
- To evaluate changes in hepatic metabolic indices and liver function markers, including ALT, AST, and HSI.
- To evaluate changes in anthropometric parameters and obesity phenotype, including body mass index and waist circumference where available.
- To investigate associations between adipokine changes and thyroid, lipid, glucose-insulin, hepatic, inflammatory, and anthropometric variables.
- To explore whether early thyroid-axis recovery is synchronous or asynchronous with adipose-metabolic remodeling.

3. Study Design

This is a single-center, prospective observational cohort study. Participants are evaluated before treatment initiation and after 8 weeks of standard-of-care levothyroxine replacement therapy. All participants receive levothyroxine as part of routine clinical care; no investigational drug, device, randomized allocation, or protocol-mandated experimental intervention is used.

The study uses a within-patient pre-treatment and post-treatment comparison design. Each participant serves as her own control, allowing assessment of early changes in adipokine concentrations and metabolic parameters after levothyroxine replacement.

4. Study Setting

The study is conducted at the Internal Medicine outpatient clinic of Basaksehir Cam and Sakura City Hospital, University of Health Sciences, Istanbul, Türkiye. Laboratory and biospecimen processing procedures are performed according to the study protocol and institutional laboratory standards.

5. Study Population

The study population consists of premenopausal female patients aged 18 years or older who were newly diagnosed with primary hypothyroidism and had not previously received levothyroxine or any other thyroid hormone replacement therapy. Participants are evaluated at baseline before treatment and again after 8 weeks of levothyroxine therapy. Only participants who completed both baseline and follow-up assessments are included in the final analysis.

6. Eligibility Criteria

6.1 Inclusion Criteria

- Female patients aged 18 years or older.
- Premenopausal status.
- Newly diagnosed primary hypothyroidism, defined by elevated serum TSH above the reference range with low free T4.
- No previous use of levothyroxine or other thyroid hormone replacement therapy.
- Ability and willingness to provide written informed consent.
- Completion of both baseline and 8-week follow-up assessments.

6.2 Exclusion Criteria

- Known hypothalamic or pituitary disease.
- Previous thyroid hormone replacement therapy.
- Pregnancy or lactation.
- Menopause.
- History of thyroid surgery or radioactive iodine therapy.
- Active malignancy.
- Acute or chronic inflammatory disease that may affect adipokine or metabolic biomarker levels.
- Severe hepatic or renal disease.
- Diabetes mellitus.
- Use of medications known to significantly affect thyroid function, glucose metabolism, lipid metabolism, or adipokine levels.

- Inability or unwillingness to provide informed consent or comply with follow-up requirements.

7. Sampling Method and Recruitment

A non-probability sampling method is used. Consecutive or eligible patients presenting to the Internal Medicine outpatient clinic and meeting the inclusion and exclusion criteria are invited to participate. Written informed consent is obtained before any study-specific biospecimen collection or data recording related to the research protocol.

8. Levothyroxine Treatment and Follow-up

Levothyroxine replacement therapy is prescribed as standard-of-care treatment according to routine clinical practice and current guideline-based clinical judgment. Dose selection and dose adjustment are performed by the treating physician based on body weight, baseline thyroid function tests, clinical status, and routine care considerations. The study does not assign participants to treatment arms and does not test an investigational drug strategy.

Participants are reassessed after 8 weeks of therapy. This time frame is selected to capture early biochemical recovery of the thyroid axis while evaluating whether adipokine and metabolic parameters show parallel or divergent changes during the same period.

9. Study Assessments and Schedule

Assessment	Baseline	Week 8
Informed consent	Yes	No
Eligibility assessment	Yes	No
Clinical and anthropometric assessment	Yes	Yes
Thyroid function tests	Yes	Yes
Routine biochemical parameters	Yes	Yes
Fasting glucose and insulin	Yes	Yes
Lipid profile	Yes	Yes
Serum adipokine sampling	Yes	Yes
Calculated metabolic indices	Yes	Yes

10. Biospecimen Collection, Processing, and Retention

Fasting venous blood samples are collected at baseline and after 8 weeks of levothyroxine replacement therapy. Samples are obtained in the morning, preferably between 08:00 and 09:00, after at least 8 hours of fasting to reduce preanalytical variability.

Serum samples are processed under standardized conditions. Blood samples are collected into serum separator tubes, centrifuged at 4000 rpm for 15 minutes at +4°C, aliquoted into Eppendorf tubes, and stored at -80°C until analysis. Retained samples consist of serum only and are intended for adipokine and metabolic biomarker analyses. DNA extraction or genetic analysis is not planned.

