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PERSPECTIVES IN NUTRITIONAL SCIENCE

Oxygen – the forgotten nutrient

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Abstract

Oxygen is essential for the maintenance and growth of aerobic animals, similar to the essentiality of what are classically considered nutrients. Nevertheless, O_2 is not customarily regarded as a nutrient, this reflecting the route by which it enters the body – through the lungs or gills in vertebrates, rather than via the mouth and gastrointestinal tract. A relative deficiency of O_2 occurs at high altitudes and during deep-sea diving, to which distinct adaptations occur. Deficiency is also evident in lung diseases such as emphysema. Without O_2, mitochondrial respiration and oxidative phosphorylation cannot take place. At a molecular level, cells adapt to O_2 deficiency by switching from oxidative metabolism to anaerobic glycolysis and there are changes in the expression of a multiplicity of genes, driven by hypoxia-sensitive transcription factors, particularly HIF-1. It is argued that O_2 should be fully included within the remit of nutritional science alongside the other essential macro-nutrients.

149 words

The core of nutritional science has long been the provision of macro- and micro-nutrients, the processes by which they are taken up by the body, the metabolic, molecular and cellular systems with which they are involved, and the consequences for the maintenance of health and the prevention of disease of either under- or over-provision. The macro-nutrients are customarily defined as encompassing proteins, carbohydrates and lipids, while micro-nutrients refer to the multiplicity of vitamins, minerals and trace elements that are required for normal physiological function. Perusal of any textbook of nutrition will show chapters devoted to each of these groups of nutrients. However, one major macro-nutrient that does not feature is oxygen – other than indirectly in relation to energy expenditure and metabolic rate in the context of energy balance and substrate utilization (RQ). Indeed, O_2 may not even be listed in the index, reflecting the fact that many would not consider it to be a nutrient as such. This then raises the question of what is a nutrient?

The *Oxford English Dictionary* defines a nutrient as "a substance that provides nourishment for the maintenance of life and for growth"; other definitions include "any substance or matter that is needed for the life and growth of living things" (*Webster's*). O_2 is self-evidently an essential requirement for all aerobic organisms, and given such definitions it is unambiguously a nutrient. The explanation for why O_2 is invariably ignored as a critical nutrient lies in the route by which it is obtained - nutrients are regarded as being delivered from the diet through the mouth and via the gastrointestinal tract. O_2, in contrast, is procured by a distinctly different route – from the ambient air via the lungs in terrestrial vertebrates, and from the surrounding water through the gills in fish (Table 1).

This article considers O_2 as a nutrient (macro-nutrient) and the similarities and dissimilarities that are evident in comparison with the recognised, classical nutrients.

Provision and delivery of oxygen

In mammals, and other higher animals, there is a requirement for what is in effect the continuous, or virtually continuous, delivery of O_2. This contrasts with other nutrients, the provision of which is episodic, most mammals being "periodic feeders though constant metabolisers". A relative lack of O_2 occurs in specific situations, either continuously in the case of terrestrial species living at high altitudes, or acutely and temporarily such as with aquatic animals undergoing deep-sea dives. Even some terrestrial animals living at sea level may be periodically exposed to low levels of O_2, such as the naked mole-rat in its underground burrows[(1)]. When acute or chronic O_2 deficiency is part of the ecological niche or environmental circumstances to which a species is customarily exposed, selective adaptations have evolved. For

example, in the naked mole-rat resistance to near anoxic conditions is sustained by utilising fructose as a fuel in glycolysis, thereby bypassing the key regulatory glycolytic enzyme phosphofructokinase[1].

Once taken up by the lungs, O_2 is distributed in essence immediately and directly to tissues and cells throughout the body, needing no prior processing before being made available. In contrast, the classical nutrients normally require release from the complex structures of the foods in which they are present, as well as processing to a form that can be transferred out of the interior of the gastrointestinal tract (as with polysaccharides and triglycerides). In addition, in many cases once food has been digested the nutrients released are in many cases transported from the gastrointestinal tract via substrate-specific transporters (e.g. Na+-dependent glucose transporter and amino acid transporters) rather than moving passively across cell membranes.

