Commentary

The LIME Framework: A Novel Conceptual NIRS Approach for Quantifying Muscle Metabolic Flexibility via Mitochondrial Oxidative Efficiency

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Metabolic flexibility (MF) defines an organism's capacity to shift between fat and carbohydrate oxidation in response to changes in substrate availability and energetic demand. However, most existing systemic and muscle-specific methods for assessing MF—such as indirect calorimetry (RER) and near-infrared spectroscopy (NIRS; k_HHb = 1/τ_HHb)—focus on static metrics and do not evaluate the dynamic, adaptive processes occurring at the tissue level. This concept paper advances a framework that shifts from a metabolic "state" to the dynamic "process" of adaptation. Three NIRS-derived indices are proposed for the non-invasive, local assessment of MF: LIMELip (Local Index of Mitochondrial Efficiency—Lipid), LIMECarb (Local Index of Mitochondrial Efficiency—Carbohydrate), and LIMEFlex (Local Index of Metabolic Flexibility). A pilot study would involve a comparison of metabolically healthy and insulin-resistant adults. NIRS-derived k_HHb (s^{-1}) would be obtained after brief arterial occlusion under fasting (lipid-dominant) and post-glucose (carbohydrate-dominant) conditions to probe oxygenation kinetics via the CO_2-mediated Bohr effect. Primary index definitions: LIMELip = k_HHb in the fasting state; LIMECarb = k_HHb 90 min post-glucose; LIMEFlex (%) = [(LIMECarb − LIMELip) / LIMELip] × 100. Operationally, "mitochondrial efficiency" is defined as faster post-occlusion reoxygenation; thus, a higher k_HHb (s^{-1}) denotes higher oxidative efficiency. LIMEFlex would be hypothesized to be lower in insulin-resistant individuals and to correlate positively with ΔRER. Additionally, state-specific k_HHb (LIMELip, LIMECarb) would correlate negatively with τ_PCr and positively with 1/τ_PCr (^{31}P-MRS), supporting k_HHb as a surrogate for in vivo mitochondrial capacity. If these indices are validated successfully, they could provide an accessible, non-invasive

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toolset for quantifying local metabolic adaptability, enabling earlier diagnosis and refined metabolic monitoring.

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Introduction

Metabolic flexibility (MF) is a core property of energy homeostasis in living organisms, indicating the ability to switch between fat and carbohydrate oxidation as substrate availability and demand change. This capacity is supported by mitochondria, hormonal regulation, and microcirculation. Loss of MF—metabolic inflexibility—is an early marker of insulin resistance, obesity, and type 2 diabetes[1][2][3] (Smith et al., 2018).

Skeletal muscle is central to MF: it disposes of most circulating glucose during insulin-stimulated states and contains the bulk of cellular mitochondria. Each fiber functions as an autonomous energy unit in which the rate of oxidative phosphorylation determines insulin sensitivity. Reduced mitochondrial activity in skeletal muscle limits the switch from fat to carbohydrate oxidation and contributes to insulin resistance. Thus, muscle not only reflects systemic metabolism; it helps shape it[4][1][2][3] (Smith et al., 2018).

Methodological Gap

Modern instrumental biochemical and biophysical methods for assessing metabolic flexibility have limitations. Systemic metrics such as the respiratory exchange ratio (RER) integrate across organs and cannot localize defects[4][3]. Local techniques offer specificity—phosphorus-31 MRS (^{31}P-MRS) is a reference for oxidative capacity—but broad use is limited by cost and complexity[5]. Near-infrared spectroscopy (NIRS) is non-invasive and tracks muscle oxygenation; the deoxyhemoglobin resaturation rate constant (k_HHb = 1/τ_HHb) correlates with τPCr, supporting NIRS as a functional surrogate of mitochondrial activity[4][6][7]. Nevertheless, most NIRS protocols assess a single metabolic state rather than the dynamic adaptability that defines MF[8][6].

