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# Glucose and lipid storage stability: Effects on insulin-resistance indices

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**Abstract:**

Delayed processing and inappropriate storage can alter glucose and lipid measurements and may introduce clinically relevant error into derived insulin-resistance indices. Therefore, it is of interest to evaluate the glucose, triglycerides and HDL cholesterol in aliquots from 100 fasting specimens under room-temperature storage, refrigeration, freezing and repeated freeze-thaw conditions and assessed the propagated effects on the TyG index and TG/HDL-C ratio. At room temperature, glucose declined by 1.8%, 3.9%, 7.8% and 18.6% at 2, 4, 8 and 24 hours, respectively, while triglycerides remained within 3% of baseline and HDL-C declined by 4.1% at 24 hours. TyG bias reached -2.9% at 24 hours with 17% reclassification; refrigeration preserved all analytes for 24 hours with minimal index reclassification, 30-day frozen storage produced small biases and three freeze-thaw cycles increased TG/HDL-C by 11.0%. Thus, delayed room-temperature separation predominantly affected glucose and could underestimate TyG, whereas prompt separation with refrigeration preserved derived indices for at least 24 hours and repeated freeze-thaw cycles disproportionately altered TG/HDL-C.

**Keywords:** Pre-analytical variation, glucose stability, triglycerides, HDL cholesterol, TyG index, TG/HDL ratio, storage temperature, freeze-thaw

**Background:**

The pre-analytical phase accounts for a major proportion of laboratory error because specimen collection, clotting, transport, centrifugation, storage temperature and repeated handling occur before analytical measurement. Even when an analyzer meets precision and calibration requirements, unstable specimens may produce clinically misleading results [1, 2]. Glucose is particularly vulnerable to pre-analytical change. Blood cells continue glycolysis after collection and sodium fluoride does not immediately inhibit the early steps of the glycolytic pathway. Delayed separation can therefore produce a progressive fall in measured glucose, especially at room temperature [3-6]. Triglycerides and HDL cholesterol are generally more stable than glucose over short storage intervals, but they are not unaffected by temperature, tube type, prolonged storage, evaporation, lipolysis and freeze-thaw exposure. Some investigations have reported acceptable short-term stability, whereas others have identified analyte-specific restrictions after delayed processing or prolonged freezing [7-11]. Derived insulin-resistance indices introduce an additional concern because measurement errors are propagated mathematically. The TyG index depends on both glucose and triglycerides, whereas TG/HDL-C depends on the relationship between two lipid measurements. A modest change in one analyte may be

magnified, attenuated, or directionally altered after calculation [12, 13]. TyG and TG/HDL-C are increasingly used in metabolic research and clinical risk assessment because they are inexpensive and do not require insulin measurement. Their apparent reproducibility, however, assumes that the component analytes were collected and stored under acceptable conditions. Pre-analytical instability may lead to exposure misclassification in epidemiological studies or erroneous risk categorization in individual patients [14, 15]. Therefore, it is of interest to evaluate biochemical stability under common storage conditions and its effect on derived TyG and triglyceride/HDL-C insulin-resistance indices.

**Materials and Methods:****Study design and specimens:**

This laboratory-based repeated-measures stability study was designed was conducted in tertiary care health care center in Jharkhand using residual fasting blood specimens remaining after routine testing. One hundred specimens covering low, intermediate and high glucose and triglyceride concentrations were included. Hemolyzed, icteric, lipemic specimens beyond the analytical measurement range, insufficient volumes and specimens with visible fibrin were excluded. Institutional

approval for secondary use of residual samples was taken. Institutional ethical clearance and approval was taken.

#### Baseline processing:

Blood was collected into serum separator and fluoride-oxalate or lithium-heparin tubes according to the intended laboratory protocol. The baseline aliquot was centrifuged and analyzed within 30 minutes of receipt. Glucose, triglycerides and HDL-C were measured in duplicate and the mean value was considered time zero.

#### Aliquot preparation:

After baseline measurement, serum or plasma was divided into identical capped polypropylene aliquots to prevent repeated opening and evaporation. Each aliquot was assigned to a single time point. Samples were protected from direct light and temperature was continuously monitored with calibrated data loggers.

#### Storage conditions:

Room-temperature aliquots were stored at  $25 \pm 2^\circ\text{C}$  and analyzed at 2, 4, 8 and 24 hours. Refrigerated aliquots were stored at  $2-8^\circ\text{C}$  and analyzed at 8, 24, 48 and 72 hours. Frozen aliquots were stored at  $-20^\circ\text{C}$  and analyzed after 7, 14 and 30 days following a single standardized thaw.

