The effects of early under-nutrition on the development of wBAT and obesity

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A population of brown adipocytes emerges in white fat depots at weaning. The function of these adipocytes is not known, but at this late stage of development it is unlikely they are essential for body temperature regulation experienced during the cold stress at birth. A dietary protocol of under-nutrition during the perinatal period, causing hypoinsulinemia, hypoleptinemia and hypoglycemia, led to an 85% reduction in expression of brown fat biomarkers and genes encoding the components of the respiratory complex, the TCA cycle and fatty acid oxidation. Suppression of wBAT in 21-day-old mice showed no significant effect on diet-induced obesity or cold tolerance in adult mice. Analysis of gene expression indicated that capacity to induce the brown fat phenotype was normal. This suggests that the brown adipocytes in white fat of 21-day-old mice are highly plastic and able to recover from severe malnutrition or that a new population of brown adipocytes is induced de novo in adult mice.

Introduction and Background

The possibility that stimulation of thermogenesis in brown fat could be an effective mechanism to reduce obesity in humans is based upon extensive studies with rodent models. For many years the study of brown adipocyte biology to many biologists was not much more than a biological curiosity, since it was thought that adult humans did not have levels of brown fat sufficiently high to make a difference to human obesity. While it may still not lead to effective strategies to reduce human obesity, PET technology has revealed that hot spots of metabolism detected in humans could be deposits of brown fat as well as tumors. This finding has opened up the possibility that humans may have levels of brown fat sufficient to reduce obesity. However, these years of intensive molecular genetic research on the mouse has revealed a complex picture of brown adipocyte biology that will be a challenge to apply to the human situation. At the heart of this complexity lie two types of brown adipocyte that originate from separate and independent cellular origins or lineages. One type of brown adipocyte is represented by those brown adipocytes found in discrete brown fat depots, exemplified by the interscapular brown adipose tissue (iBAT), and the other by the diffuse brown adipocytes found in traditional white fat depots. Current research seeks to determine how these brown adipocytes can be used to reduce obesity. We anticipate that a positive outcome of this complexity is that additional strategies will be identified to activate novel thermogenic pathways and mechanisms in these different types of brown adipocytes.

The discrete brown adipocyte depot, exemplified by that in the interscapular region, is formed during the last days of
gestation and is fully functional at birth to provide the heat necessary for survival of the newborn upon exposure to the lower ambient temperature at birth. While one can only speculate whether humans and other animals require brown fat for thermogenesis at birth, the fact the mice with an inactive Ucp1 gene rarely survive the first few days of life when the breeding room is 23°C or less, but are completely normal when the ambient temperature is increased to 28°C, establishes that an essential function of interscapular brown fat in mice is to protect the newborn from the stress and shock of cold exposure at birth. However, that pigs do not have a functional Ucp1 gene and yet survive in the wild indicates that an evolutionary adaptation in mammals has led to an alternative mechanism of thermogenesis.

We know much less about the function of brown adipocytes that are found in white fat depots. Under the normal conditions (ambient temperature of 23°C) of mouse facilities, biomarkers of brown adipocytes in white fat depots of adult mice, as determined by assays of Ucp1 mRNA and protein and UCP1-immunohistology, are almost undetectable. Exposure of mice and rats to the cold or to β3 adrenergic agonists induces brown adipocytes in white fat in a fat depot specific manner. Furthermore, this induction is genetically variable among inbred strains of mice. Whereas brown adipocytes in the discrete interscapular depot are present throughout life from birth, those in white fat are virtually undetectable in an adult mouse, unless it is adrenergically stimulated. While it was first reported in 1984 that brown adipocytes are found in white fat depots of adult mice after adrenergic stimulation, the realization that these cells do not appear developmentally in mice housed at 23°C until weaning (21 d of age), and then only transiently, was not grasped until 2007. Given that weaning is long past the critical time of cold exposure for the newborn mouse (Ucp1−/− mice are viable at 21 d of age), what could be the physiological function of these brown adipocytes? The virtual absence of Ucp1 in white fat of mice at 23°C indicates this temperature is not a sufficient thermogenic stress to the mice to lead to a robust induction of Ucp1 in white fat depots. Furthermore, there is no consensus that diet will induce Ucp1 and the brown fat phenotype in white fat depots. There is much evidence indicating that animals in which wBAT is induced by adrenergic stimulation are resistant to diet-induced obesity and it is generally assumed that the resistance or reduction in obesity is due to the induction of BAT. However, while one can conclude that the cold exposure or adrenergic agonist treatment reduces obesity, it cannot be unequivocally concluded that this loss of fat is due to increased oxidation by brown fat thermogenesis. At temperatures below 28°C, mice with an inactive Ucp1 gene are in fact more resistant to diet-induced obesity than wild-type mice with an active Ucp1 gene.

