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Popular Article

Biomarkers of Vitamin Status

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Introduction

Nutrient status is defined as the balance between the nutrient supply of the diet and the nutrient requirements of an animal or a population of animals. Nutrient status can be determined directly by measuring the concentrations of nutrients in blood or specific tissues or by measuring quantitative net nutrient retention in the body. The latter is often measured indirectly in balance studies in which nutrient input via the diet and nutrient losses via faeces, urine and expired air and as heat, in case measurement of energy retention, is considered. Nutrient retention is calculated as nutrient intake via the diet minus its excretion via faeces, urine and expired air or otherwise, in case of energy as heat. Alternatively, concentrations of nutrients, their metabolites or biological compounds or expression of genes involved in their metabolism can be measured as indicator of the nutrient status in either specific pools, organs and tissues in the body or in excretal products. Quantitative techniques for measuring nutrient status require facilities and equipment to quantitatively collect faeces, urine, expired air, and heat loss mostly over a period of several days. These techniques are therefore time consuming and costly but generally provide proper estimates for nutrient retention. Alternatively, nutrient retention in the animal itself and in the animal end product can also be measured directly by analysing the nutrient of interest in homogenized sample of the whole body of small animals and, if applicable, in the mentioned end products. Also this approach is generally time consuming and costly, and in certain cases, such as in dairy cows and large ruminant species, not feasible given the size of the animal.

Nutritional Biomarkers

In general terms, a nutritional biomarker can be defined as any biological specimen that is an indicator of nutritional status with respect to intake or metabolism of dietary constituents. It can be biochemical, functional or clinical index of status of an essential nutrient or another dietary

constituent. They can be used to monitor the process, to take action in time to manage the process, or to predict the reaction of the process during an intervention. For example: If a nutrient is difficult or expensive to measure the consequences of changing the content of that nutrient in the feed may only become visible after a long time (e.g. as a consequence of long time undersupply). When a biomarker is available the reaction of the metabolism to the change of the feed composition can be followed in real time allowing to monitor the effect of the feed directly in the metabolism of the animal, which may allow the prediction of the long-term consequences, which can be used to manage the experiment.

An exemplary nutritional marker should

- quickly acknowledge the variations caused owing to consumption of nutrients
- be unaffected by presence of other diseases
- be easily and accurately tested with easily available equipment in the hospitals
- be affordable for the patients

Further, the biomarker must have the attributes as short biological half-life, occur in a proportionately tiny pool, have a catabolic rate which can be foreseen, and should be sensitive to specific nutrient intake.

Biomarkers of nutritional status are not necessarily nutrients. Examples include:

- Methylmalonic acid levels are elevated in a deficiency state of vitamin B12 and therefore serve as a biomarker of vitamin B12 status.
- Homocysteine levels are elevated in the absence of enzymes to metabolise it to cysteine or methionine. The enzymatic reactions require vitamins including vitamin B6, vitamin B12, and folic acid, and thus elevated homocysteine may indicate the lack of any of these nutrients.

Specimen collection

Nutritional biomarker methods rely upon biological specimen being collected from the participant. Examples of biological specimens include:

- **Serum and plasma**: Reflect the short term intake from a few days to one month.
- **Erythrocytes:** Reflect longer-term intake than serum and plasma, because of their half-life (approximately 120 days), but shorter than adipose tissue.
- **Adipose tissue:** Reflects long-term intake, most useful to assess exposure to fat-soluble vitamins and essential fatty acids.
- **Urine:** Reflects short-term intake. Samples collected can be 24-hour samples, overnight samples or spot samples. 24-hour samples are suitable for recovery biomarkers such as nitrogen, potassium and sodium or prediction biomarkers such as sucrose plus fructose.
- **Hair and nails:** Reflect long-term intake. They are easy to collect and store, but it is possible that environmental contamination occurs (particularly for hair).
- **Stool/faeces:** Difficult to collect, higher burden for the participants.

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Other bio-specimens may include leucocytes, milk, saliva, sweat, and any biopsied tissue.

Vitamin A biomarkers

Serum RBP - Not released from the liver when retinol is limiting. Used as a proxy for serum retinol in identification of vitamin A deficiency.

Serum/plasma retinol - Most commonly used biomarker. Correlates with the prevalence and severity of xerophthalmia and may change in response to interventions.

Retinol isotope dilution - Although technically challenging, it is the most sensitive test to measure vitamin A status and intervention impact on vitamin A reserves. It is minimally invasive and accurate.

Milk retinol Status - Good indicator of vitamin A status in areas where breastfeeding is common until 6 months of age. Milk retinol varies with milk fat; measurement of milk fat is recommended.

Retinyl esters Status - Validated qualitative measure of hypervitaminosis A. It may also be confounded by liver disease at the individual level.

Vitamin B12 biomarkers

Biomarker	**Indication**	**Interpretation**
Total vitamin B12	Global vitamin B12 status	↓ In vitamin B12 deficiency ↑ Myeloid cell proliferation ↓ Pregnancy
Holotranscobalamin	Vitamin B12-bound to transcobalamin or active B12	↓ In vitamin B12 deficiency ↓ In TC deficiency
Methylmalonic acid	Functional marker of vitamin B12 deficiency	↑ Vitamin B12 deficiency ↑ Renal dysfunction
Homocysteine	Functional marker of vitamin B12 deficiency	↑ Vitamin B12 deficiency ↑ Renal dysfunction ↑ Folate deficiency

Vitamin C biomarkers

Plasma ascorbic acid may be a reliable biomarker for dietary intake of vitamin C from all feed sources.

Vitamin D biomarkers

- **Dihydroxy vitamin D_3 [1,25(OH)(2)D_3]-** this biomarker – besides controlling calcium and phosphate – regulates the genes that control cell protein-coding, cell differentiation, and cell proliferation.
- **25-hydroxyvitamin D3 [(OH)(D3)]** has been found to influence cancer, cardiovascular disorder, the immune system and its associated disorders.

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Conclusion

In terms of nutrition, biomarkers are the cornerstone of research that establishes the functional effects of nutrition on the health-disease relationship. Currently, given the complex relationship between feed intake and health/disease status, a more integrative understanding of the concept of biomarker in relation to nutritional status and health is being developed, by focusing in nutritionally-regulated biomarkers of health. The development of such new types of biomarkers with an integrative trait, integrative nutritional biomarkers, recognizes the intimate connection between nutrition and metabolism. They could be indicative of both intake and of effects on the body and could even reflect health/disease state. Biomarkers may reflect the effects of nutrient intake or a lack thereof and in certain cases, they can also act as an intermediate biomarker that indicates the potential risk of developing a pathology associated with either excess or deficit of the nutrient to which it is linked.

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