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Through fat and thin - A journey with the adipose tissues

The Gowland Hopkins Award Lecture 2019

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1 **Abstract**

2 The present paper is based on the lecture that I gave on receiving the Nutrition Society's
3 inaugural Gowland Hopkins Award for contributions to Cellular and Molecular Nutrition. It
4 reviews studies on the adipose tissues – brown and white – conducted by the groups that I have
5 led since entering nutrition research in 1975. The initial focus was on exploring metabolic factors
6 that underpin the development of obesity using animal models. This resulted in an interest in
7 non-shivering thermogenesis with brown adipose tissue being identified as the key effector of
8 facultative heat production. Brown fat is less thermogenically active in various obese rodents,
9 and major changes in activity are exhibited in physiological conditions such as lactation and
10 fasting consistent with a general role for the tissue in nutritional energetics. My interests moved
11 to white adipose tissue following the cloning of the *Ob* gene. Our initial contributions in this area
12 included demonstrating nutritional regulation of *Ob* gene expression and circulating leptin levels,
13 as well as a regulatory role for the sympathetic nervous system operating through β_3 -
14 adrenoceptors. My interests subsequently evolved to a wider concern with the
15 endocrine/signalling role of adipose tissue. Inflammation is a characteristic of white fat in
16 obesity with the release of inflammation-related adipokines, and we proposed that hypoxia
17 underlies this inflammatory state. O₂-deprivation was shown to have substantial effects on gene
18 expression and cellular function in white adipocytes. The hypoxia studies led to the proposition
19 that O₂ should be considered as a critical – essential - macronutrient.

20 Background

21 I am greatly honoured to receive the Gowland Hopkins Award from the Nutrition Society, and
22 indeed to be the first recipient. I never met Sir Frederick Gowland Hopkins OM PRS, who
23 received the Nobel Prize for Physiology or Medicine in 1929 for the discovery of vitamins, being
24 born a year after he died. There is, however, a tangential link in that my entry into nutrition from
25 a basic science background in physiology and biochemistry came through joining the MRC Dunn
26 Nutritional Laboratory in Cambridge in 1975. The Dunn had been founded in 1927 when
27 Gowland Hopkins was Dunn Professor of Biochemistry in the University, and the original intent
28 was that he should be directly involved in the research. In practise, because of extensive other
29 commitments, including as President of the Royal Society, his primary role with respect to the
30 Dunn was as an advisor and member of the Management Committee. Gowland Hopkins has, of
31 course, a close association with the Nutrition Society as one of the principal figures behind its
32 foundation.

33 My research over the past 40+ years since entering nutrition has centred on the adipose
34 tissues – first brown and then white. This began following my initial studies at the Dunn where I
35 had been recruited by Philip James as a member of the then newly formed Energy Group (Fig.
36 1). The group had been established in recognition of obesity beginning to emerge as a public
37 health problem. At the time, the incidence of obesity was considerably less than now - in 1980,
38 for example, 6% of adult males and 8% of adult females in the UK were classified as obese⁽¹⁾ on
39 the basis of a body mass index ≥ 30 , while by 2017 the figure was approximately 30% of all adults
40 (<https://www.worldobesitydata.org/country-profiles/>). Obesity is, of course, important
41 primarily because of the increased risk of several associated diseases, particularly type 2 diabetes,
42 hypertension, coronary heart disease and certain cancers^(2,3).

43 The ethos prevailing when we began in the mid-1970's was that obesity is the product of
44 'gluttony and sloth', but our focus was on exploring whether there are important metabolic
45 factors which underpin the development of the disorder. My remit was to investigate the
46 fundamentals of the regulation of energy balance using animal models. The animal of choice was
47 the genetically obese *ob/ob* (*Lep^{ob}/Lep^{ob}*) mouse, and a colony of the Aston strain of these
48 mutants was set-up. The attraction of *ob/ob* mice, which at the time were the most widely used
49 animal model in obesity research, was that not only is the obese state extreme with body weight
50 being up to three times that of lean siblings but that it is reducible to a mutation in a single
51 recessively inherited gene⁽⁴⁾. The link to a mutant gene meant that the obesity of *ob/ob* mice
52 results from a change in just one protein - and that protein must play a critical role in the
53 regulation of energy balance.

54 The *ob/ob* mouse is not, of course, the only rodent in which obesity is the result of a single
55 gene mutation, and we subsequently established colonies of the other major obese mutants - the
56 Zucker *fa/fa* (*Lep^{fa}/Lep^{fa}*) rat and the diabetic-obese *db/db* (*Lep^{db}/Lep^{db}*) mouse⁽⁴⁾. We were
57 later able to house colonies of the Adipose mouse (*Ad*), golden hamsters (*Mesocricetus auratus*) and
58 Djungarian hamsters (*Phodopus sungorus*), each as a specific model within our energy regulation
59 studies. This was based on the August Krogh Principle that “for a large number of problems
60 there will be some animal of choice or a few such animals on which it can be most conveniently
61 studied”⁽⁵⁾. As I have noted previously, the ability to maintain multiple colonies of experimental
62 animals at the Dunn without direct cost to the investigator was remarkable⁽⁶⁾.

