Serum Fibroblast Growth Factor-21, Irisin, and Selenium-Related Antioxidant Proteins (GPx4, HO-1, SELENOP, SELENBP1) in Lactating versus Non-Lactating Women: A Case–Control Study of Oxidative and Metabolic Adaptation

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Abstract

Background: Lactation is one of the most challenging metabolic and oxidative periods, and the metabolic and selenium-dependent antioxidant mediators' behaviour in the period of lactation is not well known, especially in regional populations. The fibroblast growth factor-21 (FGF21) regulates lipid mobilization and energy expenditure, while glutathione peroxidase-4 (GPx4), heme oxygenase-1 (HO-1), selenium-binding protein-1 (SELENBP1), and selenium (Se) are involved in redox balance and selenium handling. Hypotheses: a) There will be no significant differences in serum levels of these six markers between lactating and non-lactating women, and b) these markers will be related to each other and to their ability to discriminate between lactating and non-lactating women. In this case–control study, 120 women aged between 20 and 40 years (60 lactating and 60 non-lactating) were recruited. ELISA was used to quantify serum biomarkers. Independent-samples t-tests, Cohen's d and Bonferroni correction were used to test group differences, Pearson correlation associations, and receiver-operating-characteristic (ROC) analysis discriminatory performance. Key Findings: Both groups were well matched with respect to body-mass index (BMI) and age ($p>0.93$). The SELENOP levels were significantly higher in lactating women (76.2±9.03 ng/mL vs 67.2±9.54 ng/mL; $p<0.001$, surviving Bonferroni correction, Cohen's d 0.76–0.97) while there were no differences in HO-1 and SELENBP1 ($p>0.24$). The AUC value for SELENOP was the highest (0.755), with moderate discrimination for FGF21 and GPx4 (AUC=0.738 each). FGF21 was positively associated with irisin and GPx4. Conclusions: Lactation is associated with a coherent and simultaneous increase in a subset of metabolic regulators, as well as in the constitutive arm of

the selenium-dependent antioxidant system, without affecting the levels of those that are inducible by stress; this is consistent with, but not proof of, a supply-driven pre-emptive adaptation. Further assessment of oxidative and selenium status is needed for confirmation.

Keywords: *breast-feeding; metabolic adaptation; redox homeostasis; selenium transport; energy expenditure; myokine; reproductive physiology; case–control.*

1. Introduction

Lactation represents one of the most energetically demanding phases of the female life cycle, requiring a sustained redirection of nutrients and energy toward milk synthesis. This physiological state is sustained by extensive endocrine and metabolic remodeling, including enhanced lipid mobilization from maternal stores and a shift in substrate partitioning between adipose tissue and the liver [1]. The metabolic mediators that orchestrate this adaptation, and the antioxidant systems that protect maternal tissues during this period of elevated metabolic flux, have attracted growing interest as candidate markers of maternal metabolic health [2].

Fibroblast growth factor-21 (FGF21) is a hepatically derived endocrine regulator of glucose and lipid metabolism whose circulating concentration rises sharply in states of intense lipid utilization [3]. In high-yielding dairy cows, plasma FGF21 is nearly undetectable in late pregnancy, peaks at parturition, and remains chronically elevated through the energy deficit of early lactation [1], implicating it directly in the lipid-mobilizing economy of milk production. In rodents, hepatic FGF21 acts on the hypothalamus to mediate the protective metabolic legacy of prolonged breast-feeding [4], and FGF21 is also secreted into milk, where it shapes neonatal intestinal function [5]. Maternal serum FGF21 also varies across pregnancy and the puerperium [6]. Irisin, a myokine cleaved from FNDC5, promotes energy expenditure and insulin sensitivity and is present in both serum and human milk, where its concentration is modulated by maternal nutritional status [7] and is altered in gestational diabetes [8]. Together, these two mediators provide a plausible axis linking lactational energy demand to systemic metabolic signalling.

