Clin Chem Lab Med 2012;50(3):419–421 © 2012 by Walter de Gruyter • Berlin • Boston. DOI 10.1515/cclm-2012-0035

Editorial

The never-ending search of an acceptable compromise for pancreatic lipase standardisation

Mauro Panteghini

Human pancreatic lipase (HPL) is a single-chain glycoprotein with a molecular weight of 48,000 Da (1). Its concentration in the pancreas is approximately 9000-fold greater than in other tissues, and the concentration gradient between pancreas and serum is approximately 20,000-fold (2). For full catalytic activity and greatest specificity, the presence of bile salts (such as sodium deoxycholate) and a cofactor called colipase, a small protein secreted by the pancreas, are required. HPL activity depends on the substrate, which in vivo conditions are glycerol esters of long-chain fatty acids (triglycerides), being present as an emulsion. Thus, the biological reaction does not obey classical Michaelis kinetics and the enzyme activity depends not on substrate concentrations but rather on interface area. Colipase, aided by the presence of bile salts to stabilise the triglyceride emulsion, binds to HPL to form a complex. This association produces a conformational change for HPL, such that the latter can more efficiently bind to the substrate to produce lipolytic products (3).

HPL measurement on serum has been used for several years to diagnose acute pancreatitis and its elevation is considered a more specific diagnostic finding than increases in serum amylase catalytic activity (4). A number of substrates and complex auxiliary and indicator systems are currently used in commercial HPL methods (1, 3). In general, long-chain triglyceride substrates have demonstrated a correlation of results with the biological and clinical state that is superior to that seen with methods using other substrates (5). In 1994, Lessinger and Férard first pointed out considerable inter-assay discrepancies in the plasma HPL catalytic activities according to different measurement procedures, possibly leading to incorrect interpretation of test results (6). In order to improve comparability of HPL measurements and in agreement with a similar approach already recommended for other clinically important enzymes, they proposed the development of a reference measurement system for HPL catalytic activity, including both a reference measurement procedure and suitable reference materials, based on the concept of metrological traceability (7).

In applying the reference system theory, enzymes represent a special class of analytes (8, 9). As they are defined in terms of the "catalytic amount", their numerical results depend entirely on the experimental conditions under which measurements are made. Therefore, a reference measurement procedure, which defines the conditions under which the catalytic activity of a given enzyme is measured, occupies the highest level of the metrological traceability chain (10). On the basis of the seminal work performed by Tietz et al. (11), the kinetic titrimetric method employing an automated potentiometric titrator (an instrument commonly referred to as a "pH-stat") has long been considered as a candidate reference method for HPL. Lessinger and coworkers developed an optimised titrimetric procedure at constant pH for HPL using triolein-based emulsion as substrate, a standardised mode of substrate emulsification, and optimisation of HPL effectors, such as sodium deoxycholate, calcium ions and colipase, and proposed its use for certification of reference materials (12, 13). Problems affecting the type of emulsification procedure and the reproducibility and stability of the emulsion were examined thoroughly and the feasibility of interlaboratory transferability of the method under controlled conditions was demonstrated by an international exercise (14). The production of two reference materials for HPL was also described (15, 16). These enzyme preparations were characterised in terms of catalytic properties, homogeneity, stability, and commutability (16). Particularly, the reference material containing HPL purified from human pancreatic juice (coded BCR 693) was found fully commutable between the mentioned standardised titrimetric procedure and a widely used colorimetric commercial method, thus potentially serving to transfer trueness from titrimetric procedure (taken as reference) to field methods, through the assignment of traceable values to commercial calibrators, and improve comparability of HPL results (Figure 1).

With this titrimetry-based reference system in place from 2004, why we are still discussing about the best practical way for standardising HPL measurements? The concept of a reference measurement system is valid only if the reference procedure and corresponding lower-order routine methods have similar analytical specificities toward the specific enzyme (7, 17). In the case of HPL, commercial methods employ different measurement principles that may reflect differences in analytical specificity (5). Furthermore, a number of suboptimal routine methods leading to interference by esterases or nonpancreatic lipases are still available in the market (18–20), even if no significant differences were found in their diagnostic efficiency when compared to more specific HPL assays (21). Consequently, the idea of relating commercial assays to each other through the use of the highly, but differently specific titrimetry-based reference system could create some insurmountable problems in the establishment of traceability of commercial methods. Theoretically, these assays