This creates a practical gap: the absence of a tool to quantify the adaptive process of substrate switching at the tissue level. The concept advanced here is intended to bridge this gap by moving from static measurement of metabolic states to dynamic, local assessment of the adaptation process in skeletal

muscle, enabled by muscle NIRS. Importantly, it should be noted that while local and systemic measures each have inherent strengths and limitations, their combined use may offer a more nuanced and integrated understanding of metabolic inflexibility and its relevance for clinical practice. Table 1 summarizes and contrasts these approaches.

Method	Level	Measured parameter	Physiological meaning	Limitations
Indirect calorimetry	Systemic	RER (ΔRER)	Assesses whole-body substrate switching/flexibility[2]	Cannot localize muscle dysfunction; global metric
Lactate/FA Ox/CHO Ox Exercise test	Functional	Lactate, FATox, CHOox	Indirect marker of substrate switching and mitochondrial adaptation[9]	Requires exercise; low specificity for muscle site
^{31}P-MRS (phosphocreatine recovery)	Local	PCr recovery constant (τ_PCr)	Quantitative mitochondrial oxidative capacity[10]	High cost, low accessibility, large muscles only
NIRS (existing protocols)	Local	τ_HHb (resaturation time constant)	Surrogate of mitochondrial efficiency in one state[4][6][7]	Does not assess adaptability; affected by perfusion, fat, temperature
NIRS (proposed: LIMELip, LIMECarb, LIMEFlex)	Local/ systemic integration	State-specific k_HHb (LIMELip/LIMECarb), and normalized LIMEFlex	Dynamic kinetics of local O_2 utilization shift in response to substrate switching (present work)	Empirical validation/correlation with systemic indices required

Table 1. Methods for assessing MF and skeletal muscle mitochondrial function.

Abbreviations:

RER – respiratory exchange ratio,

FA Ox – fatty acid oxidation,

CHO Ox – carbohydrate oxidation,

^{31}P-MRS – phosphorus-31 magnetic resonance spectroscopy,

PCr – phosphocreatine,

τ – recovery time constant,

LIMELip – lipid phase NIRS index,

LIMECarb – carbohydrate phase NIRS index,

LIMEFlex – metabolic flexibility index.

NIRS – near-infrared spectroscopy

Methodological framework and physiological rationale

This concept paper introduces a framework of NIRS-derived indices for the non-invasive assessment of local MF, comprising two state-specific measures (LIMELip, LIMECarb) and a normalized flexibility index (LIMEFlex). The indices are derived from the HHb recovery rate constant k_HHb = 1/τ_HHb, a validated surrogate of local oxidative capacity, measured during a standardized vascular occlusion test over the brachioradialis muscle[4][6][7]. The protocol is designed to probe two distinct metabolic states: an overnight fast (lipid-dominant) and a postprandial condition following a 75-g oral glucose load (carbohydrate-dominant).

The methodological framework is grounded in a physiological cascade linking substrate selection to oxygenation kinetics: a shift toward carbohydrate use would increase mitochondrial CO_2/H^+ output, strengthen the Bohr effect, and right-shift the oxyhemoglobin dissociation curve[4][11]. This, in turn, would facilitate O_2 unloading and accelerate HHb recovery, captured by NIRS as the HHb recovery rate constant k_HHb = 1/τ_HHb (s^{-1}). Accordingly, rather than emphasizing oxygen delivery, the framework focuses on oxygen utilization driven by mitochondrial metabolism and regulated via CO_2/pH.

Interpretation

Higher values of LIMELip and LIMECarb indicate faster deoxyhemoglobin resaturation in the respective metabolic states, reflecting efficient muscle oxygen utilization and healthy microvascular function. Conversely, lower values imply slower resaturation, which may signal impaired oxygen uptake or disruptions in local muscle metabolism.

A positive LIMEFlex value reveals preserved metabolic flexibility, signifying the muscle's ability to increase oxygen use when switching from lipid to carbohydrate metabolism. Values close to zero or

negative suggest metabolic inflexibility, as seen in insulin resistance or prediabetes. The specific diagnostic threshold for LIMEFlex will require further validation.