63

64 **Energy balance and thermogenesis**

65 Hyperphagia is part of the basis for the obesity of the *ob/ob* mouse, and indeed that of the other
66 obese mutants, food intake being greater than in lean siblings⁽⁴⁾. However, studies where young
67 *ob/ob* mice were either directly pair-fed to the *ad libitum* intake of their lean siblings, or otherwise
68 given restricted amounts of food, indicated that obesity still develops without hyperphagia^(7,8).
69 Our own work, in which full energy balance studies were performed, clearly illustrates the point;
70 young *ob/ob* mice pair-fed to the *ad libitum* intake of lean siblings at room temperature (23°C)
71 exhibited a rate of energy deposition 2.3 times that of the lean⁽⁷⁾. The study was conducted at
72 four different environmental temperatures – 33° (thermoneutral for the mouse), 28°, 23° and
73 17°C. At each temperature, the energy gain of the obese animals was greater than the lean, but
74 the lower the temperature the higher the excess gain⁽⁷⁾.

75 The capacity for excess energy deposition in the absence of hyperphagia indicated that one
76 or more components of energy expenditure is reduced in the *ob/ob* mutants. Of the main
77 components of expenditure, facultative (or adaptive) non-shivering thermogenesis (NST) was
78 particularly attractive as the key element. Not only is thermoregulatory thermogenesis a major
79 part of total expenditure in small mammals in order to maintain body temperature, but reduced
80 expenditure on thermogenesis was being advocated by Miller and Stock as a causal factor in the
81 development of obesity^(9,10). Furthermore, some 25 years earlier impaired homeothermy had
82 been noted in *ob/ob* mice⁽¹¹⁾. This appeared counter-intuitive given the improved insulation
83 provided by the additional body fat of the obese animals, and was suggestive of a reduced
84 capacity to generate heat. In our own studies, core temperature fell rapidly - to as low as 15°C -
85 just 3 h after exposure of *ob/ob* mice to 4°C, whereas lean siblings maintained their temperature
86 above 35°C⁽¹²⁾.

87 Direct measurements of NST from the peak increase in resting metabolic rate at
88 thermoneutrality following the administration of noradrenaline indicated that the capacity for
89 this form of heat production was 2-fold lower in *ob/ob* mice than lean siblings⁽¹²⁾. Furthermore,
90 resting metabolic rate – expressed ‘per animal’, as should be done in energetic studies – was
91 reduced in the obese mice relative to the lean at every temperature examined below
92 thermoneutrality, indicating a lower expenditure on NST⁽¹²⁾.

93

94 **Brown adipose tissue**

95 An immediate question raised by these physiological studies was the nature of the molecular and
96 cellular mechanisms of NST. Several possibilities were under consideration at the time, including
97 protein turnover, the α -glycerophosphate shuttle, Na^+ transport across the plasma membrane
98 mediated by Na^+ - K^+ ATPase, and futile/substrate cycles such as that between fructose-6-
99 phosphate and fructose-1,6-bisphosphate⁽¹³⁻¹⁹⁾; in practise, most are in effect a form of energy-
100 consuming substrate cycle. There were, however, substantial reservations with each of these
101 mechanisms⁽²⁰⁾ in that it seemed unlikely that they had the potential to generate sufficient
102 quantities of heat and to do so acutely without disrupting normal metabolic control. In addition,
103 there was a central issue of tissue localisation - several of the mechanisms, particularly protein
104 turnover and Na^+ transport, being essentially universal rather than restricted to a specific tissue
105 site.

106 The question of the tissue basis for NST subsequently centred on brown adipose tissue
107 (BAT, or brown fat), which had first been described by Conrad Gessner in 1551. Although
108 different roles had been proposed for this tissue, including as an endocrine organ, the principal
109 function was resolved in the early 1960s - as a thermogenic organ with heat as the primary
110 product⁽²¹⁾. The tissue is prominent in hibernating species, in the newborn of many mammals
111 (including humans) and in rodents acclimated to the cold^(22,23). The quantitative importance of
112 BAT in adaptive NST in rodents was demonstrated in influential studies by Foster and
113 Frydman⁽²⁴⁻²⁶⁾. These authors mapped regional blood flow to different tissues using radioactively-
114 labelled microspheres in rats in which NST was maximally stimulated following either cold-
115 acclimation or the administration of noradrenaline. From the measurements of regional blood
116 flow, together with the cardiac output and the oxygen extraction across the interscapular depot,
117 BAT was estimated to account for 60% of NST in cold-acclimated rats⁽²⁵⁾. Our own studies on
118 mice using the same approach suggested a broadly similar figure⁽⁷⁾.

119 In parallel with the identification of BAT as the principal locus for NST, the unique
120 bioenergetic properties of the tissue’s mitochondria were being elucidated. Heat was shown by

121 Nicholls to be generated by a regulated uncoupling of oxidative phosphorylation, the energy
122 inherent in the proton gradient across the inner mitochondrial membrane being dissipated as
123 heat rather than coupled to ATP synthesis⁽²²⁾. This process is controlled by the 32,000 M_r
124 mitochondrial uncoupling protein-1 (UCP1) discovered by Ricquier^(22,27). Acute stimulation of
125 BAT thermogenesis leads to an activation of UCP1, while chronic stimulation results in an
126 increase in the amount of the protein – through a combination of a higher concentration in the
127 mitochondria and through mitochondriogenesis⁽²³⁾. These changes, both acute and chronic, are
128 primarily driven by the release of noradrenaline from the extensive sympathetic innervation of
129 BAT, acting mainly via β_3 -adrenoceptors^(28,29).

