Supplementary Materials

SM1. Second-derivative intensity vs. band area

To evaluate the feasibility of using the second derivative of Raman spectra for the quantitative estimation of specific spectroscopic markers, we focused on the spectral region associated with the H1 marker, defined in the main manuscript as the ratio between the Raman intensities at 1655 cm^{-1} and 1577 cm^{-1}. This region, spanning approximately 1490–1730 cm^{-1}, is characterized by a high density of vibrational contributions that partially overlap, complicating the direct quantification of individual spectral components in the original Raman profiles.

In the conventional analysis, raw Raman spectra were first corrected for baseline contributions to remove fluorescence and slowly varying background signals. Subsequently, the areas of the bands centred at 1655 cm^{-1} and 1577 cm^{-1} were estimated using a peak-fitting procedure, in which overlapping components were modelled to isolate the contribution of each spectral feature. The H1 marker was then calculated as the ratio of the integrated band areas corresponding to these two peaks.

In parallel, the same spectral region was analyzed using the second derivative of the Raman profiles. Derivative spectroscopy is known to enhance spectral resolution and suppress baseline contributions, thereby facilitating the discrimination of overlapping bands. In the second-derivative spectra, the minima associated with the 1655 cm^{-1} and 1577 cm^{-1} features were directly identified, and their intensities were used to compute the H1 ratio without the need for explicit baseline subtraction or peak-fitting procedures.

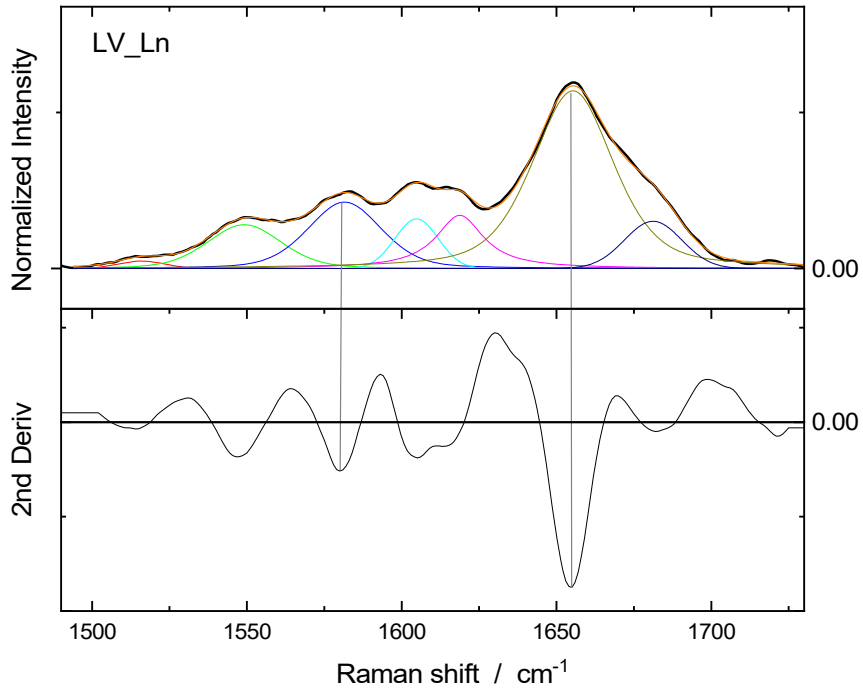


Figure S1: ***H1 Spectroscopic marker in Ln samples.*** *Mean spectral data from the measurements referred to the left ventricle of the control group. 1490-1730 cm^{-1} region of Raman spectrum (upper panel) and second-derivative profile (lower panel). Vertical lines indicate the bands at 1577 and 1655 cm^{-1} used to estimate the H1 spectral marker. Upper panel also show the eight components used in the fitting procedure to reproduce the spectrum in this frequency range.*