Normalizing LIMEFlex to the lipid state (LIMELip) enables adjustment for individual baseline muscle function, ensuring the index reflects true substrate-switching dynamics rather than differences in resting-state physiology.

Proposed Experimental Protocol

The following section outlines a detailed experimental protocol proposed for future validation and potential application of the LIME framework. This protocol is conceptual and intended to demonstrate both the feasibility and the versatility of the proposed indices in relevant research or clinical settings. No empirical data have yet been collected; all procedures are described as recommendations for possible future implementation and further methodological development.

Study Design

A single-visit protocol would assess two metabolic states in each participant: fasted and 90 minutes after glucose ingestion. In a separate test–retest subsample (n≈8–10), the full protocol would be repeated on a second visit (7–14 days later) to assess between-visit reliability of k_HHb-based indices (ICC, CV, Bland–Altman), consistent with established NIRS protocols and repeatability reports[4][5][6].

A schematic overview of the proposed experimental workflow is presented in Figure 1.

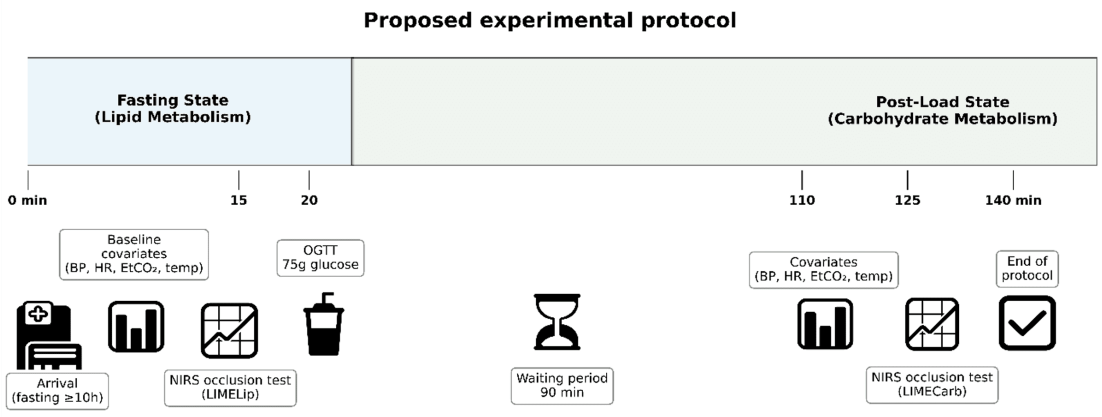

Figure 1. Schematic of the proposed two-state protocol: lipid-dominant vs. carbohydrate-dominant. This figure illustrates a hypothetical protocol; no data were collected.

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Participant Selection and Individualization

Two groups would ideally be defined:

1. metabolically healthy adults (BMI 20–25 kg/m², HOMA-IR <2.0),
2. insulin-resistant adults (BMI 27–35 kg/m², HOMA-IR ≥2.5 or HbA1c 5.7–6.4%).

These cutoffs represent widely used criteria for clinical and physiological studies.

Both groups would be matched for age (20–45 years) to control for age-related effects.

Key exclusion criteria would be diagnosed diabetes, significant cardiovascular disease, pregnancy, recent use of metabolism-modifying drugs, recent smoking (≤6 months), and contraindications to arterial occlusion.

It would be advisable to measure subcutaneous adipose tissue thickness at the sensor site using calipers and, where feasible, ultrasound. Participants whose subcutaneous fat would exceed the device's technical limit (1.5–2 cm, e.g., PortaMon) should either be analyzed separately or excluded, as this could significantly affect signal quality and physiological validity[5].

Standardization

Participants should refrain from vigorous exercise and alcohol for 48 hours and caffeine for 12 hours and should achieve ≥7 hours of sleep before testing. Testing would occur at a consistent time of day and menstrual cycle phase for women. Hydration would be standardized, with ad libitum water intake permitted up to 2 hours before testing. Room temperature, forearm position, and sensor location would need to be controlled throughout.