130 My group at the Dunn, in parallel with several other groups, began to explore the potential
131 role of BAT in energy balance and the development of obesity. Two seminal observations were
132 pivotal; in the first, Himms-Hagen and Desautels in Ottawa demonstrated reduced GDP
133 binding to BAT mitochondria in *ob/ob* mice relative to lean siblings, this reflecting a reduction in
134 the thermogenic proton conductance pathway of the tissue⁽³⁰⁾. Our own blood flow studies
135 indicated that the reduced NST and consequent lower energy expenditure of the obese mutant is
136 entirely due to decreased metabolic activity in BAT⁽³¹⁾. In the second key study, Rothwell and
137 Stock in London proposed that BAT is the locus of the diet-induced thermogenesis that they
138 were observing in rats overfed through the provision of a cafeteria diet⁽³²⁾. In follow-up studies
139 with our group, key molecular indices of BAT thermogenic activity were demonstrated in the
140 cafeteria-fed animals – increased mitochondrial mass and GDP binding, as well as GDP-
141 sensitive respiration⁽³³⁾.

142 These initial reports were followed by a series of studies in which the thermogenic activity
143 of BAT was shown to be reduced in a variety of obese rodents. They included other single gene
144 mutants – *fa/fa* rat and *db/db* mouse – and rodents with experimentally-induced obesity such as
145 that following lesioning of the ventromedial hypothalamus, the administration of gold
146 thioglucose, and treatment with corticosteroids^(20,34,35). Along with the studies on obese animals,
147 the role of BAT in nutritional energetics was further explored in a range of physiological and
148 pathophysiological situations in which body fat and energy flux change (Fig. 2). These included
149 the reproductive cycle – pregnancy and lactation – hibernation, photoperiod, cancer cachexia and
150 nutritional manipulations such as fasting/refeeding and the provision of a low protein diet^(34,35).

151 Lactation was a physiological stress of particular interest to us at the Dunn. The energy
152 cost of lactation is high in small mammals, and energy intake is increased by approximately 3-
153 fold in lactating rats, for example, compared to virgin animals^(36,37). Our studies in mice showed
154 that BAT thermogenesis is suppressed in lactation, the suppression being maximal in late

155 lactation when milk production peaks⁽³⁸⁻⁴⁰⁾. Mitochondrial mass and GDP binding are both
156 markedly reduced in BAT of lactating mice, the latter to the same level as virgin animals at
157 thermoneutrality^(38,39). The concentration of UCP1 in the mitochondria is also reduced relative to
158 that of virgin mice, with the total UCP1 content of the interscapular pad at late lactation being
159 <10% of the virgin animals (Fig. 3)⁽³⁹⁾. These changes in BAT activity effectively lead to a
160 substantial energy saving, helping to meet the high energy cost of milk production. However, this
161 adaptation essentially reflects the limited scope for heat dissipation in the face of the high
162 metabolic heat generation associated with milk synthesis rather than a specific energy saving
163 mechanism as such, as convincingly argued in a recent review on BAT in lactation⁽⁴⁰⁾.

164 Perhaps the strongest illustration of the link between energy expenditure and BAT
165 thermogenesis comes from the changes in the tissue that occur when small rodents are
166 acclimated to the cold. In mice, energy expenditure and food intake are increased 3-fold between
167 thermoneutrality and 4°C, reflecting the energy cost of generating heat for homeothermy^(12,41). In
168 rats acclimated at 4°C, the mitochondrial content, mitochondrial GDP binding and UCP1
169 concentration were each substantially higher than in rats acclimated to thermoneutrality (29°C),
170 while the total UCP1 content of the interscapular BAT depot was increased by >100-fold⁽⁴²⁾.

171

172 **Brown adipose tissue in humans**

173 By the beginning of the 1990s, the importance of BAT in nutritional energetics had been firmly
174 established across a range of obesity models and in other conditions in experimental animals in
175 which energy flux is altered. In the case of humans, interest in the tissue had been driven to a
176 considerable extent by the concept that reduced thermogenesis is a key factor in the
177 development of obesity in humans and that BAT is a potential therapeutic target for the
178 treatment of the disorder.

179 Although brown fat was widely recognised to be an important locus of heat production in
180 the human neonate, the tissue appeared – on the basis of histological appearance – to be absent
181 after the first few years of life. The presence of BAT in adult humans was confirmed, however,
182 by immunological studies identifying UCP1 in fat depots, including in some elderly subjects⁽⁴³⁻⁴⁵⁾.
183 In addition, expression of the *UCP1* gene was evident through detection of the encoded
184 mRNA⁽⁴⁶⁾. Activation of the tissue in patients with pheochromocytoma was also
185 demonstrated^(47,48).

186 Despite the clear evidence for the presence of BAT in adults, with the capacity for
187 adaptive changes, the prevailing view was that the tissue was of little, or no, significance in
188 human energetics other than in neonates and during the first years of life. Interest in BAT then

189 declined markedly, with the notable exception of those groups (particularly that of Cannon and
190 Nedergaard in Stockholm⁽²³⁾) whose principal focus was on understanding the fundamental
191 biology of the tissue. Since 2009 there has, however, been renewed interest on BAT in humans
192 following the application of fluorodeoxyglucose positron emission tomography⁽⁴⁹⁻⁵¹⁾. This has
193 firmly demonstrated active BAT in adults, activity being reduced in obesity and with ageing, for
194 example, while being stimulated on cold exposure and by the administration of a selective β_3 -
195 adrenoceptor agonist⁽⁴⁹⁻⁵⁵⁾.