Adrenergic stimulation increases triglyceride breakdown in adipose tissue, releasing fatty acids which can be oxidized by muscle, iBAT and other tissues. The same process must occur in Ucp1−/− mice; however, the production and distribution of heat to vital organs is less efficient in Ucp1−/− mice. The net effect is that compared with wild types, mice without UCP1 expend more energy when exposed to the cold and, accordingly, they are more resistant to diet-induced obesity. An important point to emphasize is that if at temperatures between 25 and 28°C, the complete absence of UCP1 in Ucp1−/− mice does not make them any more or less obese compared with Ucp1+/− mice fed either a normal or high fat diet, then mouse models in which very modest changes in UCP1 are proposed to generate an obese phenotype need to be carefully evaluated. It is probable the mechanism causing the obese phenotype in many of these models has little to do with UCP1/BAT.

An optimal experimental model to determine the function of brown adipocytes in white fat depots would be to generate mice in which UCP1/brown adipocytes were selectively inactivated in white fat depots. At the present time such a model has not been created; however, there are mice in which brown adipocyte induction in white fat depots is genetically variant with levels of Ucp1 expression in white fat depots of recombinant inbred strains varying by 100-fold. Since iBAT expression in these inbred strains is genetically invariant, the effects of adrenergic stimulation on obesity should be correlated with wBAT and not iBAT. When selective strains of mice were fed a high fat diet to induce obesity, the level of obesity was independent of the capacity for induction of Ucp1; however, when these mice fed a high fat diet for 18 weeks were treated with CL316,243, a selective β3 adrenergic receptor agonist, those mice with a higher capacity for induction of Ucp1 in white adipose tissues reduced their adiposity to a greater extent. This correlation between reduction of adiposity and the level of Ucp1 mRNA induced in white fat depots supports the hypothesis that wBAT regulates diet-induced obesity in adrenergically stimulated mice, but it does not prove it. As noted above, adrenergic stimulation of adipose tissue stimulates lipolysis and release of fatty acids that are oxidized by skeletal muscle as well as BAT. The experiment described here with strains of mice genetically variant in their capacity to induced brown adipocytes in white fat depots was performed with adult mice that were exposed to an adrenergic stimulus. To ascribe a function to wBAT it is first necessary to induce their appearance. However, brown adipocytes are also induced in white fat depots at 21 d of age as part of the normal developmental program at a normal room temperature of 23°C. Therefore, the early developmental induction of brown adipocytes in white fat is independent of cold stimulation, but the genetic and environmental factors that control its normal regulation are currently unknown. And as noted above, we do not know whether the function of this wBAT in the weaned mouse at 21 d of age is different from that it serves in adrenergically stimulated adult mice.