Measurement Protocol

A continuous-wave NIRS optode (e.g., Artinis PortaMon) should be positioned over the brachioradialis (one-third of the distance from the lateral epicondyle to the radial styloid) following manufacturer guidance. The probe should be secured with an elastic wrap and a light-shielding barrier. An upper-arm pneumatic cuff (10–12 cm wide) would be inflated to ≥50 mmHg above systolic blood pressure for 3 min to induce arterial occlusion. Testing should be performed first in the fasted state and then repeated 90 ± 5 min after ingestion of 75 g of glucose (in 250–300 mL of water, consumed within 5 min) to approximate an insulin-mediated, carbohydrate-dominant state. Each condition would include a 2-min baseline, 3-min occlusion, and 3–5-min reperfusion; trials contaminated by motion or optical artifacts should be

repeated to ensure signal quality. At least three NIRS reperfusion curves should be recorded in each metabolic state per participant.

The proposed optode placement over the brachioradialis is shown in Figure 2.

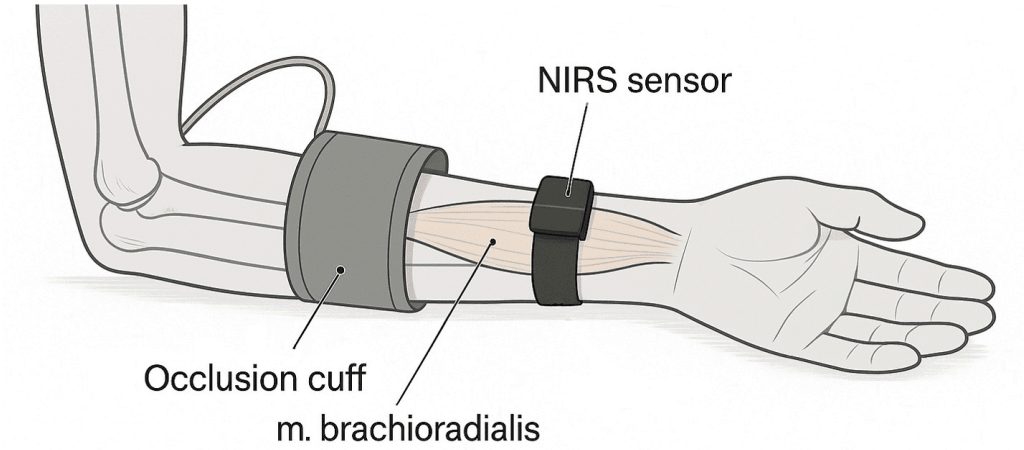

Figure 2. Placement of NIRS sensor and occlusion cuff on the forearm (m. brachioradialis).

Physiological Covariates

Baseline measurements should ideally include blood pressure, heart rate, respiratory rate, SpO_2, end-tidal CO_2 (nasal cannula), and 5-minute heart rate variability (RMSSD). Hydration status (hemoglobin, hematocrit, urine specific gravity) and blood lactate should also be measured. Plasma non-esterified fatty acids (NEFA) would be recorded at baseline as a covariate in between-subject analyses to account for variability in lipid availability; given its role in the causal pathway from substrate selection to k_HHb, NEFA would not be included in the primary within-subject model but would be examined in sensitivity analyses.

Data Acquisition and Processing

NIRS signals (O_2Hb, HHb) would be acquired at 10–50 Hz. Baseline stability and quality should be monitored in real time. Data would be filtered using a zero-phase Butterworth filter (0.2–0.5 Hz); brief artifacts should be interpolated, and detrending performed relative to the pre-occlusion baseline.

The primary outcome, k_HHb (the rate constant, where $k\_HHb = 1/\tau\_HHb$), would be obtained by mono-exponential fitting of the post-occlusion HHb recovery. The primary indices (LIMELip, LIMECarb,

LIMEFlex) would be based on unadjusted k_HHb. From the same fit, secondary metrics would be derived —half-time ($t_{1/2} = \ln(2)/k$) and initial slope ($k \cdot (y_\infty - y_0)$). As sensitivity analyses only, perfusion-adjusted variants (e.g., LIMELip/LPI, LIMECarb/LPI) would be explored to assess robustness to between-state differences in O_2 delivery; here, LPI denotes a simple perfusion proxy (e.g., the early reperfusion slope of tHb)[5].

