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Two Desiccation Methods to Measure Tissue Water Content in Rats with Trauma and Hemorrhage

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Introduction

- Tissue edema is a key outcome measure for evaluating trauma-induced endotheliopathy (EoT). However, standardized and reliable methods for quantifying tissue edema in either laboratory or clinical settings are lacking.
- The drying incubator is a method to measure moisture and water content in tissues and are based on convection heating of the sample by circulating hot air. This method is very easy to perform but is a very slow determination period and is prone to errors due to excessive handling, reading and calculations.
- The halogen moisture analyzer (HMA) consist of a heating unit, precision weighing balance, a temperature control system, and a moisture sensor. The analyzer use the principles of thermogravimetric analysis (TGA)(1). The sample is heated by the integral halogen heating module and during the drying process the analyzer continually measures the weight of the sample. After the drying is completed, it compares the initial weight vs the weight achieved after drying. The analyzer then calculates the moisture content based on the loss of weight and reports the moisture content as a percentage(2). HMA are used in industries, including food and beverages, pharmaceuticals, cosmetics, chemicals and more. However, is uncommon to use the HMA on tissue samples.

Objectives/Hypothesis

- The purpose of this study was to compare a traditional drying oven with a halogen moisture analyzer for measuring tissue water content (TWC).

Methods

- Isoflurane anesthetized male Sprague-Dawley rats were used either as healthy controls (blood donors (BD), n = 12-15) or were subjected to polytrauma/hemorrhage (PTH, n = 12-17). The BD rats were euthanized immediately after blood collection. The PTH rats underwent polytrauma (comprising laparotomy, multi-organ crush injuries, and femur fracture) followed by a 40% hemorrhage , while under constant anesthesia, and were euthanized at 4 hr. after trauma. The tissue samples (averaging 0.3 grams) were collected from the heart, lung, liver, kidney, spleen, skeletal muscles, brain, stomach, and intestine immediately after euthanasia and dissected into two respective tissue sets. TWC was measured by either weighing wet and dry weights prior and after oven-drying (60 ^{0}C) or by directly analysis with the HMA (200 ^{0}C).
- Water content was determined in all tissues by the dry-wet method and by halogen moisture analysis. One set of tissues averaging 0.3 g were rapidly excised, weighed in pre-weighed aluminum bowls and placed in the incubator (Fig 2.) to dried for 14 days at 60 ^{0}C. The second set of tissues were placed in cryotubes to minimize evaporation before testing. Tissue samples were then cut into few small pieces and placed in moisture analyzer. Weighing and analysis were made using the moisture analyzer (Fig 1).

Data Analysis:

Data was expressed as mean + SD. Two-way ANOVA was used to compare the differences in the tissue water content between the groups. Differences were considered significant when p was <0.05.