196

197 **White adipose tissue – the discovery of leptin**

198 As interest in BAT declined, my own research focus changed and abruptly so following the
199 cloning of the *Ob* (*Lep^{ob}*) gene and the identification of the encoded protein⁽⁵⁶⁾. Within days of the
200 report in *Nature* on 1 December 1994, my group at the Rowett in Aberdeen (where I had
201 relocated in 1988) had designed and validated oligonucleotide probes to examine *Ob* gene
202 expression. This move reflected the fact that some two years earlier a consortium of us in the
203 UK, which included Michael Stock and John Stirling, had sought funding to identify the
204 defective genes in the obese mouse mutants. We were, however, unsuccessful since it was argued
205 (correctly) that at least one group in the United States was well-advanced in the goal and that it
206 was unlikely that we could be competitive. My response was that once the *Ob* gene had been
207 identified, our strategy would be to explore the physiology of the protein product.

208 The *Ob* gene was reported to be expressed in white adipose tissue (WAT) and the protein
209 – initially termed ‘OB’, and then leptin – to act as a lipostatic signal⁽⁵⁶⁻⁵⁸⁾. Subsequently, the
210 hormone was found to be produced by several tissues, including BAT⁽⁵⁹⁾ and the placenta⁽⁶⁰⁾,
211 although WAT is the major source. Similarly, the functions attributed to leptin quickly expanded
212 and it became regarded as a pleiotropic factor⁽⁶¹⁾. The early studies of my group at the Rowett
213 demonstrated that expression of the *Ob* gene is nutritionally regulated, the mRNA level in WAT
214 of lean rodents rapidly decreasing on fasting with a restoration on refeeding⁽⁶²⁾. The circulating
215 levels of the hormone change in parallel with the alterations in gene expression⁽⁶³⁾.

216 We then showed that acute exposure of mice to cold led to a strong inhibition of *Ob*
217 expression, and a fall in circulating leptin level, both of which are rapidly reversed on return to a
218 warm environment^(64,65). The cold-induced reduction in *Ob* mRNA level was mimicked by the
219 administration of noradrenaline and by the β -adrenoceptor agonist isoprenaline. From these
220 observations we proposed that the sympathetic system plays a key role in the regulation *Ob* gene
221 expression⁽⁶⁴⁾. Subsequent observations indicated that this operates primarily through β_3 -
222 adrenoceptors^(65,66). Further studies on leptin at the Rowett included the demonstration by *in situ*

223 hybridisation that the receptor, and particularly the long form responsible for signalling, is
224 strongly expressed in regions of the hypothalamus, consistent with being an adipocyte-derived
225 signal for appetite⁽⁶⁷⁾.

226

227 **Adipokines and the secretory function of white adipocytes**

228 Leptin quickly became a major area in research on obesity and its associated disorders. One of
229 the key outcomes of the discovery of the hormone was a radical change in perspective on the
230 functions of white adipocytes and therefore of WAT itself. Adipocytes were recognised as
231 endocrine cells with WAT as a major signalling organ⁽⁶⁸⁻⁷¹⁾. Although secreted protein factors
232 had been identified previously, this had not led to the conceptualisation of white adipocytes as
233 endocrine and signalling cells. The secreted proteins known prior to leptin were adipisin
234 (complement factor D)⁽⁷²⁾, which is a serine protease, the cytokine TNF α ⁽⁷³⁾, and lipoprotein
235 lipase. Lipoprotein lipase is, of course, released from adipocytes to catalyse the breakdown of
236 circulating triacylglycerols to enable the uptake of fatty acids into adipocytes; it was not, however,
237 regarded as a fat cell secretory protein as such.

238 The 'secretome' of adipocytes, and of WAT as a whole, is extensive (Fig. 4). Quantitatively,
239 fatty acids are the largest secretory product, but there are several other lipid groups released from
240 the cells. Some, such as specific prostaglandins and the endocannabinoid anandamide, are
241 synthesised *de novo* within adipocytes, while others, including cholesterol and vitamin A, are taken
242 up, stored, and subsequently released⁽⁶¹⁾. A question raised by the discovery of leptin was
243 whether there are a range of protein hormones and signals synthesised and secreted by fat cells.
244 The answer is very much in the affirmative and one of the earliest of these adipokines, as they
245 are termed, identified was another major adipocyte hormone, adiponectin, whose functions
246 encompass insulin sensitising, angiogenic and anti-inflammatory actions (Fig. 3)⁽⁷⁴⁻⁷⁸⁾.

247 The search for novel adipokines became a core focus of my group, both at the Rowett and
248 later at the University of Liverpool to where I moved in 2002. Among the several adipokines that
249 we discovered were: the neurotrophic signal nerve growth factor⁽⁷⁹⁾; specific metallothioneins⁽⁸⁰⁾,
250 these having metal binding actions; and the lipolytic/cachetic factor zinc- α_2 -glycoprotein^(81,82).
251 Nerve growth factor was found to be linked to the inflammatory response in WAT, secretion of
252 the protein being strongly stimulated by TNF α ⁽⁷⁹⁾. As in mice, zinc- α_2 -glycoprotein expression
253 increased substantially in WAT of patients with cachexia associated with gastrointestinal
254 cancer⁽⁸³⁾.

255 An extensive range of individual adipokines has now been identified, and proteomic
256 studies and *in silico* analysis suggest that there are several hundred in total⁽⁸⁴⁻⁸⁶⁾. The wide-ranging

257 secretory function of white adipocytes established over the past two decades has in part served
258 as a model for other cell types which were not previously regarded as having a significant
259 endocrine or signalling function. Myocytes, for example, are now known to release a range of
260 proteins signals - myokines^(87,88) - while another example is hepatocytes which secrete multiple
261 hepatokines⁽⁸⁹⁾.