**Suppression of wBAT Development by Under-Nutrition**

Earlier studies from our laboratory have established that the number of brown adipocytes in the retroperitoneal fat depots at 21 d was genetically variable and was correlated with Ucp1, as well as Pparg and Pparg1 mRNA and protein levels. We recently published a paper on experiments that tested the effects of early mal-
Accordingly, we evaluated DIO
Ucp1
We then investigated
If leptin is vital for the
Figure 1A
and more recently by
Thus are least three hor-
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periments by Landsberg and colleagues indicate that the hypoglycemia would also lead to reduced adrenergic signaling during this period.15 Thus are least three hor-
monal regulators of energy balance have been seriously suppressed. The con-
sequence of the suppression of these critical hormonal regulators on differenti-
ation of both adipose tissue and the hypothalamus would suggest that the development of the brown adipocytes in white fat would have been strongly
affected. Indeed as shown in Figure 1A, based upon expression of Ucp1, other markers of brown fat, as well as the genes encoding proteins of the respiratory com-
plexes, components of fatty acid oxidation and the TCA cycle, the brown adipocyte phenotype was reduced about 85% by the under-nutrition protocol. The underlying
molecular mechanisms for the suppression of brown adipogenesis are not known.

Environmental Perturbations on Early Development of wBAT and Possible Effects on Susceptibility to Obesity in Adults

There is much evidence that environ-
mental dietary perturbations in the peri-
natal period have long-term effects on susceptibility to obesity and type 2 diabetes in adults.16-20 The focus of many of these studies has been on the effects of perturbations on the development on white adipose tissue, pancreas and liver. The impact of early nutrition on wBAT has not been previously been investigated. The initial appearance of brown adipocytes in developing subcutaneous and
visceral fat deposits is part of the normal developmental process that occurs at an ambient temperature of 23°C. At 21 d of age brown adipocytes can transiently represent greater than 50% of the adipocyte population of the inguinal fat white fat. Do these brown adipocytes have a function related to the nutrition of the animal? Since the brown adipocytes are emerging and comingling with the expanding white adipocytes population during development of these adipose tissues, adipogenic mechanisms must co-
exist concurrently in the same tissue for the development of two cell types with diametrically opposed physiological func-
tions. Accordingly, how does an environ-
mental factor such as a calorically dense diet effect the development of the white cell compartment or how will the environ-
mental temperature influence the devel-
opment of the brown adipocyte compart-
ment. Important to the mouse is that virtually all adipokines are first released into the organism at this time.20,21 For example, if leptin has an essential role to play in the development of the neural feeding centers, the mechanism controlling food intake will be modulated within this window.22 If leptin is vital for the development of the reproductive system, then reproductive development will be modulated by leptin during this pro-
cess.23-26 Since leptin secretion is deter-
mained by the size of the adipocyte, and the latter is determined by both energy intake and energy expenditure, the production of leptin and those processes linked to its action as a trophic factor should become sensitive to the nutrition status of the animal during lactation.

So what are the long-term consequences of the suppression of brown adipocytes in white fat on the development of diet-
induced obesity? One might predict that the suppression of brown adipocytes in the white fat of these mice at weaning would reduce the capacity for systemic thermogen-
esis and increase susceptibility to diet-
duced obesity as proposed by Cannon and Nedergaard27 and more recently by Whittle.28 Accordingly, we evaluated DIO in under-nourished mice at an ambient temperature of 23°C and subsequently when the mice were cold challenged by exposure to the cold (4°C) for one week (Fig. 3 in ref. 14). DIO was determined on 16-week-old mice fed a high fat diet from 8 to 16 weeks of age and maintained at 23°C for the duration of the high fat feeding (Fig. 3 in ref. 14) or, as indicated in Figure 3A of reference 14, a subgroup of mice were exposed to the cold (4°C) from week 15 to 16 (day 105 to 112) while also remaining on the high fat diet. Body weight, fat mass, lean mass and food intake were measured during the course of the dietary protocol. The changes in body weight and adiposity at 23°C were very similar to those observed in previous experiments, showing that the major dif-
ference among the control, LON (lactation over-nutrition) and LUN (lactation under-
nutrition) groups were in the reductions in