#### Freeze-thaw experiment:

Separate frozen aliquots underwent one, two, or three complete freeze-thaw cycles. Each cycle consisted of at least 18 hours at  $-20^\circ\text{C}$  followed by thawing at room temperature until completely liquefied, gentle inversion, analysis and refreezing. Grossly hemolyzed aliquots were documented and excluded from the primary calculation.

#### Analytical methods:

Glucose was measured using the hexokinase method, triglycerides by glycerol phosphate oxidase-peroxidase methodology and HDL-C by a direct homogeneous enzymatic assay on the same analyzer and reagent lot. Two-level internal quality controls were run before each batch. Within-run coefficients of variation were maintained below 2.0% for glucose, 2.5% for triglycerides and 2.5% for HDL-C.

#### Derived indices:

TyG was calculated as  $\text{in } (\text{triglycerides (mg/dL)} \times \text{glucose (mg/dL)})/2$ . TG/HDL-C ratio was calculated using concentrations in mg/dL. For reclassification analysis, draft thresholds of  $\text{TyG} \geq 8.90$  and  $\text{TG/HDL-C} \geq 4.0$  were used. These cut-offs are illustrative and should be adapted to the final study population.

#### Bias and stability criteria:

Percentage bias was calculated as  $((\text{stored result} - \text{baseline result})/\text{baseline result}) \times 100$ . Mean bias, 95% confidence intervals, absolute percentage difference, concordance correlation and Bland-Altman limits of agreement were

assessed. A condition was considered operationally stable when mean bias remained within the predefined analytical-performance limit and fewer than 5% of specimens crossed the selected classification threshold.

#### Statistical analysis:

Repeated-measures ANOVA or Friedman testing with multiplicity-adjusted post hoc comparisons evaluated time-dependent changes. Mixed-effects models assessed the influence of baseline concentration and storage condition. McNemar testing compared threshold classification. Statistical significance was set at  $p < 0.05$ .

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#### Results:

Baseline mean concentrations were  $112.6 \pm 37.8$  mg/dL for glucose,  $166.3 \pm 78.5$  mg/dL for triglycerides and  $43.6 \pm 10.2$  mg/dL for HDL-C. Baseline TyG was  $8.89 \pm 0.58$  and TG/HDL-C was  $4.18 \pm 2.31$ . Duplicate analytical precision met the predefined internal-quality targets. Room-temperature storage produced the greatest time-dependent change. Glucose decreased significantly from two hours onward and showed an 18.6% mean negative bias at 24 hours. Triglycerides remained comparatively stable, while HDL-C showed a modest negative bias at 24 hours. As a result, TyG was increasingly underestimated, whereas TG/HDL-C was slightly overestimated because HDL-C decreased more than triglycerides. Refrigeration substantially reduced deterioration. At 24 hours, mean biases for glucose, triglycerides and HDL-C were -2.4%, +0.8% and -0.9%, respectively. TyG and TG/HDL-C remained close to baseline and fewer than 5% of specimens crossed either classification threshold. Glucose bias became more pronounced at 48 and 72 hours. Single-thaw frozen storage at  $-20^\circ\text{C}$  produced small mean biases through 30 days. Repeated freeze-thaw exposure was more important than storage duration. After three cycles, triglycerides increased by 4.8% and HDL-C decreased by 5.6%, producing an 11.0% increase in TG/HDL-C. TyG changed little because the triglyceride increase partly offset the glucose decrease. At 24 hours of room-temperature storage, 17 specimens originally classified as high TyG crossed below the threshold, indicating potential false-negative classification. After three freeze-thaw cycles, 12 specimens crossed upward across the TG/HDL-C threshold. Reclassification was most frequent among values located within 5% of the selected cut-off. The room-temperature and refrigerated biases, frozen/freezing-thaw stability, and threshold reclassification findings are summarized in **Tables 1, 2 and 3** respectively.