In parallel, the heightened metabolic rate of lactation increases the potential for reactive-oxygen-species generation, placing a premium on antioxidant defence [9]. Glutathione peroxidase-4 (GPx4) is a selenocysteine-containing enzyme that uniquely reduces phospholipid hydroperoxides within membranes and is the central gatekeeper against ferroptotic, iron-dependent lipid peroxidation [10,11]. Heme oxygenase-1 (HO-1), the inducible, NRF2-regulated rate-limiting enzyme of heme

catabolism, is a stress-responsive cytoprotective and antioxidant effector [12,13]. The KEAP1–NRF2 axis coordinately governs HO-1 and the GSH–GPx4 system, linking these defences within a single regulatory network [14]. Selenoprotein P (SELENOP), the principal plasma selenium transporter, is an established biomarker of selenium status and is positively associated with antioxidant capacity and erythrocyte GPx activity while being inversely related to lipid-peroxidation markers [15,16]. Selenium-binding protein-1 (SELENBP1), although not a selenoprotein, functions as a copper-dependent methanethiol oxidase and a marker of cellular differentiation and selenium handling [17,18]; copper availability further modulates selenoprotein synthesis and activity, situating SELENBP1 at the copper–selenium interface [26]. Maternal selenium requirements rise during gestation and lactation, and selenium supply governs the expression of both SELENOP and the glutathione peroxidases [19,20]. These proteins collectively map the selenium-dependent and stress-inducible arms of maternal redox defence.

Despite the physiological plausibility of these pathways, direct human data on FGF21, irisin, GPx4, HO-1, SELENOP, and SELENBP1 during lactation are scarce, and regional evidence from Iraqi women is almost absent. Prior local work has characterized adipokines, conventional oxidant/antioxidant markers, and inflammatory mediators in lactating and non-lactating women [21], in obese women [22], and in women with unexplained infertility [23], but none has examined the newer metabolic and selenium-related markers studied here. Human-milk antioxidant capacity and its determinants have been described [24,25], yet the maternal serum compartment for these specific proteins remains largely uncharacterized during lactation. This gap motivates the present work. We tested the hypothesis that lactating women exhibit higher antioxidant and selenium-related proteins together with altered FGF21 and irisin relative to non-lactating women, reflecting a coordinated metabolic and redox adaptation to lactation. The aim was to compare the serum levels of the six biomarkers between groups, to examine the relationships between the biomarkers, and to assess their discriminatory power between lactating and nonlactating groups.

2. Materials and Methods

2.2 Sample selection and plan

A total of 120 women aged 20-40 years attending Tikrit Teaching Hospital and primary health-care centres in Salah al-Din Governorate, Iraq, from March to July 2025 were included in this case–control study. 60 lactating women and 60 non-lactating women were assigned to the two groups.

All study participants signed informed consent forms, and the study respected the principles of the Declaration of Helsinki.

2.2 Inclusion and exclusion criteria

The women were aged 20–40, had no history of chronic disease and gave written consent. The exclusion criteria were diabetes mellitus, thyroid diseases, hepatic and renal diseases, autoimmune disease, smoking, current pregnancy, and antioxidant supplement use within the last three months to avoid confounding of redox and metabolic parameters. All lactating women were eligible, regardless of their stage of lactation within the first 18 months after childbirth, as long as they were still breastfeeding at enrolment.

2.3 Lactation-period assessment

The lactation period was described as the number of months of uninterrupted breastfeeding completed by the participants at the time of sampling (determined by a structured maternal interview) for lactating participants. In the present cohort, the lactation period was 1-18 months (mean 9.6 ± 4.9 months and median 10 months), encompassing early, mid and established lactation. In within group correlation analysis, this variable was treated as a continuous covariate and not used to split the sample.

2.4 The sample collection and biochemical analysis runs

The 5 mL venous blood samples were collected from each individual in Gel Separator Tubes. For labile proteins, the serum was separated by centrifugation (3000 ×g, 10 min) and subsequently stored at −20 °C, and the assays were all performed before plasma was repeated frozen and thawed, and before the expiration of the storage time. Commercial human-specific sandwich enzyme-linked immunosorbent assay (ELISA) kits (SunLong Biotech Co., Ltd, catalogue number listed in Table 1, Hangzhou, China) were used for measurements of serum FGF21, irisin, GPx4, HO-1, SELENOP, and SELENBP1. Each sample was tested in duplicate, and absorbance was measured at 450nm; absorbance was interpolated from a standard curve created on each plate. If the OD of a sample was above the highest standard, then the sample was diluted in the validated linear range of the assay, as indicated by the manufacturer, and the result was multiplied by the dilution factor. The assay catalogue number, detection range and intra-assay and inter-assay coefficient of variation (CV) for

each kit are summarized in Table 1. Duplicate readings were only accepted if within 20% of the mean.