Following transfer from the lungs (or gills) into the circulation the handling of O_2 becomes more similar to that of other nutrients. O_2 is transported to tissues and cells by the specific iron-containing metalloprotein, haemoglobin, located in the cytoplasm of erythrocytes in vertebrates. This has parallels with the delivery of a number of other nutrients, such as lipids and retinol, to the sites where they are required. Not all nutrients are transferred directly to the site of action - many, including glucose (as glycogen), fatty acids (as triglycerides), and vitamins such as retinol and vitamin D, are first stored prior to being delivered to the sites where they are required - with the liver and white adipose tissue being key storage organs. O_2 is stored to a limited extent in muscle, bound to myoglobin, for local use only within the tissue, and this is especially evident in marine mammals that undergo apnoea when diving[2].

Metabolic functions

Despite not being considered within the remit of nutritional science in whole-body terms, at a cellular level O_2 is recognised as a critical factor without which respiration and other key metabolic processes cannot take place. Oxidative metabolism, particularly the catabolism of fatty acids and glucose with the production of ATP through oxidative phosphorylation and mitochondrial respiration, requires a continuous supply of O_2. Metabolic pathways central to this include glycogenolysis, glycolysis and lipolysis, and involve cytochrome enzyme systems within the mitochondria[3]. The number of mitochondria in a cell, and whether there is a highly developed cristae structure within these organelles, varies according to the extent to which each cell type undergoes oxidative metabolism and consumes O_2.

Brown adipocytes in rodents adapted to the cold, for example, have large numbers of mitochondria with densely packed cristae, reflecting the exceptionally high levels of fatty acid oxidation and O_2 consumption needed for thermoregulatory heat production (thermogenesis)[4].

Oxygen deficiency states

The complete absence of O_2 leads to death within minutes in man and other mammals. In addition to environmental circumstances in which a relative lack of O_2 occurs related to the ecological niche of a species, there are certain disease states, primarily lung diseases such as pulmonary fibrosis and emphysema, where the provision of O_2 to the body as a whole is impaired[5]. There are also states of cyclic O_2 lack, as in obstructive sleep apnoea, which is one of the disorders particularly associated with obesity[6]. In each of these cases the overall availability of O_2 is limited, though not necessarily to a specific tissue. O_2 deficiency can be ameliorated, both acutely and chronically, whether in lung disorders such as chronic obstructive pulmonary disease or in medical emergencies, by increasing the provision through O_2 therapy.

The O_2 tension (pO_2) of inspired air at sea level is 160 mmHg and in alveolar blood it is approximately 104 mmHg, while the general level of oxygenation in tissues is of the order of 40-50 mmHg[7-9]. However, some tissues have a markedly lower pO_2, examples including the retina, thymus and spleen, with a pO_2 of 2-25, 10 and 16 mmHg, respectively[7-9].

As well as low levels of O_2 being characteristic of certain tissues under normal circumstances, local deprivation also occurs in specific pathological situations. These include the site of wound healing, in the heart in ischemic disease, in tumours, and in white adipose tissue depots in obesity[7-10]. The pO_2 of solid tumours can be so low that those cells at the centre may be effectively anoxic. In the case of white fat, a reduced pO_2 has been documented in white adipose tissue depots of obese rodents[11-13], the O_2 tension being >3-fold lower than in lean animals[11,14]. Adipose tissue hypoxia in obesity is considered in part to reflect the considerable size of enlarged white adipocytes in relation to the normal diffusion distance of O_2 in tissues[9,15]. This hypoxic state is linked to inflammation and fibrosis, and is considered to be a key factor underlying the changes in adipose tissue function that lead to the development of the major obesity-associated diseases, particularly insulin resistance and the metabolic syndrome[9,15-16].

Metabolic and cellular adaptations to oxygen deficiency

Part of the response to a chronic deficiency of O_2 in a tissue is the stimulation of angiogenesis in order to extend the vasculature. At the level of the cell, a local deficiency of O_2 leads to extensive metabolic changes[7-8,17-18] (Fig. 1). Glucose and lipid oxidation, oxidative

phosphorylation and mitochondrial respiration fall, and there is a compensatory increase in substrate flux through anaerobic pathways[7-8,18]. In particular, the rate of glycolysis is greatly increased with lactate being the end product rather than pyruvate[7-8,18]; under aerobic condition pyruvate is oxidised via acetyl CoA and the Citric Acid cycle. Elevated rates of glycolysis are driven by increases in glucose uptake through the recruitment of GLUT1, the basal facilitative transporter, and raised levels of key glycolytic enzymes[7-8]. Tumours have, of course, long been recognised to produce substantial quantities of lactate, reflecting their marked hypoxic state[7,19]. Similar observations have been made on white adipocytes maintained under hypoxic conditions[9,20].