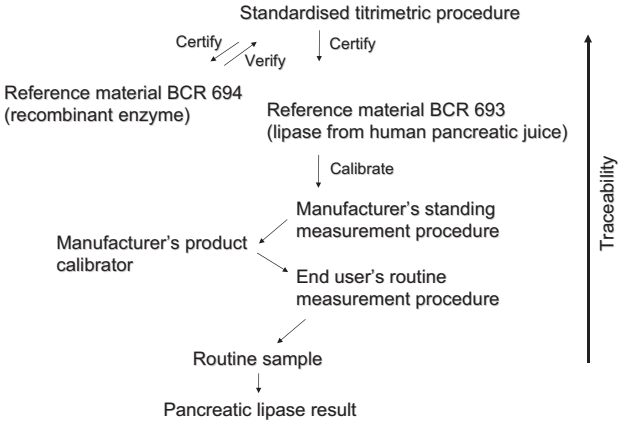

Figure 1 The reference measurement system for pancreatic lipase measurement proposed by Lessinger and coworkers (13, 16).

should be replaced by analytical methods where the traceability of calibration to the selected reference measurement procedure has been experimentally proved. Unfortunately, at present proof-of-concept comparison studies specifically evaluating the correlation between the standardised titrimetric procedure and the most popular commercial assays are still lacking. In principle, high specificity for HPL is an essential requisite for a reference method, but, if the second requisite is good comparability with field methods, in the case of HPL the two requirements could be incompatible. An additional practical issue was that the implementation of the titrimetric method needs specific instrumentation and expertise, which are different from those usually available in enzyme reference laboratories (9). This represented a substantial hindrance in the adoption of the methodology by the accredited reference services.

Because of previously discussed issues, the IFCC Committee on Reference Systems for Enzymes recently started to debate on a concept for the development of a new reference procedure for HPL, still favouring the selectivity of the method for HPL and the reproducibility of substrate preparation, but using spectrophotometry as the measurement technique (22). Similarly, the Japanese Society of Clinical Chemistry (JSCC) became active in searching for a reference measurement procedure for HPL. The paper published in this issue of *Clinical Chemistry and Laboratory Medicine* represents the first outcome of the work commissioned by JSCC (23). The proposed substrate for HPL is the 1,2-dioleoylglycerol (DODG) and the increase of NADPH, after a complex reaction scheme using four auxiliary reactions, is spectrophotometrically recorded in the indicator reaction of the method. Limitations of diglycerides, such as that used in this study, as substrates for HPL, have been known for a long time. They have been used in routine assays because of their increased solubility, but usually they seem not specific enough for HPL to be proposed as substrates in reference methods (5). In the Iizuka's study, no demonstration of the specificity of the proposed method for HPL is actually available, e.g., no correlation with a triglyceride-based physiological substrate assay, such as titrimetry; no interference experiments using carboxyl esterase or other nonpancreatic lipases; or evaluation of residual HPL activity after sample heating. The only (indirect) data related to the analytical specificity of the proposed assay that can be derived from the study are those reported in the Iizuka's Table 3, in which seems to be evident that the DODG method displays similar specificity of another commercial 1,2-diglyceride method (i.e., the intercept of the regression equation is close to zero), but different specificity when compared with the 1,2-*o*-dilauryl-*rac*-glycero-3-glutaric acid (6′-methylresorufin) ester (DGGR) method (positive intercept) (23). On the other hand, as already demonstrated for quite similar substrates (18, 20), a marked positive interference in samples collected after intravenous heparin administration was noted. The presence of this interference (considered to reflect activity of lipoprotein lipase released by heparin from its endothelial binding sides) in the proposed method is important, as it may denote a lack of assay optimisation. Demanet et al. (18), following the indications previously published by Tietz and coworkers (11), successfully eliminated the post-heparin interference in a HPL assay that uses 1,2-dilineoylglycerol (a substrate similar to DODG) by replacing deoxycholic acid with sodium glycocholate.

The need for a sample blank because of occasional nonspecific absorbance increase is another potential drawback of the DODG method, as its need may imply problems in analytical specificity of the assay, hampering the application of the traceability concept. Basically, the IFCC eliminated sample blanking from all the currently recommended reference methods of enzymes, because this step is impractical to be transferred to routine methods (9). Result imprecision was also an issue for the proposed assay, especially in between-laboratory experiments (23). The recommendation to perform multiple measurements on the same sample or use automated platforms when HPL values are lower than catalytic concentrations around the upper reference limit is not in line with the characteristics of narrow variability that should be a major feature of a reference procedure. In general, for reference procedures much better performance than field assays is requested, conventionally lower than or equal to a quarter of the desirable goal for routine methods (24). Using the goal derived from biological variability of HPL in serum (25), an expanded uncertainty <3% is then expected.