2.5 Statistical analysis

The SPSS 26 package was used for the analysis of data. Continuous variables are presented as mean ± standard deviation (SD). All biomarkers in both groups met the criteria of approximate normality, which was confirmed using the Shapiro–Wilk test, and therefore, between-group comparisons were performed with an independent-samples t-test, with the equal-variance assumption suggested by Levene's test, and the effect size calculated using Cohen's d. The Bonferroni correction (0.05/6 = $p<0.0083$) was used for multiple comparisons across the six biomarker comparisons. Pair-wise correlation was done using Pearson correlation coefficients, and multiple linear regression was used to determine the factors associated with FGF21. The discriminatory capacity was assessed using receiver operating characteristic (ROC) analysis, and optimal cutoffs were based on the Youden index. Statistically significant differences were determined by a $p\leq0.05$ (or Bonferroni, as specified) value.

3. Results

3.1 Assay characteristics

The analytical characteristics of the six ELISA kits are presented in Table 1. All assays had a precision that was in line with the pre-established precision criteria, which gives confidence in the measured concentrations.

Table 1. Immunoassay characteristics for the biomarker quantifications.

Biomarker	Catalogue No.	Detection range	Intra-assay CV	Inter-assay CV
FGF21	EL0216Hu	31.25–2000 pg/mL	2.7–4.8%	3.1–6.1%
Irisin	SL2020Hu	0.5–30 ng/mL	<10%	<12%
GPx4	SL2273Hu	0.6–40 ng/mL	<10%	<12%
HO-1	SL0839Hu	0.06–4 ng/mL	<10%	<12%

Biomarker	Catalogue No.	Detection range	Intra-assay CV	Inter-assay CV
SELENOP	SL1566Hu	0.2–10 ng/mL	<10%	<12%
SELENBP1	SL3330Hu	31.25–1600 pg/mL	<10%	<12%

All kits were supplied by SunLong Biotech Co., Ltd (Hangzhou, China). CV = coefficient of variation. For FGF21, the manufacturer reports measured intra- and inter-assay CV ranges across three concentrations; for the remaining assays, the manufacturer specifies precision limits of intra-assay CV <10% and inter-assay CV <12%. Detection limits (sensitivity) were 0.19 pg/mL (FGF21), 0.1 ng/mL (irisin, GPx4), 0.01 ng/mL (HO-1), 0.05 ng/mL (SELENOP), and 10 pg/mL (SELENBP1).

3.2 Demographic comparability

The two groups were demographically well matched. Mean age was 30.3±4.48 years in lactating women and 30.2±3.97 years in non-lactating women (p=0.935), and body-mass index was virtually identical (26.4±3.51 vs 26.4±2.98 kg/m², p=0.980). This comparability means that biomarker differences are unlikely to be attributable to age or adiposity (Table 2).

Table 2. Demographic and biomarker profile of lactating and non-lactating women (mean ± SD) with group comparison.

Variable	Lactating (n=60)	Non-lactating (n=60)	t	p	Cohen's d
Age (years)	30.3 ± 4.48	30.2 ± 3.97	0.08	0.935	0.015
BMI (kg/m²)	26.4 ± 3.51	26.4 ± 2.98	0.03	0.980	0.005
FGF21 (pg/mL)	240.2 ± 44.1	199.1 ± 46.4	4.98	<0.001	0.909
Irisin (ng/mL)	7.21 ± 1.02	6.41 ± 1.07	4.18	<0.001	0.763
GPx4 (ng/mL)	19.7 ± 3.21	16.2 ± 4.04	5.21	<0.001	0.952
HO-1 (ng/mL)	5.37 ± 1.07	5.13 ± 1.22	1.16	0.249	0.212
SELENOP (ng/mL)	76.2 ± 9.03	67.2 ± 9.54	5.31	<0.001	0.97
SELENBP1 (ng/mL)	12.1 ± 2.36	11.6 ± 2.29	1.09	0.278	0.199

Note: Independent-samples t-tests (df=118). Bonferroni-corrected significance threshold for the six biomarkers = p<0.0083.

