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Biological Variation Of Oxyntomodulin Assays In The Follow-Up Of Patients With Diabetes: A Commentary

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Abstract

Oxyntomodulin (OXM), a 37-amino-acid proglucagon-derived peptide co-secreted with GLP-1 from intestinal L-cells, has emerged as a molecule of substantial translational interest due to its dual agonism at GLP-1 and glucagon receptors. While endogenous OXM is increasingly proposed as a potential biomarker for monitoring enteroendocrine function, bariatric surgery outcomes, and incretin-based therapies in patients with diabetes, its clinical utility for serial patient follow-up remains unverified. Meaningful interpretation of longitudinal changes requires robust Biological Variation (BV) data-specifically within-subject (CVI)) and between-subject (CVG) coefficients of variation-to establish reference change values (RCVs) and analytical performance specifications. Currently, no appraised BV estimates exist for OXM in diabetic populations. This commentary highlights major barriers to generating reliable OXM BV data, including cross-reactivity with glicentin and glucagon in commercial immunoassays, analytical imprecision at low picomolar concentrations, and pre-analytical degradation by dipeptidyl peptidase-4 (DPP-4). Furthermore, diabetes-specific confounders such as altered gastric emptying, pharmacotherapy, bariatric surgery, and renal impairment complicate variance structures. Before OXM can transition from a research biomarker to a clinical monitoring tool, investigators must validate assay specificity-potentially utilizing immunoaffinity liquid chromatography–tandem mass spectrometry-and conduct rigorous BV studies adhering to European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) standards.

Keywords: Oxyntomodulin; Biological variation; Reference change value; Diabetes mellitus; Immunoassay cross-reactivity.

Introduction

Oxyntomodulin (OXM) has moved from a physiological curiosity to a molecule of genuine translational interest [1]. A 37-amino-acid product of proglucagon processing, it is released from intestinal L-cells alongside GLP-1, GLP-2, glicentin and peptide YY in response to nutrients [1,2]. It activates both the GLP-1 receptor and the glucagon receptor, reducing appetite, increasing energy expenditure and modulating glucose handling [1,2]. That dual action has inspired a generation of unimolecular co-agonists, several of which are now in clinical development or use for obesity and type 2 diabetes [2]. It is therefore natural to ask whether endogenous OXM could serve as a biomarker in the follow-up of people with diabetes, whether to characterise enteroendocrine function, to monitor response to bariatric surgery or incretin-based therapy, or to stratify patients [3,4]. A biomarker is only as useful as our ability to interpret a change in its value. That interpretation depends on the interplay of analytical imprecision, within-individual biological variation and between-individual variation [5]. For OXM, this framework has barely been applied. This commentary argues that, before OXM measurements are used for serial monitoring, the field needs rigorous Biological Variation (BV) data generated with assays whose analytical limitations are understood. Without them, apparent changes in OXM in a patient may reflect noise rather than physiology or treatment.

Why Biological Variation Matters for Serial Monitoring

BV studies estimate the within-subject Coefficient of Variation (CVI) and the between-subject Coefficient of Variation (CVG), usually from repeated sampling of individuals under standardised conditions, followed by variance decomposition that separates analytical from biological components [5]. These estimates drive three practical quantities. The first is the Reference Change Value (RCV), the minimum difference between two consecutive results that exceeds expected analytical plus within-subject variation at

a chosen probability [5]. It is commonly calculated as the square root of two multiplied by a z-score and by the combined analytical and within-subject CV. The second is the index of individuality, the ratio of within- to between-subject variation. A low index means individuals occupy narrow personal ranges within a wide population range, so population reference intervals are insensitive and serial, subject-based comparison is preferable [5]. A high index means population-based decision limits work well. The third is the set of analytical performance specifications, conventionally derived so that imprecision consumes only a modest fraction of within-subject variation [5,6]. For diabetes follow-up, where decisions are made by comparing a patient with their own previous values, the RCV is the quantity that matters most. If the RCV for OXM is very large, a clinically plausible response to a drug or surgery may be indistinguishable from random fluctuation in an individual patient, even when group-level effects are clear.

The Analytical Problem: What is an OXM Assay Actually Measuring?

OXM is difficult to measure [7]. Circulating concentrations are low, typically in the picomolar range in the fasting state, with postprandial excursions of limited magnitude [7]. More importantly, OXM shares its sequence with glucagon, with an eight-residue C-terminal extension [1,7]. Antibodies raised against OXM may cross-react with glicentin, which contains the whole OXM sequence, with other proglucagon-derived peptides, or with glucagon itself depending on epitope [7,8]. Antibodies targeting the shared glucagon-like region cannot distinguish OXM from larger or smaller fragments [7]. Historical radioimmunoassays often reported what was effectively a mixture of enteroglucagon-like immunoreactivity, and different commercial ELISAs have shown markedly different absolute values and, in some comparisons, poor agreement [7,8]. This matters for BV in two ways. First, assay specificity determines what is being varied. A method that partly detects glicentin will combine the biological variation of two peptides that are co-secreted but differ in clearance and metabolism [8]. Second, imprecision near the lower limit of quantification inflates the analytical component, and at low concentrations CVA may dominate. If (CVA) exceeds roughly half of (CVI), the performance of the assay is inadequate for monitoring regardless of how stable the analyte is [6]. Mass spectrometry offers a route to better specificity, particularly with immunoaffinity enrichment and targeted detection of OXM-specific peptides [3,7]. Such methods remain technically demanding, and sensitivity at physiological concentrations is a persistent challenge [7]. Whichever platform is used, BV estimates are method-specific: a (CVI) derived on one immunoassay cannot be transferred to another, and reports should identify the antibody pair, calibrator and any extraction step [9].