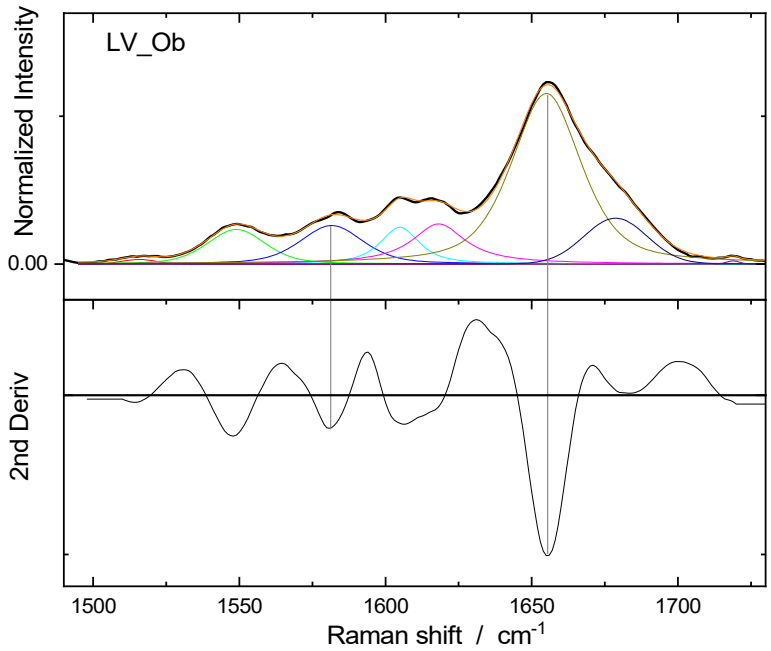


Figure S2: ***H1 Spectroscopic marker in Ob samples.*** *Mean spectral data from the measurements referred to the left ventricle of the obese group. 1490-1730 cm^{-1} region of Raman spectrum (upper panel) and second-derivative profile (lower panel). Vertical lines indicate the bands at 1577 and 1655 cm^{-1} used to estimate the H1 spectral marker. Upper panel also show the eight components used in the fitting procedure to reproduce the spectrum in this frequency range.*

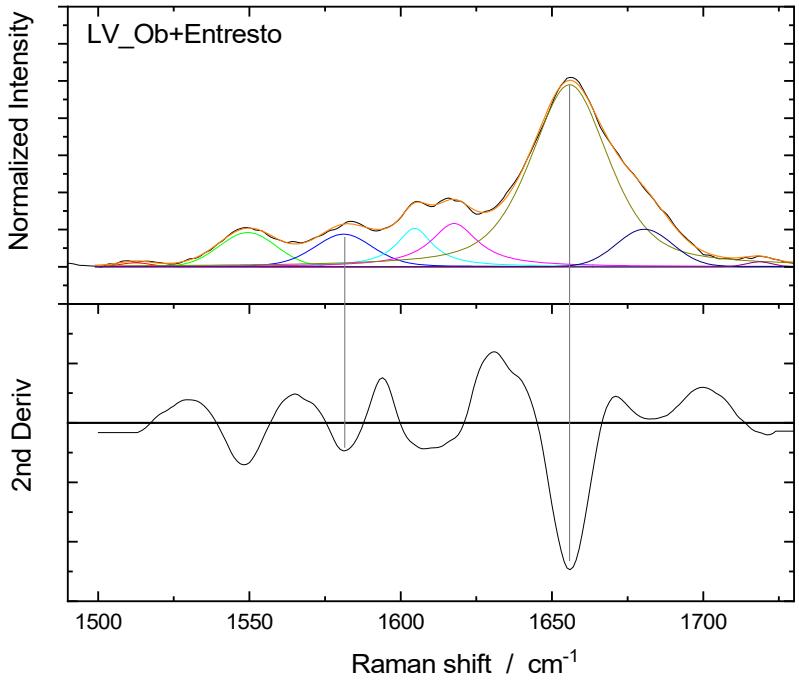


Figure S3: ***H1 Spectroscopic marker in Ob+En samples.*** *Mean spectral data from the measurements referred to the left ventricle of the Ob+En group. 1490-1730 cm^{-1} region of Raman spectrum (upper panel) and second-derivative profile (lower panel). Vertical lines indicate the bands at 1577 and 1655 cm^{-1} used to estimate the H1 spectral marker. Upper panel also show the eight components used in the fitting procedure to reproduce the spectrum in this frequency range.*

A direct comparison between H1 values obtained from band-area analysis of baseline-corrected spectra and those derived from second-derivative intensities demonstrated a strong agreement between the two approaches.