Statistical Analysis

Data normality should be assessed by the Shapiro-Wilk test. Within-participant contrasts would ideally use paired t-tests or Wilcoxon; between-group comparisons should use independent t-tests or Mann-Whitney. Correlations with reference measures (^{31}P-MRS, RER, HOMA-IR) should be analyzed by Pearson or Spearman coefficients (95% CI). Diagnostic performance could be evaluated using ROC analysis (AUC, sensitivity, specificity, Youden's J). Reproducibility would be described by ICC and CV for both within- and between-day testing[5]. Results should be reported as mean ± SD or median [IQR], with $\alpha = 0.05$ and Holm-Bonferroni adjustment for multiple comparisons.

Upon implementation, all data and scripts should be deposited in open repositories (e.g., Harvard Dataverse)[5].

Additional Notes

There is currently no established international standard for NIRS protocols assessing metabolic flexibility; the present framework should draw on best practices from published research on muscle mitochondrial function and validated NIRS protocols[5][6][7].

Hypothetical Trajectories and Interpretation

The proposed experiment is expected to yield characteristic kinetic HHb recovery profiles across fasted and post-glucose metabolic states, allowing for the derivation of three indices: LIMELip (k_HHb_fasted), LIMECarb (k_HHb_glucose), and LIMEFlex = [(LIMECarb − LIMELip)/LIMELip] × 100%. In metabolically healthy individuals, glucose administration typically increases whole-body RQ, accelerates mitochondrial kinetics, and thus raises k_HHb (LIMECarb > LIMELip), reflecting preserved metabolic flexibility[4][3][11] (Smith et al., 2018). In insulin-resistant adults, both fasted and post-glucose recovery are blunted and nearly overlap, indicating metabolic inflexibility.[1][12]. Accordingly, positive LIMEFlex

signals effective substrate switching, while near-zero or negative values indicate metabolic inflexibility. Group-wise differences and state-dependent kinetics are visualized in Figure 3.