Results

Fig 1. Mettler Toledo, Moisture Analyzer Excellence Plus HX204

Fig 2. Fisher Scientific, Heratherm Incubator

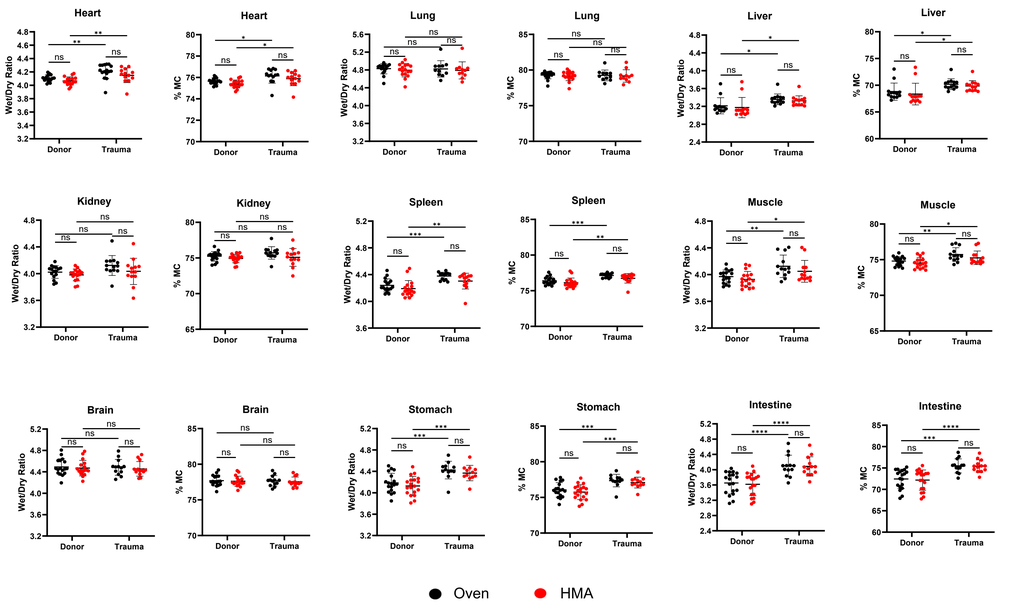

Fig 3. MC: Moisture Content. Comparison of wet/dry ratio and percent MC in normal and trauma rat tissues using both desiccation methods. Paired t-test. *: p<0.05, **: p<0.01; ***: p<0.001; ****: p<0.0001

Correlation Analysis of Wet/Dry Weight Ratio By HMA and Oven

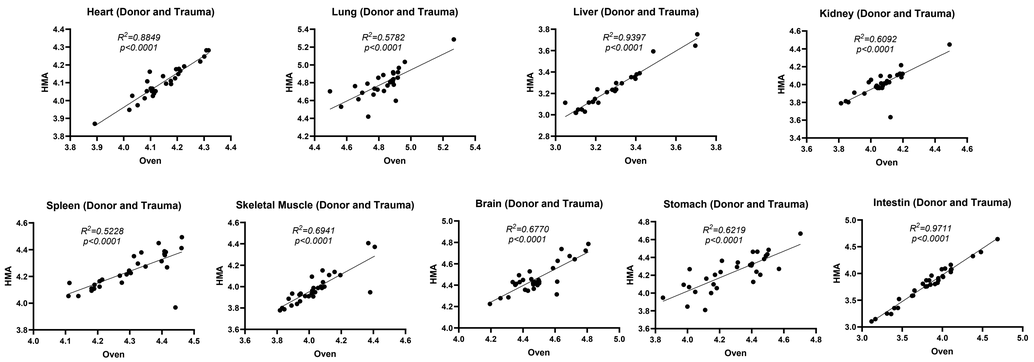

Fig 4. There was a significant linear correlation of wet/dry weight ratio measured by HMA and oven method.

Summary

- The Wet/Dry ratio and percent moisture content in rat tissues was determined by drying incubator and thermogravimetric analysis. The HMA provided tissue water content results of both blood donors and trauma samples in minutes was significantly faster than the oven-drying method, which required 10 to 14 days.
- No significant differences were found in tissue water content measured by the oven and HMA methods in the tissues from either blood donors or trauma groups, and the TWC between BD and PTH. However, water contents were significantly elevated in the heart, liver, spleen, muscle, stomach, and intestine of the traumatized animals compared to the donor animals.
- A significant linear regression was observed for the W/D weight ratios measured by the HMA and the oven method.

Conclusions

- Both tissue drying techniques are comparable for measuring tissue water content in various organs from both healthy and trauma/hemorrhage models.
- Although the traditional oven method to measure TWC is validated by the automatic HMA in this study, the HMA provides a more robust and efficient procedure for use in animal studies under current laboratory settings.

References

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Disclaimer: Research was conducted in compliance with Animal Welfare Act, the implementing Animal Welfare regulations, and the principles of the Guide for the Care and Use of Laboratory Animals. The Institutional Animal Care and Use Committee approved all research conducted in this study. The facility where this research was conducted is fully accredited by the AAALAC International.

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