The range of metabolic changes resulting from low pO_2 extends well beyond the augmentation of glycolysis. In the specific case of white adipose tissue, microarray studies have indicated that the expression of approximately 1,300 genes is altered in adipocytes exposed to 1% O_2 (Fig. 1) compared with those incubated under 'normoxic' conditions (21% O_2)[21]. In addition to glucose utilisation, lipolysis and lipid oxidation, the pathways and functions altered in fat cells in response to low pO_2 include cell-to-cell signalling and interaction, amino acid metabolism, and cell death[21]. This is reflected in changes in the amounts of encoded transporters, enzymes, and key proteins such as adipokines - including those associated with the inflammatory response[9,20]. Cells not only respond to major differences in pO_2, but again as illustrated in adipocytes, they appear to carefully titrate small variations in O_2 tension with alterations in gene expression and glucose utilisation[22].

The cellular sensing of O_2 deficiency is initiated at the cell membrane primarily through potassium ion channels[23] and the intracellular response is transmitted by hypoxia-sensitive transcription factors which regulate the expression of hypoxia-sensitive genes[7-8,17-19]. The most important of these transcriptional signals are the hypoxia-inducible factors (HIFs), particularly HIF-1 which is termed the "master regulator of O_2 homeostasis"[18]. HIF-1 consists of two subunits - HIF-1β, which is constitutively expressed, and HIF-1α which is continuously synthesised and degraded but is stabilised when O_2 tension is low, this enabling the formation of the functional transcription factor[8,17-18]. The transcription of multiple genes is directly regulated by HIF-1, including GLUT1, glycolytic enzymes, vascular endothelial growth factor (VEGF), angiopoietin-like protein 4 and the adipocyte hormone leptin[7-9,20]. VEGF is, of course, a key angiogenic signal, the growth of the vasculature being central to the delivery of O_2 as well as of other nutrients.

Coda

O_2 has been a forgotten, or at the very least highly neglected, nutrient. It is absolutely critical for all aerobic animals, and for most higher species is required on a continuous basis. It is essential for cellular respiration and for a host of other metabolic processes. States of deficiency are recognised and can be ameliorated. Cells have the ability to adjust to acute or chronic changes in O_2 availability, this involving alterations in the expression of a multiplicity of hypoxia-sensitive genes regulated by key transcription factors.

Despite the similarities between O_2 and other nutrients, there are some differences beyond the route of delivery. There is no meaningful equivalent of the RDA, and in most circumstances O_2 is both abundant and freely available, and requires no prior processing. In contrast to many other nutrients, excess is difficult to achieve though toxicity is evident in artificially induced hyperoxaemia. It is argued that O_2 should be viewed as firmly residing within the purview of nutritional science.