In conclusion, the idea present in the Iizuka's paper, of the possibility to propose their assay as a candidate for the reference procedure for HPL, appears to be quite premature, as the experimental evidence for supporting this proposal is inconclusive. Firstly, the assay specificity for HPL should be definitively proved. Secondly, sources of uncertainty of each reaction component should be clearly defined (9, 26). In particular, further studies should be specifically designed to obtain appropriate information fulfilling the relevant ISO 15193 standard requirements (27).

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

References

1. Panteghini M, Bais R, van Solinge WW. Enzymes. In: Burtis CA, Ashwood ER, Bruns DE, editors. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th ed. Philadelphia, PA: Elsevier Saunders, 2006:597–643.
2. Panteghini M. Electrophoretic fractionation of pancreatic lipase. Clin Chem 1992;38:1712–6.
3. Panteghini M. Laboratory evaluation of pancreatic diseases. Biochim Clin 2010;34:19–25.
4. Forsmark CE, Baillie J. AGA Institute technical review on acute pancreatitis. Gastroenterology 2007;132:2022–44.
5. Lykidis A, Mougios V, Arzoglou P. Pancreatic lipase assays with triglycerides as substrate: contribution of each sequential reaction to product formation. Clin Chem 1994;40:2053–6.
6. Lessinger JM, Férard G. Plasma pancreatic lipase activity: from analytical specificity to clinical efficiency for the diagnosis of acute pancreatitis. Eur J Clin Chem Clin Biochem 1994;32:377–81.
7. Panteghini M, Ceriotti F, Schumann G, Siekmann L. Establishing a reference system in clinical enzymology. Clin Chem Lab Med 2001;39:795–800.
8. Infusino I, Bonora R, Panteghini M. Traceability in clinical enzymology. Clin Biochem Rev 2007;28:155–61.
9. Infusino I, Schumann G, Ceriotti F, Panteghini M. Standardization in clinical enzymology: a challenge for the theory of metrological traceability. Clin Chem Lab Med 2010;48:301–7.
10. ISO 18153:2003. In vitro diagnostic medical devices – Measurement of quantities in biological samples – Metrological traceability of values for catalytic concentration of enzymes assigned to calibrators and control materials. ISO, Geneva, Switzerland.
11. Tietz NW, Astles JR, Shuey DF. Lipase activity measured in serum by a continuous-monitoring pH-stat technique – an update. Clin Chem 1989;35:1688–93.
12. Lessinger JM, Dourson JL, Férard G. Importance of standardization of lipase assays by using appropriate calibrators. Clin Chem 1996;42:1979–83.
13. Lessinger JM, Parashou S, Arzoglou P, Ramos P, Chapus C, Dufaux J, et al. Determination of lipase catalytic activity in two reference materials: BCR 693 and BCR 694 by titrimetry at constant pH. Clin Chem Lab Med 2004;42:62–6.
14. Arzoglou P, Goudoula C, Tsantili P, Lessinger JM, Férard G, Mignot V, et al. Transferability of lipase titrimetric assays: deductions from an interlaboratory study. Eur J Clin Chem Clin Biochem 1994;32:773–7.
15. Canalias F, Visvikis A, Thioudellet C, Siest G. Stable expression of enzymatically active human pancreatic lipase in V79 cells: purification and characterization of the recombinant enzyme. Clin Chem 1994;40:1251–7.
16. Lessinger JM, Arzoglou P, Ramos P, Visvikis A, Parashou S, Calam D, et al. Preparation and characterization of reference materials for human pancreatic lipase: BCR 693 (from human pancreatic juice) and BCR 694 (recombinant). Clin Chem Lab Med 2003;41:169–76.
17. Férard G, Edwards J, Kanno T, Lessinger JM, Moss DW, Schiele F, et al. Interassay calibration as a major contribution to the comparability of results in clinical enzymology. Clin Biochem 1998;31:489–94.
18. Demanet C, Goedhuys W, Haentjens M, Huyghens L, Blaton V, Gorus F. Two automated fully enzymatic assays for lipase activity in serum compared: positive interference from post-heparin lipase activity. Clin Chem 1992;38:288–92.
19. Kazmierczak SC, Van Lente F. Effect of gastric lipase on turbidimetric and dry-film methods for measuring pancreatic lipase. Clin Chem 1992;38:2555–6.
20. Dorner K, Schulze S. In vivo interference of heparin bolus injection with photometric continuous lipase determination. Eur J Clin Chem Clin Biochem 1996;34:363–4.
21. Panteghini M, Pagani F, Bonora R, Alebardi O, Ceriotti F. Diagnostic value of four assays for pancreatic lipase determination in serum: a comparative reevaluation. Clin Biochem 1991;24:497–503.
22. Lessinger JM. Theoretical bases for standardizing pancreatic lipase measurements. 2nd International Scientific Meeting "Standardization in clinical enzymology: a challenge for the theory of metrological traceability". November 25, 2008, Milano, Italy. Available at: http://users.unimi.it/cirme/public/UploadAttach/7_%20Lessinger.pdf.
23. Iizuka Y, Ueda S, Hanada T, Tani W, Adachi H, Haga R, et al. 1,2-Dioleoylglycerol method for pancreatic lipase catalytic activity in serum. Clin Chem Lab Med 2012;50:475–81.
24. IFCC external quality assessment scheme for reference (calibration) laboratories in laboratory medicine – Procedures. Rev 1.3, 2008. Available at: http://www.dgkl-rfb.de:81/IFCC_EQAS_ProcManual.pdf.
25. www.westgard.com/biodatabase1.htm. Accessed on January 10, 2012.
26. Frusciante E, Infusino I, Panteghini M. Stima dell'incertezza della misura della concentrazione di attività catalitica dell'alanina amminotransferasi (ALT) nel siero mediante il metodo di riferimento IFCC. Biochim Clin 2011;35:20–5.
27. ISO 15193. In vitro diagnostic medical devices and medical laboratories – Measurement of quantities in samples of biological origin – Requirements for content of reference measurement procedures, 2nd ed. ISO, Geneva, Switzerland, 2009.