262 The identification of a multiplicity of protein signals and factors from adipocytes indicated
263 that WAT is involved in a range of physiological and regulatory processes^(61,70,90-92). While some
264 adipokines are endocrine in function, signalling to tissues and organs distant to the adipose
265 depots, others have local paracrine and/or autocrine actions. The processes in which various
266 adipokines play a role include appetite and energy balance, lipid metabolism, vascular
267 haemostasis, blood pressure, angiogenesis and insulin sensitivity (see^(61,91)). A number of
268 adipokines are linked to immunity and inflammation, these including classical cytokines and
269 chemokines such as IL-1 β , IL-6, IL-10, and MCP-1; they also include inflammation-related
270 factors, examples being VEGF, serum amyloid A and adiponectin (see^(61,70,91)).

271 In obesity, WAT exhibits chronic mild inflammation with increased production and release
272 of inflammatory adipokines. There is a notable exception to this in that the synthesis and release
273 of adiponectin, with its anti-inflammatory action, falls^(93,94). Inflammation in expanded WAT is
274 augmented by the infiltration and activation of macrophages in particular, but also of other
275 immune cells^(92,95-97).

276

277 **Hypoxia and the metabolic response to oxygen deprivation in adipocytes**

278 Inflammation in WAT has been considered a key factor in the development of the major
279 obesity-associated disorders, particularly insulin resistance and the other components of the
280 metabolic syndrome^(3,70,98,99). The question that intrigued me in the early 2000s was why does
281 inflammation develop as adipose tissue mass expands? A 'News' article in *Science* on how cells
282 endure low oxygen⁽¹⁰⁰⁾ encouraged me to consider the possibility that hypoxia might be a key.
283 This was presented as a hypothesis in a 'Horizons' article in the *British Journal of Nutrition* in
284 2004⁽⁹⁰⁾. I am particularly proud of this paper: not only does it describe the hypoxia hypothesis,
285 but it is my most highly cited publication (>1,400 citations in the Web of Science; >2,450
286 citations in Google Scholar) as well as being the fourth most highly cited article in the Nutrition
287 Society's flagship journal (or indeed in all of its journals).

288 The hypothesis proposed that as adipose tissue mass expands with the development of
289 obesity, areas within the tissue become relatively hypoxic as the enlarging adipocytes become
290 more distant from the vasculature, this leading to major adaptive changes involving the hypoxia-

291 inducible transcription factor HIF-1. The recruitment of HIF-1 was hypothesised to lead to
292 increased expression of a series of hypoxia-sensitive genes linked to inflammation and the
293 inflammatory response in WAT. The proposition was based on the following: (i) hypoxia occurs
294 in situations such as ischaemic injury, wound healing and solid tumours leading to extensive
295 metabolic changes⁽⁹⁰⁾, (ii) blood flow to WAT is not increased in obese subjects, despite the
296 higher mass of the tissue⁽¹⁰¹⁻¹⁰⁴⁾, (iii) in contrast to lean subjects, blood flow to WAT does not
297 increase post-prandially in the obese⁽¹⁰⁴⁻¹⁰⁶⁾, (iv) large adipocytes (which may be up to 200 μm
298 diameter⁽¹⁰⁷⁾) are further from the vasculature than the normal diffusion distance for O_2 (100
299 μm)⁽¹⁰⁸⁾. These observations refer to local hypoxia, but the provision of O_2 on a whole-body
300 level is reduced in specific environmental and pathological situations, such as high altitude, deep
301 sea dives, lung diseases and obstructive sleep apnoea^(61,108).

302 In 2007, studies using two separate techniques reported that WAT depots in different
303 types of obese mouse are hypoxic, with the O_2 tension being 2- to 3-fold lower than in lean
304 mice^(109,110). Subsequent studies on mice were consistent with these observations⁽¹¹¹⁾. In contrast,
305 although some human studies have indicated that WAT depots are relatively hypoxic^(103,112)
306 others have reported either the same or an increase in pO_2 (partial pressure of oxygen)^(106,113).
307 The issue remains unresolved, but there is evidence that differences in the way in which O_2 is
308 delivered in terms of vascularisation and utilisation may occur^(106,113,114).

309 From 2004 the focus of my group in Liverpool was on examining the direct effects of
310 hypoxia on gene expression and cellular function in adipocytes. Almost all of our studies were
311 conducted on human adipocytes, differentiated in culture from fibroblastic preadipocytes. The
312 initial priority was to examine whether incubation under a low O_2 tension leads to increased
313 expression and release of inflammation-related adipokines consistent with our initial hypothesis.
314 A candidate gene approach was employed and increased production of several adipokines was
315 observed, including IL-6, VEGF and leptin⁽¹¹⁵⁾. Raised production of VEG and leptin, as well as
316 of specific matrix metalloproteinases, had been reported earlier in 3T3-F442A adipocytes (a
317 mouse cell line), reflecting a pro-angiogenic response to hypoxia⁽¹¹⁶⁾.

318 The key cellular adaptation to O_2 deficiency is a switch from aerobic to anaerobic
319 metabolism. Mitochondrial oxidative phosphorylation cannot, of course, continue when O_2 is
320 severely limited, and there is instead increased anaerobic glycolysis. As expected, adipocytes
321 exhibit greater glucose uptake under hypoxic conditions, as demonstrated by 2-deoxy-D-glucose
322 uptake studies⁽¹¹⁷⁾ and by measurement of glucose in the culture medium⁽¹¹⁶⁾. This is mediated
323 through increased synthesis of the GLUT1 facilitative glucose transporter, driven by a marked
324 stimulation of *GLUT1* gene expression⁽¹¹⁷⁾. The expression of several genes encoding glycolytic

325 enzymes is also raised, glucose-6-phosphate isomerase and phosphofructokinase for example^{(118,}
326 ¹¹⁹⁾. Lactate release is augmented in hypoxic adipocytes^(116,120), reflecting the increased glucose
327 utilisation and glycolytic flux, this being mediated by increases in the synthesis of the
328 monocarboxylate transporter, MCT1⁽¹²⁰⁾.