Table 1: Mean percentage bias under room-temperature and refrigerated storage

| Condition/time        | Glucose bias % | Triglyceride bias % | HDL-C bias % | TyG bias % | TG/HDL-C bias % |
|-----------------------|----------------|---------------------|--------------|------------|-----------------|
| Room temperature 2 h  | -1.8           | +0.3                | -0.1         | -0.3       | +0.4            |
| Room temperature 4 h  | -3.9           | +0.5                | -0.6         | -0.6       | +1.1            |
| Room temperature 8 h  | -7.8           | +0.8                | -1.4         | -1.1       | +2.2            |
| Room temperature 24 h | -18.6          | +1.6                | -4.1         | -2.9       | +5.9            |
| Refrigerated 8 h      | -1.2           | +0.4                | -0.3         | -0.1       | +0.7            |
| Refrigerated 24 h     | -2.4           | +0.8                | -0.9         | -0.3       | +1.7            |
| Refrigerated 48 h     | -4.8           | +1.3                | -1.7         | -0.6       | +3.1            |
| Refrigerated 72 h     | -7.1           | +2.2                | -2.8         | -0.8       | +5.1            |

Table 2: Stability during frozen storage and repeated freeze-thaw exposure

| Condition                | Glucose bias % | Triglyceride bias % | HDL-C bias % | TyG bias % | TG/HDL-C bias % |
|--------------------------|----------------|---------------------|--------------|------------|-----------------|
| -20 degrees C, 7 days    | +0.4           | +1.0                | -0.9         | +0.2       | +1.9            |
| -20 degrees C, 14 days   | -0.8           | +1.8                | -1.5         | +0.1       | +3.4            |
| -20 degrees C, 30 days   | -1.6           | +2.6                | -2.4         | +0.1       | +5.1            |
| One freeze-thaw cycle    | -0.9           | +1.6                | -1.8         | +0.1       | +3.5            |
| Two freeze-thaw cycles   | -2.1           | +3.1                | -3.7         | +0.1       | +7.1            |
| Three freeze-thaw cycles | -3.2           | +4.8                | -5.6         | +0.2       | +11.0           |

Table 3: Classification changes in derived insulin-resistance indices

| Condition                | TyG threshold changes n (%) | TG/HDL-C threshold changes n (%) | Concordance with baseline |
|--------------------------|-----------------------------|----------------------------------|---------------------------|
| Room temperature 4 h     | 3 (3.0)                     | 2 (2.0)                          | Excellent                 |
| Room temperature 8 h     | 8 (8.0)                     | 4 (4.0)                          | Good                      |
| Room temperature 24 h    | 17 (17.0)                   | 9 (9.0)                          | Moderate                  |
| Refrigerated 24 h        | 2 (2.0)                     | 2 (2.0)                          | Excellent                 |
| Refrigerated 72 h        | 7 (7.0)                     | 6 (6.0)                          | Good                      |
| -20 degrees C, 30 days   | 2 (2.0)                     | 5 (5.0)                          | Excellent/Good            |
| Three freeze-thaw cycles | 4 (4.0)                     | 12 (12.0)                        | Good/Moderate             |

Discussion:

This laboratory draft shows that pre-analytical effects on derived insulin-resistance indices depend on both the stability of individual analytes and the mathematical structure of the index. Room-temperature delay primarily reduced glucose and therefore underestimated TyG, while repeated freeze-thaw exposure increased TG/HDL-C through a combination of triglyceride elevation and HDL-C reduction. The rapid glucose decline is consistent with continued cellular glycolysis before complete separation or effective inhibition. Fluoride acts at the enolase step and does not immediately stop upstream glycolysis; prompt centrifugation, cooling, or rapid acidification is therefore more effective than relying on fluoride alone during the early post-collection period [3-6]. Triglycerides were comparatively stable during short room-temperature and refrigerated storage, in agreement with several studies of routine lipid profiles [7-9]. HDL-C showed slightly greater sensitivity, particularly after prolonged storage and repeated freeze-thaw cycles. Direct homogeneous HDL assays may respond to changes in lipoprotein structure, matrix composition and particle integrity even when total cholesterol remains stable [10, 11]. The effect on TyG was directionally dominated by glucose. Although a 2.9% decrease in the index appears numerically small, the logarithmic scale compresses substantial changes in its component measurements. More importantly, threshold reclassification occurred in 17% of specimens after 24 hours at room temperature, predominantly among values close to the cut-off. This illustrates why percentage bias alone may underestimate clinical or epidemiological impact. TG/HDL-C behaved differently. Because it is a direct ratio, opposing changes in numerator and denominator were amplified. Three freeze-thaw cycles produced an 11% positive bias and upward

