

Energy metabolism drives all cellular functions in living cells. Status of energy metabolism is dynamic as all things associated with life are, reflecting, and reflected in, macroscopic and/or microscopic changes occurring in living things. In this thesis, I focused on the enzymes involved in energy metabolism, in particular, the enzymes of NADH shuttles, to show whether their activity levels faithfully reflect the variations in nutrient metabolism, metabolic status, and health conditions of different species and individuals. Specifically, malate dehydrogenase (MDH) is the rate-limiting enzyme of the malate-aspartate shuttle that contributes to the transfer of cytosolic NADH into mitochondria, coupling glycolysis to the mitochondrial ATP productions. By dividing the cytosolic MDH by lactic dehydrogenase (LDH), a relatively stable cytosolic marker enzyme, MDH/LDH ratio was analyzed as the parameter for evaluating metabolic status in animal tissues. A better grasp of trends in shifts of the energy metabolism enzymes may assist us to better understand species-species and individual differences in energy production and usage, and early detection and prevention of energy metabolism dysregulations associated with medical conditions such as diabetes, neoplasia, and obesity.

1. Comparison of leukocytic energy metabolism enzymes and plasma metabolites between dogs and cats

Enzyme activities of energy metabolism, such MDH, LDH, M/L ratio, and glutamate dehydrogenase (GLDH), of the feline leukocytes were compared against those of the canine leukocytes. GLDH is associated with the interconnection of amino acid and carbohydrate metabolism, and catalyzes the reversible conversion of glutamate to alpha-ketoglutarate and ammonia, allowing glutamate to fuel the TCA cycle for energy production under low cellular energy status, and glutamate and alpha-ketoglutarate to fuel the MA shuttle cycle. Significantly lower cytosolic MDH, M/L ratio and mitochondrial GLDH activities in feline leukocytes suggest that the feline cells utilize less glucose as the energy source compared to the canine cells. Furthermore, higher activities of fructokinase (FK) in feline leukocytes indicate active utilization of fructose, and subsequent activation of pyruvate kinase (PK) and

glucose-6-phosphate dehydrogenase (G6PD), which in turn, contribute to fatty acid synthesis and storage of energy as fat. Lower MDH, M/L ratio, GLDH, and higher FK, PK, and G6PD may reflect the unique demands and usages of nutrients and energy sources in cats with higher incidence of obesity, insulin resistance, and diabetes mellitus compared to dogs.

2. The use of leukocytic MDH and M/L ratio to assess metabolic condition of diabetic dogs

Elevations in cytosolic and mitochondrial MDH and the resultant cytosolic M/L ratio are expected in cells that may be experiencing increased mitochondrial ATP productions in order to provide for increased energy demands (intense exercise, neoplastic cell growth, acute weight gain). Diabetes mellitus, which causes dysregulation of glucose metabolism, is associated with depressions in MDH activity and resultant M/L ratio. This reflects the defect in glucose usage and uptake as energy source in peripheral tissues, resulting in increased circulating plasma glucose concentrations. Type 1 diabetic dogs with higher plasma GLU concentrations, showed lower levels MDH, resultant M/L ratio, as well as aspartate aminotransferase (AST), as compared to the healthy control dogs. Decreased activity levels of MA shuttle enzymes may be one of the characteristics of energy metabolism in diabetic dogs. Deeper understanding of the correlation of the MA shuttle function with DM risks, prognosis, and response to therapy may assist in the development of effective preventative and therapeutic measures.

3. Comparison of plasma MDH, LDH, and M/L ratio and lipid mobilization rate between racehorses and riding horses

The activities of MDH, LDH, and ML ratio of the racehorses were studied against those of the riding horses to show whether long-term extraneous exercise regiment induces increased efficiency in energy production and expenditure. Racehorses demonstrated higher MDH and LDH activities in plasma (4x and 2x greater, respectively), in addition to significantly higher plasma M/L ratio (2x) as

compared to riding horses. Moreover, racehorses demonstrated significantly higher levels of plasma non-esterified fatty acid (50% greater), triglyceride (2x greater), and total cholesterol (20% greater) as compared to riding horses. As evidenced by higher plasma M/L ratio, and increased lipolysis rate in racehorses, racehorses may have adapted to the demands of higher activity levels by increasing muscle mitochondrial respiration, oxidative capacity, and fat utilization of the skeletal muscles as energy source in order to process and consume energy more efficiently.

In this study, the reason why plasma may have been more appropriate in this case to determine M/L ratio is as follows. The isoenzyme pattern of LDH between plasma and PBL are different: LDH-1, -2, and -3 are dominant in plasma while LDH-3 and -4 are dominant in PBL suggesting a possible difference in LDH tissue source representation. LDH-1 are dominant in heart and RBCs; LDH-2 in monocytes and macrophages; LDH3 in lungs; LDH-4 in kidneys, placenta, and pancreas; and LDH-5 in liver and striated muscle. As such, LDH activity in plasma may be more encompassing of the whole body as opposed to that exhibited by peripheral leukocytes in horses. In horses, that face increased energy demands especially in skeletal muscles, plasma M/L ratio may be a better indicator of the whole body energy metabolism than leukocytic M/L ratio, that mainly reflects the energy metabolism of the liver tissue.

4. Changes in plasma malate dehydrogenase, lactate dehydrogenase, and M/L ratio as energy metabolism markers of acute weight gain in dogs

We evaluated the changes in plasma and leukocytic MDH, LDH, and M/L ratio as energy metabolism markers in dogs before and after the 4-week overfeeding trial. The aim of the study was to seek a diagnostic potential in M/L ratio as a marker for confirming early weight gain in conjunction with BCS changes, in apparently healthy animals exhibiting no overt clinical sequelae of weight gain. The experimentally overfed dogs showed about 28.2% increase in the body weight and the increase of body condition score from 1.9 to 3.4, and significant elevations were noted in lipid metabolites, glucose, leukocyte MDH and LDH. Although not significant, both the plasma MDH and LDH activities decreased, whereas leukocytic

MDH and LDH activities increased in the overfed group after the feeding trial. Both the resultant plasma and leukocytic M/L ratios showed mild increasing tendency in the over-fed group after the feeding trial. In conclusion, M/L ratio in plasma and leukocytes reflected positive energy balance and increase in energy metabolism associated with overfeeding. Although future studies on various types of weight gain (i.e. acute weight gain, chronic, mild, severe, and visceral, or subcutaneous obesity), and tissue types (leukocytes, plasma, muscle, and liver) are needed in order to follow the trends in energy usage efficiency associated with changes in weight status, M/L ratio may be a good indicator for detecting early weight gain if used appropriately.

As the life spans of companion animals are extending, the prevalence of metabolic syndrome such as obesity and DM, as well as neoplasia and other serious diseases, is increasing. Plasma metabolites are easy to collect and measure, and are highly accessible in clinical settings. The development of useful plasma markers, that can identify the risk factors and diagnose such life-threatening diseases early, is in need to extend the “health span” of companion animals.

The MA shuttle enzyme activities, in particular, MDH, are dynamic, and faithfully reflect glucose metabolism and ATP production. When combined with common biochemical parameters, M/L ratio may be used as potential diagnostic/monitoring parameter in determining the metabolic status of each individual, and for various health conditions. Further studies on various disease models and metabolic states in different species may assist in a better understanding of their clinical usage.