329 While our initial exploration of the effects of hypoxia on gene expression in human
330 adipocytes probed selective candidate genes, in subsequent studies more comprehensive
331 approaches were taken. In the first of these, PCR arrays for 84 genes linked to the hypoxia-
332 signalling pathway were employed. The expression of a number of the genes changed, with one
333 particular gene exhibiting dramatically increased expression⁽¹²¹⁾. The gene in question was *MT3*,
334 which encodes a member of the metallothionein family, metallothionein-3 (also known as
335 growth inhibitory factor). This protein binds zinc and copper, and linked to its marked induction
336 by O₂-deprivation has been implicated as an angiogenic factor and to protect against hypoxic
337 damage^(122,123).

338 PCR arrays are themselves limited in terms of the number of genes whose expression can
339 be screened and a specific pathway or metabolic system needs to be selected. DNA microarrays
340 offer an unbiased approach in which all, or almost all, the genes expressed in a tissue or cell can
341 be probed simultaneously. Our microarray studies at Liverpool, in collaboration with colleagues
342 at Unilever, indicated that the expression of >1,300 genes was altered in human adipocytes
343 cultured under hypoxic conditions, stringent criteria being used to evaluate changes⁽¹¹⁹⁾. Of these
344 genes, the expression of approximately half were up-regulated and half down-regulated under
345 low pO₂. Bioinformatic analysis showed that a number of metabolic pathways and functions are
346 altered in human adipocytes by hypoxia, these including lipolysis, lipid oxidation, glucose
347 utilisation, cell to cell signalling and cell death⁽¹¹⁹⁾.

348 It is evident from these and other studies that hypoxia results in extensive changes in gene
349 expression in adipocytes. Several important functional changes have been described, in addition
350 to increased anaerobic glycolysis (Fig. 5). These include the rapid induction of insulin resistance
351 through the direct inhibition of insulin signalling^(124,125), and the disruption of the extracellular
352 matrix within WAT that characterises fibrosis^(126,127). With respect to fibrosis, hypoxia leads to
353 changes in the expression of collagens released as components of the extracellular matrix, as well
354 as matrix metalloproteinases^(116,126) involved in tissue remodelling. The overall cellular response
355 to low O₂ is regulated by a series of hypoxia-responsive transcription factors of which hypoxia-
356 inducible factor-1 (HIF-1), consisting of two subunits (HIF-1 α and HIF-1 β), is the best
357 characterised^(108,128,129).

358 Our studies, like most that examine the response of cells to hypoxia, were undertaken by
359 comparing 1% to 20% O₂ (95% air/5% CO₂ – ‘normoxia’). However, this is an extreme,
360 effectively representing a comparison between ambient air (higher than arterial pO₂) and marked
361 hypoxia. A questions that interested me was whether there is a critical point at which adaptation
362 to reduced O₂ is initiated in adipocytes, or if there is a gradual response to falling O₂ tension.
363 Experiments in which human adipocytes were incubated with a range of O₂ levels between 20%
364 and 1% clearly demonstrated a ‘dose-dependent’ response to lowering O₂ tension with
365 differences being observed between 21% and 15% and between 15% and 10% O₂⁽¹³⁰⁾. This was
366 true for the expression and secretion of several adipokines, including leptin and VEGF, as well
367 2-deoxy-D-glucose uptake and *GLUT1* gene expression. Nevertheless, changes tended to be
368 more marked between 10% and 5% O₂. Since the pO₂ in WAT of lean mice is equivalent to
369 ~7% O₂ while in obese mice it is ~2%, it is evident that there are responses to O₂-deprivation
370 over physiologically relevant differences in tissue oxygenation between the phenotypes⁽⁹¹⁾.

371 These experiments on the effects of a range of O₂ levels demonstrate that while the
372 customarily employed protocols in hypoxia studies offer ‘proof of principle’, they lead to an
373 exaggerated view of the scale of the cellular response to relative O₂ lack under normal
374 physiological conditions. This raises a question of the extent to which our understanding of
375 cellular processes has been conditioned, or even distorted, by the routine use of 20% O₂ as the
376 gas phase in cell culture and other *in vitro* experiments. It is intriguing that careful attention is
377 paid to the pH (7.4), temperature (37°C) and the concentration of glucose and other nutrients in
378 cell culture to ensure ‘physiological’ conditions (except when they are the parameter under
379 investigation), but the O₂ tension employed is quite unphysiological and indeed reflects overt
380 hyperoxia.

381

382 **Oxygen – an overlooked macronutrient**

383 A corollary of our studies on hypoxia is that they underscore that O₂ is a key nutrient at the
384 cellular level. Indeed, investigation of hypoxia is in effect exploration of the molecular and
385 metabolic consequences of the deficiency of a nutrient. However, O₂ is not considered as a
386 nutrient as such in the context of nutritional science. Textbooks of nutrition do not contain
387 sections on O₂, and reference to it is generally restricted to discussion of metabolic rate and
388 respiratory quotient. I have argued recently that O₂ should be included alongside the other
389 elements/molecules/macromolecules that are defined as nutrients^(131,132).