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References

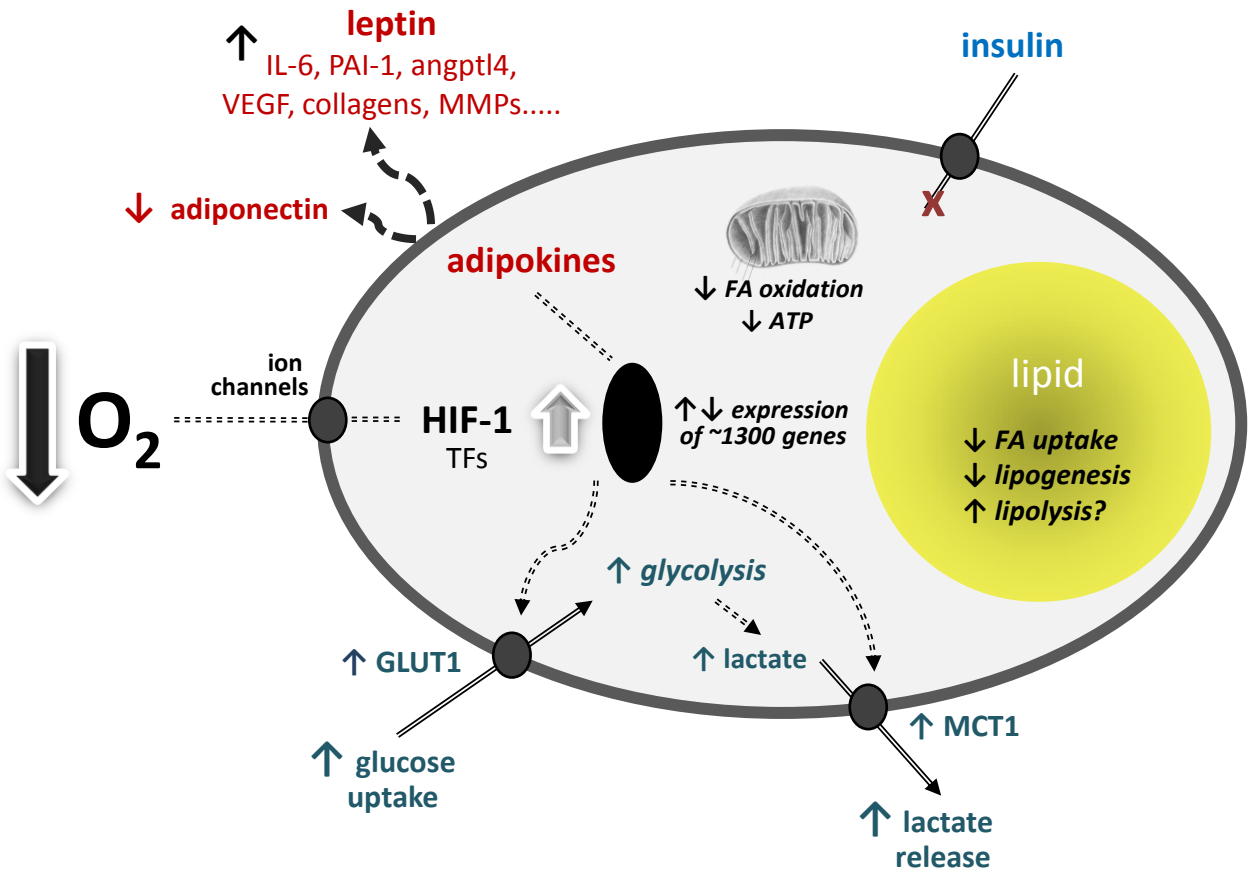
1. Park TJ, Reznick J, Peterson BL *et al.* (2017) Fructose-driven glycolysis supports anoxia resistance in the naked mole-rat. *Science* **356**, 307-311.
2. Ordway GA & Garry DJ (2004) Myoglobin: an essential hemoprotein in striated muscle. *J Exp Biol* **207**, 3441-3446.
3. Frayn KN (2010) Metabolic Regulation. A human Perspective. *3rd Edition*, pp 1-371, Chichester, Wiley-Blackwell.
4. Cannon B & Nedergaard J (2004) Brown adipose tissue: function and physiological significance. *Physiol Rev* **84**, 277-359.
5. Kulkarni T, O'Reilly P, Antony VB *et al.* (2016) Matrix remodeling in pulmonary fibrosis and emphysema. *Am J Resp Cell Mol Biol* **54**, 751-760.
6. Bonsignore MR, McNicholas WT, Montserrat JM *et al.* (2012) Adipose tissue in obesity and obstructive sleep apnoea. *Eur Resp J* **39**, 746-767.
7. Brahimi-Horn C & Pouysségur J (2007) Hypoxia in cancer cell metabolism and pH regulation. *Essays Biochem* **43**, 165-178.
8. Brahimi-Horn MC & Pouysségur J (2007) Oxygen, a source of life and stress. *FEBS Lett* **581**, 3582-3591.
9. Trayhurn P (2013) Hypoxia and adipose tissue function and dysfunction in obesity. *Physiol Rev* **93**, 1-21.
10. Semenza GL, Agani F, Feldser D *et al.* (2000) Hypoxia, HIF-1, and the pathophysiology of common human diseases. *Adv Exp Med Biol* **475**, 123-130.
11. Ye J, Gao Z, Yin J *et al.* (2007) Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of *ob/ob* and dietary obese mice. *Am J Physiol Endocrinol Metab* **293**, E1118-1128.
12. Hosogai N, Fukuhara A, Oshima K *et al.* (2007) Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. *Diabetes* **56**, 901-911.
13. Rausch ME, Weisberg SP, Vardhana P *et al.* (2008) Obesity in C57BL/6J mice is characterised by adipose tissue hypoxia and cytotoxic T-cell infiltration. *Int J Obesity* **32**, 451–463.
14. Sun K, Halberg N, Khan M *et al.* (2013) Selective inhibition of hypoxia-inducible factor 1α ameliorates adipose tissue dysfunction. *Mol Cell Biol* **33**, 904-917.
15. Trayhurn P & Wood IS (2004) Adipokines: inflammation and the pleiotropic role of white adipose tissue. *Br J Nutr* **92**, 347-355.