3.3 Biomarker differences between groups

Four of the six biomarkers were significantly elevated in lactating women and remained significant after Bonferroni correction (all p<0.001 ≪ 0.0083). SELENOP showed the largest

separation (76.2±9.03 vs 67.2±9.54 ng/mL; t=5.31, d=0.97), followed by GPx4 (d=0.952), FGF21 (d=0.909), and irisin (d=0.763), all medium-to-large effects. In contrast, HO-1 (p=0.249) and SELENBP1 (p=0.278) did not differ, with negligible effect sizes (d≈0.2). This dissociation—elevation of the constitutive, supply-dependent antioxidant arm (SELENOP, GPx4) alongside unchanged inducible stress markers (HO-1)—is the central observation of the study.

3.4 Normality assessment

The Shapiro–Wilk test supported the normality assumption for every biomarker in both groups (all p>0.05), justifying parametric testing (Table 3).

Table 3. Shapiro–Wilk normality statistics for each biomarker by group.

Biomarker	W (Lactating)	p (Lactating)	W (Non-lact.)	p (Non-lact.)
FGF21	0.984	0.599	0.976	0.295
Irisin	0.968	0.111	0.976	0.276
GPx4	0.966	0.091	0.98	0.429
HO-1	0.991	0.938	0.98	0.421
SELENOP	0.976	0.287	0.985	0.673
SELENBP1	0.989	0.854	0.969	0.125

Note: All p>0.05 indicates no significant departure from normality.

3.5 Correlations among biomarkers

In the entire sample, FGF21 was positively associated with irisin (r=0.239, p=0.009) and GPx4 (r=0.220, p=0.016), suggesting a link between the metabolic-signalling and antioxidant arms. But in the lactating group, irisin and SELENOP showed a negative correlation (r=−0.359, p=0.005), and GPx4 was negatively correlated with lactation duration (r=−0.320, p=0.013), indicating possible time-dependent decreases in antioxidant enzyme activity as lactation progresses. The pairwise coefficients are listed in Table 4.

Table 4. Pearson correlation matrix of the six biomarkers (whole sample, n=120).

	FGF21	Irisin	GPx4	HO-1	SELENOP	SELENBP1
FGF21	1.00	0.24	0.22	-0.04	0.11	0.00

	FGF21	Irisin	GPx4	HO-1	SELENOP	SELENBP1
Irisin	0.24	1.00	0.18	0.03	0.06	-0.01
GPx4	0.22	0.18	1.00	0.05	0.14	0.02
HO-1	-0.04	0.03	0.05	1.00	0.11	0.08
SELENOP	0.11	0.06	0.14	0.11	1.00	0.06
SELENBP1	0.00	-0.01	0.02	0.08	0.06	1.00

Note: Values are Pearson r. FGF21–Irisin (r=0.239) and FGF21–GPx4 (r=0.220) were statistically significant (p<0.05).

III. 3.6 Determinants of FGF21

A multiple linear regression with the other biomarkers, BMI and age, to predict FGF21 had an adjusted R^2 of 0.042 and overall was not significant (F=1.75, p=0.104) and explained little variance (R^2=0.099). Only irisin was independently (B=9.142, p=0.027), with GPx4 being close to significance (p=0.059). The weak fit means that factors other than those measured in the panel control circulating FGF21.

3.7 Discriminatory performance

ROC analysis was used to measure the capability of each biomarker to discriminate lactating from non-lactating women (Table 5, Figure 1). SELENOP had the highest AUC (AUC=0.755) and specificity (93.3%) and low sensitivity (46.7%) at the cut-off, whereas the AUC values for FGF21 and GPx4 were 0.738. Irisin exhibited moderate discrimination (AUC=0.686) while HO-1 and SELENBP1 demonstrated near chance discrimination (AUC≈0.57). These AUC values are of modest discrimination, meaning the markers are physiologically informative and not diagnostic tests.

Table 5. The discriminatory performance of each biomarker for the lactational status was assessed using the ROC procedure.

Biomarker	AUC	Cut-off	Sensitivity (%)	Specificity (%)
SELENOP (ng/mL)	0.755	78.5	46.7	93.3
FGF21 (pg/mL)	0.738	200.52	83.3	55
GPx4 (ng/mL)	0.738	19.34	58.3	76.7
Irisin (ng/mL)	0.686	6.27	85	46.7
SELENBP1 (ng/mL)	0.566	11.69	63.3	60
HO-1 (ng/mL)	0.565	5.21	61.7	58.3

Note: Cut-offs from the Youden index. AUC ≈ 0.5 indicates no discrimination; values of 0.7–0.8 indicate modest-to-acceptable discrimination.