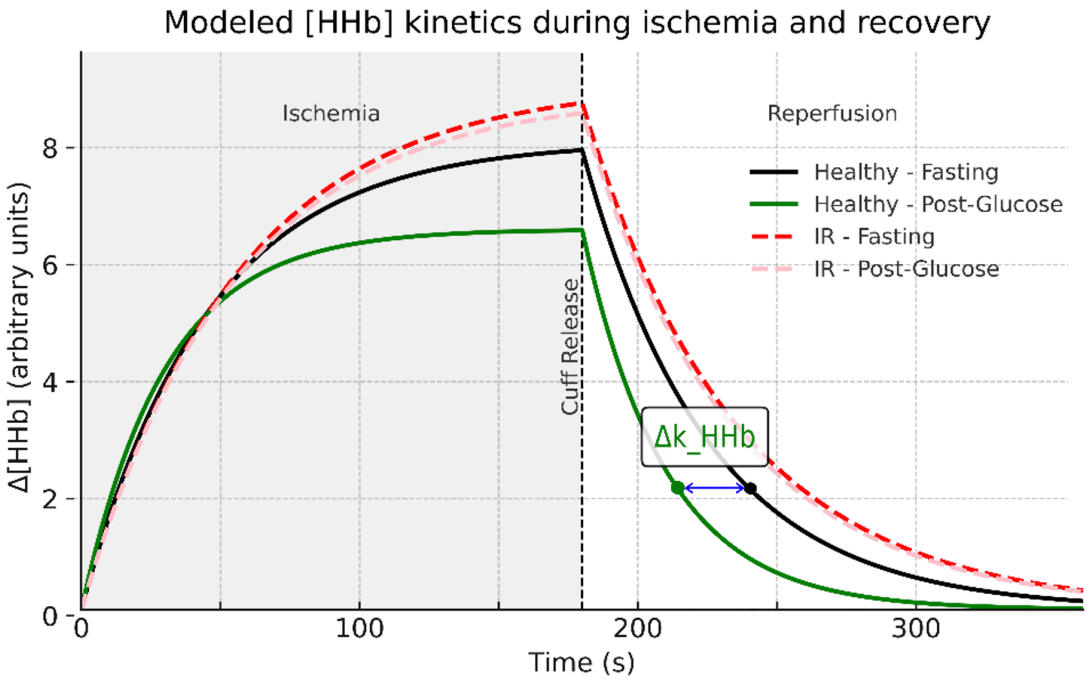

Figure 3. Hypothetical trajectories of post-occlusion HHb recovery in healthy (blue/green) vs. metabolically inflexible (red/rose) individuals. Flexible muscle shows higher k_HHb in the post-glucose state (LIMECarb > LIMELip); inflexible muscle shows minimal between-state differences. (Mechanistic rationale in text.)

Validation Plan

To establish validity, relationships with reference measures would be investigated. Criterion validity would be tested by hypothesizing a negative correlation between LIMEFlex (%) and HOMA-IR[13] and a positive correlation between LIMEFlex (%) and ΔRER (post-glucose - fasted)[3]. Concurrent validity would be assessed by hypothesizing a positive correlation between k_HHb (s^{-1}) and the PCr recovery rate constant from ^{31}P-MRS (k_PCr = 1/τ_PCr, s^{-1}), and, equivalently, a negative correlation between k_HHb (s^{-1}) and τ_PCr (s)[10]. This constellation would support k_HHb as a surrogate of in vivo mitochondrial capacity. For illustration, Figure 4 presents a hypothetical scatterplot depicting the expected negative association between LIMEFlex and HOMA-IR.

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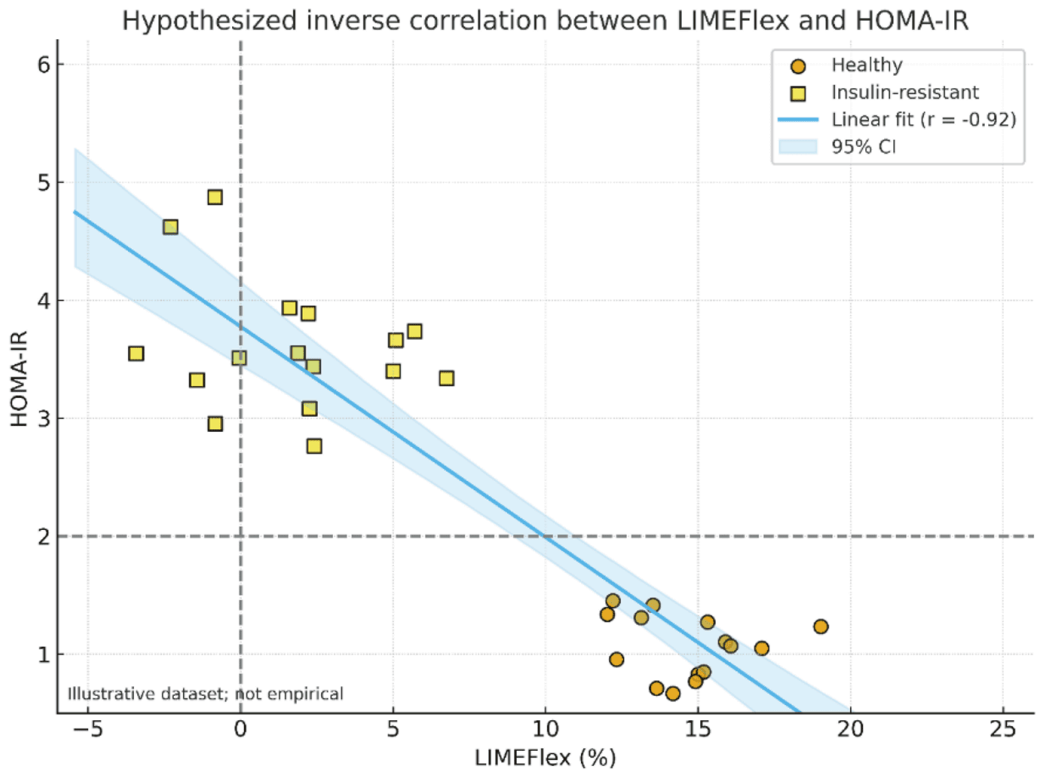

Figure 4. Hypothetical relationship between LIMEFlex and HOMA-IR: lower LIMEFlex (reduced local flexibility) would be associated with higher HOMA-IR (greater insulin resistance); points are simulated, and the line shows OLS fit with 95% CI.