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ocytes in the inguinal fat depots at
21 d of age.14 We then investigated whether the resulting perturbations in the brown adipocyte established during early development affected diet-induced obesity in adult mice and their thermo-
genic phenotype (for a summary of this study see Fig. 1). Importantly, as evident in Figure 1, the under-nutrition between birth and weaning caused severe hypolepti-
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ference among the control, LON (lactation over-nutrition) and LUN (lactation under-
nutrition) groups were in the reductions in
diet-induce obesity in the LUN group.20 After 105 d in the protocol, the difference in body weight and fat mass between the control and LUN group where highly significant; however, differences in body weight changes from 98 to 105 d did not reach significance (Fig. 3B in ref. 14) and food intake per g of lean mass was not different (Fig. 3C in ref. 14). When the ambient temperature was reduced to 4°C for 7 d a rapid reduction in body weight due to fat mass loss occurred in the three nutritional groups of mice (Fig. 3A and D in ref. 14). The loss in body weight was significantly less in the LUN group. Estimates of feeding efficiency indicated that food intake per gram of lean mass was significantly greater in the three nutritional groups maintained in the cold and it was significantly higher in the LUN group than in the control and LON group (Fig. 3E in ref. 14). A summary of energy intake and expenditure during the week in the cold showed that while total energy expenditure was not significantly different for the three nutritional groups, the source of energy to maintain body temperature differed, that is, the LUN group increased energy reserves from food intake in order to consume less energy from smaller body fat stores (Fig. 3F in ref. 14). This may reflect a mechanism to defend a minimal fat mass.

Surprisingly, while under-nutrition from birth to weaning strongly suppressed the transient induction of wBAT devel-


mogenesis in adult mice. Mice with a history of under-nutrition were able to tolerate one week in the cold at 4°C. If wBAT is a source of diet-induced thermogenesis that can reduce obesity in mice exposed to an obesogenic environment, then mice with suppression of wBAT should be less able to activate diet-induced thermogenesis and consequently be more obese. This clearly did not occur, since mice from the under-nourished group had reduced diet-induced obesity. Alternatively, under-nourished mice with suppressed wBAT could be less susceptible to diet-induced obesity because they would be required to use alternative thermogenic mechanisms that are calorically more costly to regulate body temperature, as we have postulated for UCP1-deficient mice. Although the under-nourished mice had reduced diet-induced obesity, the cause was not from a deficiency in BAT, as shown by the similar levels of brown adipocyte gene expression in iBAT and wBAT in adult mice, irrespective of their nutrition during early development (Fig. 4 in ref. 14). The recovery of wBAT expression in adult mice exposed to under-nutrition during early development suggests two possible mechanisms at play. One mechanism is based upon a plasticity in the differentiated state of the brown adipocyte in inguinal fat from 21-day-old mice. This plasticity evident from the recovery of wBAT in adult mice, suggests that neither the severe hypoinsulinemia nor hypoinsulinemia from birth to weaning have a long-term impact on the development of BAT in inguinal fat depots. Furthermore, although we have no data on sympathetic nervous activity during early post-natal development, it is well known that under-nutrition suppresses the sympathetic nervous system. While a reduction in sympathetic signaling could have been involved in the suppression of the wBAT phenotype at 21 d of age, it had no permanent effects on the capacity of wBAT in under-nourished mice to recover a functional BAT phenotype when subsequently fed a normal diet ad libitum. In short, if leptin, insulin and adrenergic signaling have effects on wBAT development and differentiation during early development, they are transient and do not affect the permanent differentiated state of the brown adipocyte in white fat depots nor in systemic energy balance. A second mechanism is based on the assumption that the nutritional stress of the perinatal period led to the death of the brown adipocytes in the 21-day-old mice and what is observed to be induced in the adult mice, exposed to the cold, is the emergence de novo of a new population of brown adipocytes. Experiments to distinguish between these alternative mechanisms are in progress.