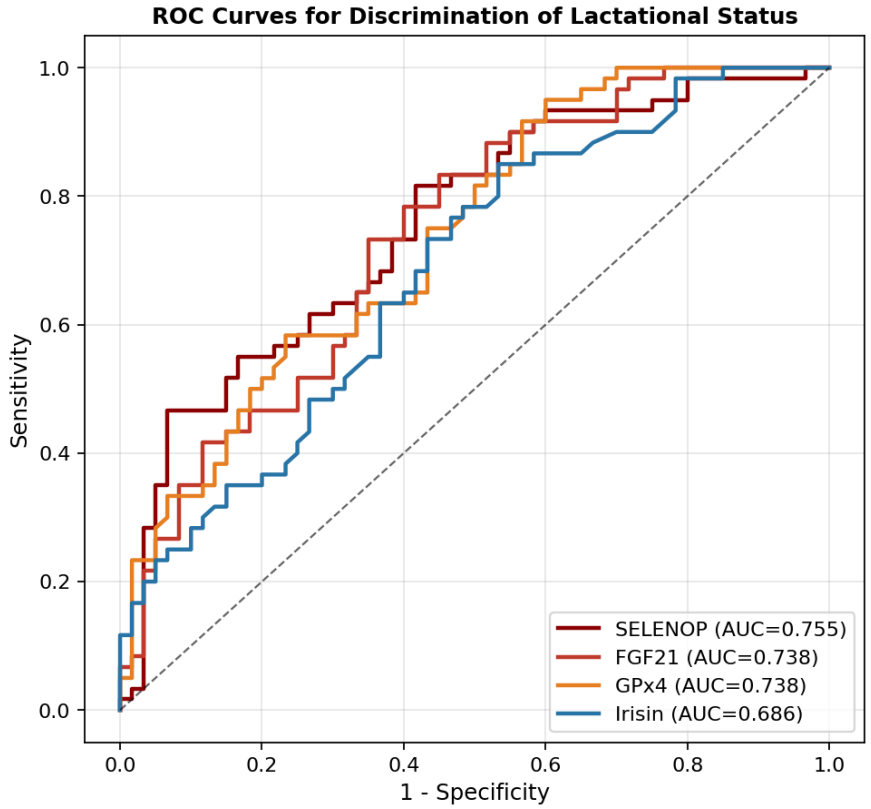

Figure 1. Receiver-operating-characteristic curves for the four biomarkers that differed significantly between groups. Areas under the curve indicate modest discrimination.

4. Discussion

IV. In a population of lactating and non-lactating women from a region where the data are lacking, this study offers one of the first comprehensive profiles of FGF21, irisin, and four antioxidant proteins that relate to selenium. The main result is a coordinated increase of SELENOP, GPx4, FGF21 and irisin during lactation, while HO-1 and SELENBP1 levels did not change, and both the latter differences remain significant after correction for multiple comparisons, between two groups that were not different in age and BMI.

The increase in FGF21 is consistent with its known functions as a sensor and effector of high levels of lipid mobilisation [3]. The bovine transition model exhibits a large surge of FGF21 at parturition, followed by a sustained elevation during the energy deficit of early lactation [1], and rodent studies place FGF21 at the heart of the long-term metabolic benefits of breastfeeding [4]. This is supported by our human data showing that lactating women have elevated circulating FGF21 levels when

adiposity is tightly matched, indicating that the increase is not due to differences in fat mass. The parallel rise in irisin and the positive correlation with FGF21 is congruent with a complementary myokine–hepatokine axis that facilitates energy expenditure during lactation [7, 8].

The most interesting findings from the antioxidant perspective are the selectivity. The selenium transporters SELENOP, which is a validated biomarker of selenium status [15] and the selenocysteine enzyme GPx4, both increased together, while the inducible stress sensor HO-1 did not. This separation is biologically significant. SELENOP and GPx4 are a supply-driven, constitutive antioxidant axis, whose capacity increases with selenium availability [19,20] and where their coordinated increase aligns with the reported positive SELENOP–GPx relationship in women with PCOS [16]. Conversely, HO-1 is very readily induced in response to oxidative or electrophilic stress that is in excess of endogenous defenses through the KEAP1–NRF2 mechanism [14]. This pattern, which involves the reinforcement of the constitutive selenium-dependent pathway in the absence of the induction of the stress pathway, is indicative of a preemptive strengthening of the antioxidant defence pathway, not a response to the additional challenge of oxidative damage. It is important to note, however, that this interpretation is only a hypothesis without any specific oxidative-damage markers.