Mauro Panteghini*
Centro Interdipartimentale per la Riferibilità Metrologica in Medicina di Laboratorio (CIRME), Università degli Studi, Milan, Italy

*Corresponding author: Prof. Mauro Panteghini, Laboratorio Analisi Chimico Cliniche, Ospedale Luigi Sacco, Via GB Grassi 74, 20157 Milan, Italy
Phone: +39 02 3904 2806, Fax: +39 02 503 19835,
E-mail: mauro.panteghini@unimi.it

The pancreas is an organ located in the abdomen. It plays an essential role in converting the food we eat into fuel for the body's cells. The pancreas has two main functions: an exocrine function that helps in digestion and an endocrine function that regulates blood sugar. Location of the Pancreas.Â The thin end is called the tail and extends to the left side. Several major blood vessels surround the pancreas, the superior mesenteric artery, the superior mesenteric vein, the portal vein and the celiac axis, supplying blood to the pancreas and other abdominal organs. Almost all of the pancreas (95%) consists of exocrine tissue that produces pancreatic enzymes for digestion. The remaining tissue consists of endocrine cells called islets of Langerhans. @inproceedings{Panteghini2012TheNS, title={The never-ending search of an acceptable compromise for pancreatic lipase standardisation}, author={M. Panteghini}, booktitle={Clinical chemistry and laboratory medicine}, year={2012} }. M. Panteghini. Published in.Â Human pancreatic lipase (HPL) is a single-chain glycoprotein with a molecular weight of 48,000 Da (1) . Its concentration in the pancreas is approximately 9000-fold greater than in other tissues, and the concentration gradient between pancreas and serum is approximately 20,000-fold (2) . For full catalytic activity and greatest specifi city, the presence of bile salts (such as sodium deoxycholate). and a cofactor called colipase , a small protein secreted by the pancreas, are required. HPLâ€¦ The pancreas is an accessory organ and exocrine gland of the digestive system. Learn now the anatomy and the functions of the pancreas at Kenhub!Â Laboratory investigations evidentiate elevated serum amylase and lipase levels. Management of acute pancreatitis involves fluid therapy, pain control, and close monitoring, and treatment of the underlying cause. On the other hand, chronic pancreatitis involves a progressive inflammation over a long period of time that causes permanent structural damage. Chronic pancreatitis manifests quite nonspecifically or even asymptomatically until the appearance of pancreatic insufficiency. At that point, the patient starts to experience pale, clay-colored stools (steatorrhea) from fat malabsorption and d