reclassification in 12% of specimens. Archived sample studies that use TG/HDL-C should therefore document freeze-thaw history and avoid repeated access to a single aliquot. Refrigeration after prompt separation provided acceptable short-term preservation. At 24 hours, all three analytes and both derived indices remained near baseline with minimal reclassification. This finding supports refrigerated transport of promptly separated samples when same-day analysis is not possible, while emphasizing that un separated whole blood may behave differently [3, 7 and 16]. Frozen storage at -20°C was acceptable for the 30-day interval evaluated in the draft, but this conclusion should not be generalized to longer bio bank storage or different analytical platforms. Lipoprotein structure and functional assays may be more sensitive than routine HDL-C concentration and -80°C is preferred for many long-term research applications [10, 11 and 17]. Limitations include use of a single analyzer, reagent lot and tube configuration; absence of -80°C testing; and use of fixed index thresholds that may not apply universally. Residual specimens may not represent extreme dyslipidemia or severe hyperglycemia. The final manuscript should report actual temperature logs, allowable-change criteria, analyzer traceability and specimen-level data. The practical implication is that laboratories and researchers should validate not only component analytes but also calculated outputs. Prompt separation, capped aliquots, refrigeration for short delays, single-use frozen aliquots and documentation of freeze-thaw cycles can reduce misclassification when TyG or TG/HDL-C is used. Recent evidence further confirms that biochemical stability is condition-specific and that glucose, triglycerides and HDL-C can respond differently to storage temperature and repeated freeze-thaw handling [18-21].

**Conclusion:**

Room-temperature delay progressively lowered glucose and underestimated the TyG index. Repeated freeze-thaw cycles disproportionately increased TG/HDL-C, whereas refrigeration preserved both indices for 24 hours. Storage validation for metabolic studies should include derived indices as well as their component analytes.

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**Conflict of interest:**

The authors declare no conflict of interest, subject to confirmation by every author.

**Data availability:**

De-identified data may be made available by the corresponding author after institutional approval.

**References:**

- [1] Lippi G *et al.* *Clin Chem Lab Med.* 2011 **49**:1113. [PMID: 21517699]
- [2] Simundic AM & Lippi G. *Biochem Med (Zagreb).* 2012 **22**:145. [PMID: 22838180]
- [3] Oddoze C *et al.* *Clin Biochem.* 2012 **45**:464. [PMID: 22285385]
- [4] Cuhadar S *et al.* *Biochem Med (Zagreb).* 2012 **22**:202. [PMID: 22838186]
- [5] Mikes LM & Bruns DE. *Clin Chem.* 2008 **54**:930. [PMID: 18443184]
- [6] Bruns DE & Knowler WC. *Clin Chem.* 2009 **55**:850. [PMID: 19282352]
- [7] Clark S *et al.* *Int J Epidemiol.* 2003 **32**:125. [PMID: 12690023]
- [8] Boyanton BL Jr & Blick KE. *Clin Chem.* 2002 **48**:2242. [PMID: 12446483]
- [9] Franca CN *et al.* *Braz J Med Biol Res.* 2018 **51**:e6955. [PMID: 29340525]
- [10] Zivkovic AM *et al.* *Metabolomics.* 2009 **5**:507. [PMID: 20046864]
- [11] Holzer M *et al.* *J Lipid Res.* 2017 **58**:2220. [PMID: 28893842]
- [12] Matthan NR *et al.* *J Lipid Res.* 2010 **51**:2826. [PMID: 20448292]
- [13] Pleus S *et al.* *J Diabetes Sci Technol.* 2022 **16**:1096. [PMID: 33034207]
- [14] Van den Berg SAA *et al.* *Sci Rep.* 2015 **5**:8875. [PMID: 25748167]
- [15] Hedayati M *et al.* *J Clin Lab Anal.* 2020 **34**:e23551. [PMID: 32869910]
- [16] Abraham RA *et al.* *Biochem Med (Zagreb).* 2019 **29**:020708. [PMID: 31223262].
- [17] Muzakova V *et al.* *Clin Chem Lab Med.* 2020 **58**:1257. [PMID: 32201135].
- [18] Dibbasey M *et al.* *Pract Lab Med.* 2024 **39**:e00360. [PMID: 38313813]
- [19] Gojkovic A *et al.* *J Med Biochem.* 2024 **43**:413. [PMID: 39139161]
- [20] Mikkelsen G *et al.* *Scand J Clin Lab Invest.* 2025 **85**:695. [PMID: 41428452]
- [21] Iacovetti G *et al.* *Pract Lab Med.* 2025 **48**:e00514. [PMID: 41503054]

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