390 O₂ undoubtedly meets dictionary definitions of a nutrient; for example “as a substance that
391 provides nourishment for the maintenance of life and for growth” (*Oxford English Dictionary*). The

392 central reason why O₂ is not considered as part of nutritional science is because of the route of
393 entry – the nose/lungs in higher terrestrial animals, rather than the mouth/gastrointestinal tract.
394 However, I argue that the route of entry should not be the critical determinant of whether O₂ is,
395 or is not, considered a nutrient, but rather its function and essentiality⁽¹³²⁾. O₂ is, of course,
396 critical to all aerobic species without which mitochondrial oxidative phosphorylation cannot take
397 place.

398 Early organisms developed under anoxic conditions, the level of O₂ in the atmosphere
399 being just 1 part in a million soon after the Earth was formed some 4.54 billion years ago^(133,134).
400 It was only after the initiation of the ‘Great Oxidation Event’ some 2.45 billion years ago that
401 considerable amounts of O₂ began to appear in the atmosphere⁽¹³⁴⁻¹³⁶⁾, the present level of 21%
402 being essentially stable over the past 600 million years⁽¹³⁴⁾. The availability of O₂ in abundance in
403 the atmosphere was critical to the evolution of life as we know it.

404

405 **Concluding perspectives**

406 An odyssey with the adipose tissues that began for me over 40 years ago has provided much by
407 way of riches and changed perspectives. The unique bioenergetic properties of BAT
408 mitochondria, through the presence of the cell-specific UCP1, was initially thought to generate
409 heat only in relation to temperature regulation. Subsequently, the link to energy balance was
410 established and the tissue has provided a theoretical target for the treatment of obesity. BAT is
411 also implicated in metabolic regulation more broadly than was originally envisaged, through roles
412 in glucose homeostasis and triglyceride clearance^(55,137-140). Whether it is a realistic target for the
413 treatment of obesity and the metabolic syndrome, as many propose^(55,137-140), remains a matter of
414 continuing debate - my own view, as noted recently, is that there are formidable barriers to this
415 concept⁽¹⁴¹⁾.

416 Perspectives on the physiological role of WAT have changed radically since the discovery
417 of leptin. An organ that appeared confined to fuel storage - a view reinforced by the histological
418 structure with a single lipid droplet taking up most of the volume of mature white adipocytes -
419 has emerged as having major endocrine and signalling functions. For specific adipose tissue
420 depots there is good evidence of local impact in relation to the organs and tissues with which
421 they abut⁽¹⁴²⁾; examples are the epicardial fat, postulated to play a role in cardiovascular
422 disease^(143,144), and dermal adipose tissue which is implicated in hair cycling and wound
423 healing^(145,146). A specific role in relation to cancer and tumour microenvironment is also evident
424 for some depots^(147,148).

425 My research has centred throughout on what are traditionally considered to be BAT and
426 WAT, both of which are defined by their respective signature cells. However, a third type of

427 adipocyte is now recognised, namely the beige or brite cell^(149,150). Beige adipocytes have some,
428 though not all, the characteristics of brown fat cells, and in particular are thermogenic through
429 the presence of UCP1. Beige adipocytes are found predominantly within what are regarded as
430 WAT depots and a number of factors lead to their recruitment, particularly cold exposure and β -
431 adrenergic stimulation^(151,152). The complexity and diversity of fat cells may be even greater with a
432 recent study reporting four distinct human adipocyte subtypes⁽¹⁵³⁾.

433 Although work on hypoxia has focused on WAT, with substantial changes in gene
434 expression and function being demonstrated in white adipocytes, a deficiency in O₂ availability
435 can also occur with BAT. BAT has an exceptionally high O₂ demand in order to fuel
436 thermogenesis and hypoxia has been noted in the tissue of normal mice exposed to cold⁽¹⁵⁴⁾.
437 Hypoxia is not evident, however, in mice acclimated to a warm environment (30°C), and studies
438 on *Ucp1* knockout animals indicate that it occurs only with thermogenesis⁽¹⁵⁴⁾. Obese mice
439 exhibit vascular rarefaction and a substantial reduction in pO₂ in BAT compared with lean mice,
440 leading to a 'whitening' of the tissue together with mitochondrial dysfunction and loss⁽¹⁵⁵⁾.

441 From the effects on hypoxia on white adipocytes, it was stressed above that as cells are
442 customarily incubated under hyperoxic conditions (20% O₂) we may have obtained a somewhat
443 distorted view of cellular processes. This may be true for many types of cell, including brown
444 adipocytes. Finally, one of the implications with the response of white adipocytes to graded
445 levels of O₂ is that cells carefully titrate small changes in the concentration of this critical nutrient
446 and this results, as with other nutrients, in the continuous modulation of cellular function.

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465

466 **Conflict of Interest**

467 The author has no conflicts of interest.

468

469 **Authorship**

470 The author has sole responsibility for all aspects of the preparation of this article.

471

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823

824

825

826 **Legends to Figures**

827

828 **Fig 1.** ‘Engaging’ with nutrition at the Dunn – Friday morning group ‘coffee and cake’. Eating
829 and drinking in the laboratory is, of course, prohibited now, but was normal in my early years as
830 a scientist.

831

832 **Fig. 2.** Schematic of different physiological and pathological conditions in experimental animals
833 in which energy flux and/or balance are altered where increases, or decreases, in brown adipose
834 tissue thermogenesis have been demonstrated. Examples of key situations in which brown fat
835 thermogenesis changes are shown. DIT, diet-induced thermogenesis; PUFA, polyunsaturated
836 fatty acids; VMH, ventromedial hypothalamus.