16. Halberg N, Khan T, Trujillo ME *et al.* (2009) Hypoxia-inducible factor 1α induces fibrosis and insulin resistance in white adipose tissue. *Mol Cell Biol* **29**, 4467-4483.
17. Semenza GL (2001) HIF-1 and mechanisms of hypoxia sensing. *Curr Opin Cell Biol* **13**, 167-171.
18. Semenza GL (2007) Oxygen-dependent regulation of mitochondrial respiration by hypoxia-inducible factor 1. *Biochem J* **405**, 1-9.
19. Semenza GL (2003) Targeting HIF-1 for cancer therapy. *Nat Rev Cancer* **3**, 721-732.
20. Trayhurn P (2014) Hypoxia and adipocyte physiology: implications for adipose tissue dysfunction in obesity. *Ann Rev Nutr* **34**, 207-236.
21. Mazzatti D, Lim F-L, O'Hara A *et al.* (2012) A microarray analysis of the hypoxia-induced modulation of gene expression in human adipocytes. *Arch Physiol Biochem* **118**, 112-120.
22. Wood I, Stezhka T & Trayhurn P (2011) Modulation of adipokine production, glucose uptake and lactate release in human adipocytes by small changes in oxygen tension. *Pflügers Archiv Eur J Physiol* **462**, 469-477.
23. Kemp PJ & Peers C (2007) Oxygen sensing by ion channels. *Essays Biochem* **43**, 77-90.

Table 1. Comparison of the characteristics of oxygen with other nutrients

	Classical Nutrient	**Oxygen**
Source	Provided by the diet	Obtained from the ambient air (surrounding water in fish)
Route of entry	Via the mouth and gastrointestinal tract	Through the lungs
Frequency of provision	Periodic	Essentially constant
Processing	Requires processing – digestion & absorption	No prior processing required
Transportation	Transport to tissues in some cases via specific transporters	Transported directly via a specific transporter (*haemoglobin*)
Storage	May be stored temporarily (e.g. glycogen in liver and muscle, lipids in adipose tissue)	Limited storage - only in muscle (*myoglobin*) for local use
Deficiency	Recognised deficiency diseases	Generally abundant, but relative deficiency at high altitudes, during diving and in lung disease. Extensive cellular adaptations to low levels
RDA	Yes	No

Fig 1. Schematic illustration of the key cellular responses to O_2 deficiency based on white adipocytes. The effect of low O_2 tension on gene expression, glucose uptake and utilisation, lipid metabolism and the production of selected adipokines is shown. angptl4, angiopoietin-like protein-4; FA, fatty acid; GLUT1, facilitative glucose transporter 1; HIF-1, hypoxia-inducible factor-1; MCT1, monocarboxylate transporter-1; MMPs, matrix metalloproteinases; PAI-1, plasminogen activator inhibitor-1; TF, transcription factors (additional to HIF-1, hypoxia-inducible factor-1); VEGF, vascular endothelial growth factor.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glucose
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lactate
release

Annex: Acceptance Information

02-Aug-2017

Dear Prof. Trayhurn,

I have now received the editorial report on your manuscript entitled **"Oxygen – the forgotten nutrient"**, including the comments from a member of the Editorial Board. I am very pleased to inform you that the paper has been accepted for publication in Journal of Nutritional Science as it stands, and I would like to congratulate you on such an interesting and well prepared manuscript.

For your information, the reports from the referees are below. However, since there is no need for revision we will now proceed to prepare your manuscript for publication.

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