11. Laboratory Measurements

Serum adipokine concentrations include asprosin, adipolin, omentin-1, and visfatin. Measurements are performed using commercially available ELISA kits according to manufacturer instructions. Thyroid function tests, lipid profile, fasting glucose, fasting insulin, liver function tests, inflammatory markers, renal function markers, and other routine biochemical parameters are recorded from baseline and follow-up assessments.

Calculated indices include HOMA-IR, TyG index, METS-IR, AIP, HSI, Castelli Risk Index I, Castelli Risk Index II, non-HDL cholesterol, and other clinically relevant cardiometabolic indices as specified in the statistical analysis plan.

12. Outcome Measures

12.1 Primary Outcome Measure

Title: Change in Serum Adipokine Levels After Levothyroxine Replacement Therapy

Description: Serum adipokine levels, including asprosin, adipolin, omentin-1, and visfatin, are measured before treatment initiation and after 8 weeks of standard-of-care levothyroxine replacement therapy. Changes in adipokine concentrations are evaluated to characterize early adipose-metabolic response during biochemical thyroid recovery.

Time Frame: Baseline and 8 weeks after levothyroxine replacement therapy.

12.2 Secondary Outcome Measures

Change in Thyroid Function Tests: Change in TSH, free T4, and free T3 from baseline to week 8.

Change in Lipid Profile: Change in total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and non-HDL cholesterol from baseline to week 8.

Change in Glucose-Insulin Metabolism and Insulin Resistance: Change in fasting glucose, fasting insulin, HOMA-IR, TyG index, and METS-IR from baseline to week 8.

Change in Hepatic Metabolic Burden: Change in ALT, AST, HSI, and related hepatic biochemical parameters from baseline to week 8.

Association Between Adipokine Dynamics and Metabolic Remodeling: Correlation and regression analyses evaluating relationships between adipokine changes and thyroid, lipid, glucose-insulin, hepatic, inflammatory, and anthropometric variables.

13. Sample Size Considerations

The planned sample size is based on a within-patient pre-treatment and post-treatment comparison framework. The primary comparison is the change in serum adipokine levels after levothyroxine replacement therapy. A priori sample size planning was performed for paired comparisons using a two-sided alpha level of 0.05 and 80% statistical power. The final analysis includes 88 participants who completed both baseline and 8-week follow-up assessments, exceeding the minimum planned sample size and allowing for evaluation of adipokine changes with paired statistical methods.

14. Statistical Analysis Plan

Continuous variables will be summarized as mean \pm standard deviation or median [interquartile range], depending on distribution. Categorical variables will be summarized as frequency and percentage. Normality will be assessed using the Shapiro-Wilk test and by visual inspection of distributional patterns where appropriate.

For pre-treatment and post-treatment comparisons, paired t-test will be used for normally distributed continuous variables, and Wilcoxon signed-rank test will be used for non-normally distributed continuous variables. Effect sizes for paired comparisons will be reported using Cohen d_z where appropriate. Categorical paired variables will be analyzed using appropriate paired categorical methods when applicable.

Correlation analyses will be conducted using Pearson or Spearman correlation coefficients according to distributional characteristics. Associations between changes in adipokines and changes in thyroid function tests, lipid parameters, insulin resistance indices, hepatic indices, inflammatory markers, and anthropometric parameters will be evaluated. Multivariable linear regression models may be used to assess independent predictors of adipokine changes, with covariates selected according to clinical relevance and statistical assumptions.

Because multiple adipokines are evaluated as primary biomarker outcomes, multiple testing correction will be applied using the Benjamini-Hochberg false discovery rate method. A two-sided p -value < 0.05 will be considered statistically significant unless otherwise specified. Analyses will be performed using standard statistical software.

15. Data Management and Confidentiality

Clinical and laboratory data will be recorded in a secure study database using coded participant identifiers. Direct participant identifiers will not be included in analytical datasets or uploaded documents. Study data will be accessed only by authorized study personnel. Individual participant data are not planned to be publicly shared due to institutional and ethical restrictions related to patient confidentiality and biospecimen-based clinical data.

16. Ethical Considerations

The study has been approved by the Clinical Research Ethics Committee of Basaksehir Cam and Sakura City Hospital. The study is conducted in accordance with ethical principles for medical research involving human participants. Written informed consent is obtained from all participants before enrollment. The study involves standard-of-care levothyroxine treatment and blood sampling for research biomarker analyses.

17. Safety Considerations

This is an observational study of standard-of-care levothyroxine treatment. No investigational medicinal product or experimental device is administered as part of the study protocol. No Data Monitoring Committee is planned because the study is low risk, non-randomized, and observational. Any clinically relevant findings identified during routine care are managed according to standard clinical practice.

18. Dissemination Plan

Study findings may be presented in academic settings and submitted for publication in peer-reviewed scientific journals. Any dissemination will use de-identified aggregate data and will not include participant names or directly identifying information.