Pre-Analytical Variation: the Hidden Component

OXM, like other proglucagon-derived peptides, is susceptible to degradation after sampling [3,7]. Dipeptidyl peptidase-4 (DPP-4) cleaves the N-terminal histidine-serine, and other proteases act on the molecule in vitro [3]. Studies of gut peptides consistently show that collection tubes without protease inhibitors, delays before centrifugation, warm storage and repeated freeze-thaw cycles all introduce error. Where protocols differ between visits or sites, pre-analytical variation can masquerade as biological variation and inflate the apparent (CVI). A credible BV study must therefore standardise and document collection: tube type with DPP-4 and protease inhibition, immediate chilling, defined centrifugation time, single-aliquot storage at ultra-low temperature, and analysis of all samples from one individual in the same run, with duplicate measurement, which allows analytical variance to be separated cleanly [9]. The tools exist: the Biological Variation Data Critical Appraisal Checklist (BIVAC) and the EFLM guidance on BV study design provide explicit requirements, and a study that fails them should not be used to derive RCVs [9,10].

Sources of Biological Variation Specific to Diabetes

BV is not a fixed property of an analyte: it depends on the population studied and the conditions under which samples are drawn [5]. For OXM in diabetes, several determinants deserve attention.

Nutrient Stimulation: OXM secretion is meal-dependent [1]. Fasting, postprandial and glucose-challenge values answer different questions and will have different variance structures. Meal composition, gastric emptying rate and timing relative to the last meal all contribute. Diabetes itself alters gastric emptying, particularly in the presence of autonomic neuropathy, so within-subject variability of postprandial OXM may be higher in patients with complications.

Glycaemic State: Hyperglycaemia and insulin resistance may modify L-cell responses, although the evidence for GLP-1 in type 2 diabetes suggests that secretory capacity is relatively preserved while the incretin effect is blunted. Whether OXM follows the same pattern is not settled, and the answer affects both mean level and variability [3,4].

Medication: Treatment is a major confounder. DPP-4 inhibitors alter the molecular forms of circulating peptides. Metformin has been associated with increased GLP-1 levels, which may reflect effects on L-cell activity or bile acid handling. GLP-1 receptor agonists, and now dual and triple agonists that are OXM-like, may cross-react in some immunoassays and may also change endogenous secretion through altered intake and weight [2,7]. Changes in medication between visits will therefore change both the signal and its variability, and BV estimates derived in untreated or stably treated individuals may not transfer to patients undergoing therapeutic change.

Surgery and Weight Change: Roux-en-Y gastric bypass and sleeve gastrectomy produce large increases in postprandial OXM, GLP-1 and PYY [3,8]. In this setting the signal is large relative to expected noise, so OXM may well be informative at the group level,

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but whether it tracks individual outcomes, such as weight loss or diabetes remission, remains unresolved and again depends on individual-level variance.

Renal function and Other Covariates: Peptides are cleared in part by the kidney, and diabetic kidney disease is common. Age, sex, body mass index, circadian timing and physical activity are additional candidates [4]. Longer-interval sampling, as occurs in routine follow-up, includes seasonal, dietary and weight-related drift that short-interval BV studies do not capture.

What is Known and what is Not

The literature on gut hormones offers some orientation. For peptides released in response to meals, within-subject variation of stimulated responses is typically substantial, and the index of individuality is often higher than for tightly regulated analytes. As far as I am aware, there is no robust, appraised estimate of (CVI) and (CVG) for OXM in people with diabetes [10]. Existing studies tend to report group means in small cohorts, use heterogeneous assays and rarely include replicate sampling designed for variance decomposition [4,7]. Readers should treat any figure quoted for OXM variability with caution unless the underlying design is explicit, and should check the current literature and the EFLM BV database before relying on a number [10]. This absence is not merely an academic gap. It means that studies using serial OXM as an outcome or marker have no principled basis for judging whether an individual change is meaningful, and that sample size calculations may be based on assumptions about variance that have not been verified.

Practical İmplications and a Proposed Design

For investigators, three recommendations follow. First, characterise the assay before characterising the biology: establish specificity against glicentin and glucagon, limits of quantification, within- and between-run imprecision at physiological concentrations, and stability under the planned handling conditions [7,8]. Second, design BV studies deliberately. Recruit adequate numbers of well-characterised participants with diabetes, stratified by treatment and, if possible, by complication status; collect repeated samples at defined intervals under standardised fasting and, separately, standardised meal-stimulated conditions; analyse in duplicate with randomised run order; test for outliers and homogeneity of variance; and use nested ANOVA or a mixed model to estimate (CVA), (CVI) and (CVG) with confidence intervals [9]. Third, report results for the specific method, population and interval, and distinguish short-term from long-term variation, since follow-up occurs over months rather than days [9].

For clinicians and trialists considering OXM measurement now, the safest position is that OXM is a research biomarker [4]. Results should not drive individual management decisions, and group-level changes in intervention studies should be interpreted alongside the measurement characteristics of the assay and not assumed to represent individual response.

Conclusion

Oxyntomodulin sits at an attractive intersection of physiology and pharmacology, and interest in measuring it in diabetes will only grow as OXM-inspired therapies expand [1,2]. But the value of any serial biomarker is bounded by its analytical specificity, its pre-analytical fragility and its biological variation [5,7]. At present, each of these is incompletely characterised for OXM. Closing the gap requires specific, validated assays, carefully controlled sampling and properly designed BV studies in the populations in which the marker will be used [7,9]. Until then, caution about the interpretation of individual changes is not pessimism but good laboratory practice.

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