837

838 **Fig. 3.** The thermogenic activity and capacity of BAT is decreased in lactation. The changes in
839 mitochondrial GDP binding, the mitochondrial concentration of UCP1 (UCP1 conc) and the
840 total UCP1 content of the interscapular BAT depot are shown for mice at late lactation (when
841 milk production is close to maximal) relative to virgin mice (virgin = 1)⁽³⁹⁾.

842

843 **Fig. 4.** The secretome of white adipocytes. Fatty acids and other lipids are secreted, together
844 with a multiplicity of adipokines (proteins); examples of some of the lipids and key adipokines
845 are shown. The major adipocyte hormones, leptin and adiponectin, are highlighted. angptl4,
846 angiopoietin-like protein-4; CETP, cholesteryl ester transfer protein; DPP4, dipeptidyl peptidase-
847 4; IGF, insulin-like growth factor-1; IL, interleukin; LPL, lipoprotein lipase; MCP-1, monocyte
848 chemoattractant protein-1; MIC-1, macrophage inhibitory cytokine-1; MIF, macrophage
849 migration inhibitory factor; MMP, matrix metalloproteinase; NGF, nerve growth factor; PAI-1,
850 plasminogen activator inhibitor-1; RBP4, retinol binding protein-4; TGF β , transforming growth
851 factor- β ; TNF α , tumour necrosis factor- α ; VEGF, vascular endothelial growth factor; ZAG,
852 zinc- α_2 -glycoprotein.

853

854 **Fig. 5.** Schematic representation of the central cellular responses to hypoxia in white adipocytes.
855 The effect of low pO₂ on gene expression, glucose uptake and utilisation, and the production of
856 selected key adipokines is shown. angptl4, angiopoietin-like protein-4; FA, fatty acid; GLUT1,
857 facilitative glucose transporter 1; HIF-1, hypoxia-inducible factor-1; IL-6, interleukin-6; MCT1,
858 monocarboxylate transporter-1; MIF, macrophage migration inhibitory factor; MMP, matrix
859 metalloproteinase; MT-3, metallothionein-3; PAI-1, plasminogen activator inhibitor-1; TF,

860 transcription factors (other than HIF-1); VEGF, vascular endothelial growth factor. Modified
861 from⁽¹³¹⁾.
862
863

For Peer Review

Fig. 1



Figure 1.

190x254mm (300 x 300 DPI)

Fig. 2

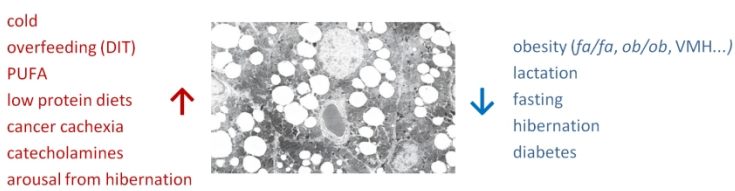


Figure 2.

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Fig. 3

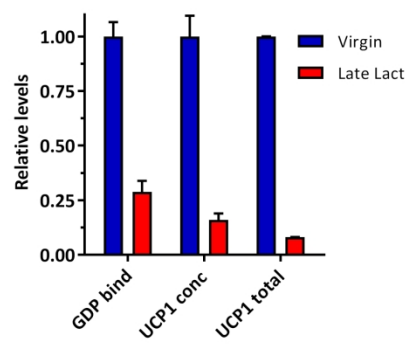


Figure 3.

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Fig. 4

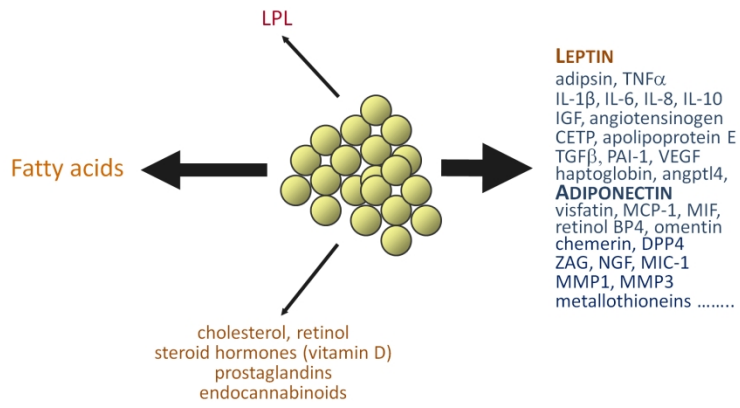


Figure 4.

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Fig. 5

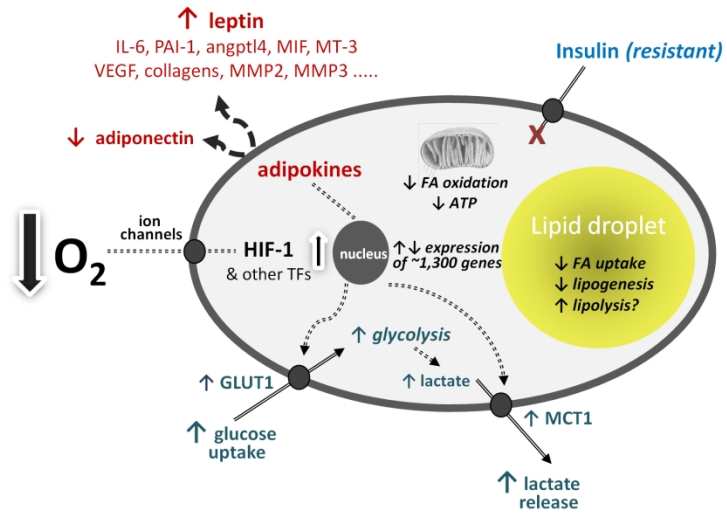


Figure 5.

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