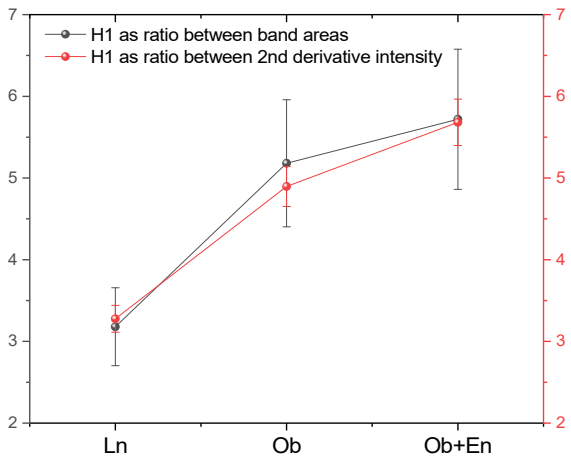


Figure S4: ***Second derivative vs. band area***. *H1 values obtained from band areas (black) and second-derivative intensities(red)*

As illustrated in Figure S4, the second-derivative method provides a more precise estimate of the H1 spectroscopic marker under these conditions because peak-fitting approaches require multiple adjustable parameters to adequately reproduce congested spectral regions, thus introducing additional sources of uncertainty.

SM2. Principal Component Analysis of Raman spectra from left ventricle

A principal component analysis (PCA) of Raman data from left ventricular myocardial tissue was performed using the 800–1700 cm^{-1} spectral range (full data are reported in ref. [19] https://doi.org/10.1186/s12967-023-04465-0).

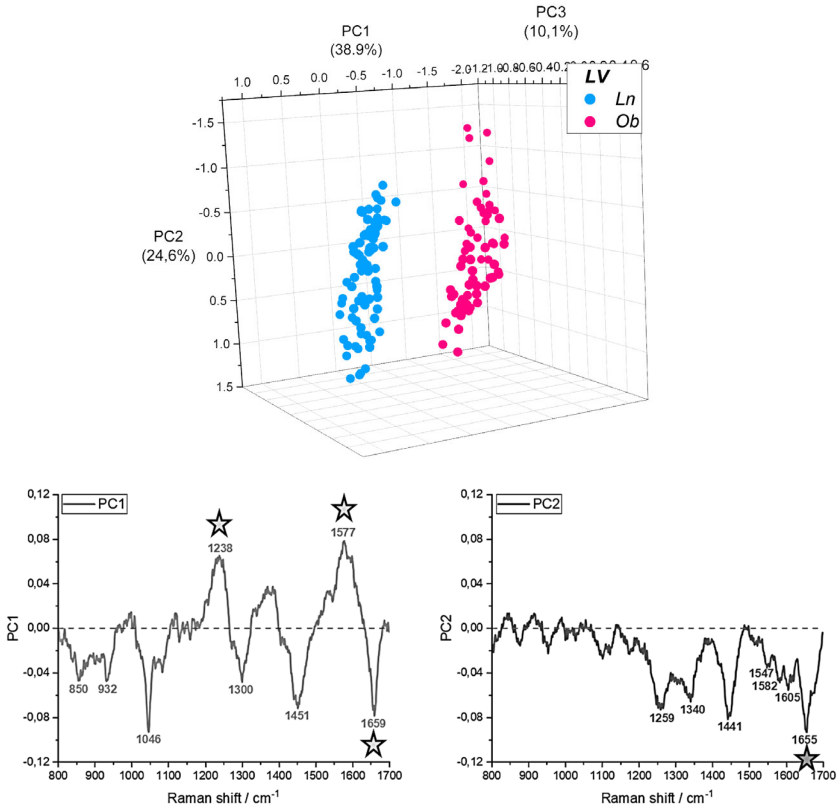


Figure S5: ***PCA of myocardial tissue****. Score plot (upper panel) and PC1- PC2 loading plots from principal component analysis of left ventricles. Stars indicate signals defining H1 and H2*