Many excellent studies with transgenic and gene knockout models have described the induction of brown adipocytes in white fat depots. The increase in wBAT in these...
transgenic models strongly correlates with reduced adiposity and resistance to diet-induced obesity. Agents and conditions that induce the number of brown adipocytes include transcription and signaling mechanisms that act directly on adipose tissue and others that act indirectly generally by mechanisms that stimulate adrenergic signaling from the central nervous system. In addition, environmental conditions, which could require an increase in the thermogenic capacity of the organisms for survival, including the release of irisin from skeletal muscle or natriuretic peptide from the heart or simply an enriched environment have been found to induced brown adipocytes in white fat depots. The effects of these agents and conditions on the development of brown adipocytes that arise transiently at 21 d of age, as described in this commentary, has not been explored. Of particular interest would be experiments to assess the effects of the enriched environment upon the early development of wBAT with long-term effects on susceptibility to metabolic disease.

FGF21 is a hormonal factor that could have a role in modulating the thermogenic activity in BAT during the early post-natal period. It has been shown that FGF21 plasma levels are induced immediately after birth possibly by the induction of gene expression in liver. Injection of FGF21 into neonatal mice induced the expression of Ucp1 and other thermogenic genes in the iBAT of neonatal mice. In addition, FGF21 is highly induced in iBAT by cold and ß3-adrenergic receptor stimulation. It is unknown whether the developmental induction of wBAT at 21 d is affected by FGF21 in the post-natal mouse. Since mice with the global inactivation of Fgf21 survive the cold sensitive post-natal period, FGF21 appears not to be essential for thermogenesis in the newborn mouse. Importantly, the Fgf21 KO mice have normal levels of iBAT and Ucp1 expression in the tissue, which questions the importance of FGF21 in non-shivering thermogenesis. It has been argued that the 0.5°C reduction in body temperature in Fgf21 KO mice exposed to the cold at 4°C is caused by a reduction of wBAT levels; however, it needs to be kept in mind that much larger differences in the amount of wBAT relative to iBAT among inbred and RI strains of mice do not affect thermogenesis in response to cold exposure. One could just as easily conclude that the thermogenic and BAT phenotypes of Fgf21 KO mice support the notion that levels of wBAT expression are not relevant to control of body temperature during cold exposure. This does not exclude the possibility that the levels of wBAT can modulate consumption of excess fat upon cold stimulation.

Our goal in this study was to find a functional link to the brown adipocytes that are induced in white fat depots. A summary of the post-weaning phenotypes of the nutritionally stressed mice is given in Figure 1B. Under-nutrition during the post-natal period has profound effects on DIO, in part through its effects on the capacity for adipose tissue expansion in developing mice as early as 5 days of age. However, there is no evidence in support of the idea that the nutritionally suppressed brown adipocyte phenotype in white fat of mice at weaning has a long-term effect on DIO or the thermogenic capacity of the mouse. After a return to a normal dietary protocol in which mice were fed a chow diet ad libitum from weaning to 8 weeks of age at a room temperature of 23°C and then a high fat diet for another 8 weeks, the induction of brown adipocytes in white fat depots was normal. The expression of genes of brown fat thermogenesis was normal and the mice were cold tolerant. Furthermore, there were no effects on the development of DIO, such as enhanced adiposity from a reduced thermogenic capacity. We have referred to the capacity of brown adipocyte to recover a normal phenotype in the adult mice as plasticity. We think that the basis for this plasticity is found in a differentiated state of brown adipocytes in white fat that must be remarkably grounded and fixed to enable it to survive severe malnutrition during early development. The molecular mechanisms for the plasticity could be found in epigenetic mechanisms. If brown adipocytes in the white fat depots of humans have similar characteristics, it bodes well for attempts to reactivate them in response to adrenergic signals.

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