Potential Limitations and Conceptual Boundaries

The interpretation of k_HHb kinetics may be influenced by factors not directly measured by NIRS, such as muscle fiber-type composition and microvascular dysfunction. The measurements are also sensitive to procedural and environmental rigor. These limitations, inherent to the methodology, delineate the boundaries of interpretation but do not invalidate the proposed framework. To mitigate these concerns, the protocol incorporates physiological covariate monitoring, perfusion correction (LPI), and stringent standardization to control for confounders and strengthen causal inference[4].

While the NIRS-derived 'deoxyhemoglobin' HHb signal in skeletal muscle reflects a combined pool of deoxygenated hemoglobin and myoglobin, all major physiological NIRS indices—including the rates of reoxygenation and their substrate dependence—are valid and reliably reflect tissue-level oxygen

utilization and microvascular function regardless of the precise molecular source. This is supported by both methodological consensus and practical application in muscle oximetry studies.[4][14]

Furthermore, non-significant correlations would not invalidate the NIRS measurement; instead, they would indicate that the present physiological interpretation of k_HHb—and/or the study's precision and power—requires reevaluation.

Clinical Implications and Future Directions

If validated, the LIME indices could provide a non-invasive toolset for assessing mitochondrial adaptability. Demonstrating that these NIRS-derived indices reflect substrate-sensitive, Bohr-mediated adaptation would position LIMEFlex as a bridge between local physiology and whole-body status, enabling early detection of metabolic rigidity and monitoring of intervention efficacy[3]. Applications span early-stage type 2 diabetes, sarcopenia, and exercise prescription personalization[12].

Clinical translation would require validation in larger cohorts against the PCr recovery rate constant (k_PCr) from ^{31}P-MRS and ΔRER (indirect calorimetry), establishing diagnostic thresholds for LIMEFlex, demonstrating intervention sensitivity, and mapping muscle-specific patterns across populations.

Conclusion

This concept paper proposes a novel NIRS-based framework to address a critical methodological gap in MF assessment. The introduced indices—LIMELip, LIMECarb, and LIMEFlex—shift the focus from static measurement of mitochondrial capacity to dynamic quantification of its adaptive function in vivo. By linking muscle oxygen kinetics to substrate competition through the physiological mediation of the CO_2-dependent Bohr effect, this approach translates a fundamental metabolic process into an accessible, non-invasive optical measurement. If validated, this methodology could be useful for the early detection of tissue-specific metabolic dysfunction, for tailoring therapeutic strategies, and for advancing research on metabolic flexibility.

Statements and Declarations

Funding

No funding was received for this work.

Conflicts of Interest

The author declares no competing interests.

Author Note

The views expressed in this manuscript are solely those of the author and do not necessarily represent those of Harokopio University.

Use of AI

The author used OpenAI's ChatGPT for language polishing and for drafting a statistical analysis plan for the proposed study (no datasets were analyzed and no inferential statistics were executed). The author retained full control over the scientific content and final decisions.

Ethics statement

This conceptual/methodological proposal reports no research involving human participants or animals.

Data availability

No new data were generated or analyzed in this study.

Code availability

All figures are theoretical/conceptual illustrations generated for explanatory purposes. No raw data or code is available/applicable.

Author contributions (CRediT)

A.G.M.: conceptualization; methodology; visualization; writing—original draft; writing—review & editing.

Acknowledgments

This is a conceptual protocol; no human participants were enrolled and no interventions were conducted.

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Declarations

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