There are regional precedents for this interpretation. In the most recent comparison, higher levels of constitutive antioxidants, such as glutathione, superoxide dismutase, and lower levels of lipid-peroxidation and inflammatory markers were seen in lactating women than in non-lactating women in Maysan [21]—the opposite direction to that seen with newer selenium-dependent mediators. Previous studies in Iraq showed disturbed leptin levels and oxidant/antioxidant balance in obese people [22] and decreased levels of selenium and antioxidant enzymes with increased levels of oxidant enzymes in patients with unexplained infertility [23]. No one measured the respective panel, emphasizing its novelty. The more general literature on human-milk antioxidant capacity, including that related to reliance on bioactive milk proteins [27] and its disruption in metabolically complex lactation (gestational diabetes) [28], provides an additional background: the maternal serum compartment we examined is likely to contribute to the supply of milk antioxidants that eventually reach the milk.

A seeming contradiction should be noted. An old human study reported a decline in plasma selenium and glutathione-peroxidase activity in lactating women compared to control women,

which was suggested to be due to the export of selenium into milk [19]. The increased levels of SELENOP and GPx4 found in lactating women might be due to differences in selenium sufficiency or the stage of lactation, to the type of selenoproteins that were measured, or to differences in selenium adequacy between populations in our cohort, which might allow for up-regulation rather than depletion. This requires selenium quantification to be directly addressed, and it is a major limitation.

There are two correlations in the same group about which it is worth noting. The inverse association of irisin with SELENOP and the reduction in GPx4 with lactation duration indicate that the antioxidant response could be dynamic and may be reduced as lactation is initiated and early peri-partum lipid flux decreases. The unchanged SELENBP1 is coherent: It is a non-selenoprotein methanethiol oxidase that is not directly linked to immediate selenium-dependent antioxidant protection, but to cell differentiation [17,18], which makes its tracking of the lactational antioxidant response less surprising.

There are some caveats to these findings. First, the regression modelling of FGF21 was weak and non-significant ($R^2 \approx 0.10$), highlighting that the concentration of FGF21 is likely to be influenced by factors not included in the measured biomarker panel, including hepatic nutritional signals, fasting status, and the timing of assay, among others, and therefore, caution must be exercised when interpreting the inter-relationships of biomarkers. Second, the cross-sectional design does not allow for causal inference and is not able to capture the temporal course of these markers throughout the course of lactation. Third, there was no assessment of direct markers of oxidative damage, or of selenium intake or status, which restricts attribution to a specific mechanism. Fourth, the AUC has large confidence intervals around it for samples of this size, and so the discriminatory estimates should be taken with a grain of salt. Lastly, the single-centre regional sample was a strength as a within-centre comparison, but it limits generalizability. Reporting the null results of HO-1 and SELENBP1 in addition to the positive results avoids selective emphasis.

5. Future Perspectives

Longitudinal designs tracking these markers from the peri-partum period through weaning would clarify whether the antioxidant elevation is transient or sustained, and would test the time-dependent attenuation suggested by our cross-sectional correlations. Pairing the present panel with direct oxidative-damage indices and quantitative selenium status would allow the supply-driven

hypothesis to be tested directly. Given the selenium-dependence of SELENOP and GPx4, interventional work on selenium adequacy during lactation is a logical next step with translational relevance for maternal health in regions where selenium intake is variable [19,20]. Integration of these circulating markers with multiomic and machine-learning approaches may ultimately help define composite signatures of healthy metabolic adaptation to lactation.

6. Conclusion

In age- and BMI-matched women, lactation was associated with significantly higher serum SELENOP, GPx4, FGF21, and irisin—differences robust to multiple-comparison correction—while HO-1 and SELENBP1 were unchanged. The coordinated elevation of the constitutive selenium-dependent antioxidant arm without induction of stress-responsive HO-1 is consistent with a pre-emptive, supply-driven adaptation supporting redox balance during lactation, although direct oxidative and selenium-status measures are needed for confirmation. SELENOP, FGF21, and GPx4 were the most physiologically informative markers of lactational status, though their modest discrimination precludes stand-alone diagnostic use. These findings establish a regional evidence base for newer metabolic and selenium-related biomarkers in lactation and motivate longitudinal, selenium-informed follow-up.

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