The finding of an elevated expression of Msi2 in these cells suggests the possibility of their complete eradication by Msi2 inhibition.

COMPETING FINANCIAL INTERESTS
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Thyroid hormones: igniting brown fat via the brain

Barbara Cannon & Jan Nedergaard

Thyroid hormones increase the energy metabolism of the body in a process called 'thyroid thermogenesis'. Its molecular mechanism, however, has been elusive. Recent findings in rats suggest that it is localized to brown adipose tissue—our one truly thermogenic organ—and is mediated via the brain (pages 1001–1008).

Humans with an overactivity of the thyroid gland become hyperthyroid and have an increased metabolism; in contrast, hypothyroidism is associated with a decreased metabolism. When mammals are treated with thyroid hormone, they increase their metabolism and heat production. This thyroid thermogenesis phenomenon has been known to exist for more than a century, but efforts to identify the cellular and molecular processes behind this effect have not been conclusive.

One reason why the nature of thyroid thermogenesis has been difficult to resolve is that the different forms of thyroid hormone—T_4, T_3 and perhaps T_2—have clear effects on gene expression and differentiation in almost every tissue in the body. In particular, if animals are experimentally made hypothyroid, there are large effects of re-added thyroid hormone on gene expression. Therefore, it has been conceivable to associate such gene expression effects with the simultaneous increases in metabolism and heat production. It has been much more complex, however, to identify the molecular mechanism of the heat production in thyroid thermogenesis. The mechanisms suggested are usually peripheral, which involves direct interaction of the thyroid hormone with cells in the body outside the central nervous system, and the earlier studies have focused primarily on muscle as the site of heat production.

At the molecular level, a role for the plasma membrane ion pump Na^+-K^+ ATPase and mitochondrial glycerol phosphate dehydrogenase has been invoked[1] (**Fig. 1**). Even one of the 'novel' uncoupling proteins, uncoupling protein-3 (UCP3), enjoyed a short career as the mediator of thyroid thermogenesis[1]. At present, the molecular mechanism most discussed for thyroid thermogenesis involves a release of Ca^{2+} from the endoplasmic reticulum in muscle cells and reuptake of Ca^{2+} into the reticulum by the Ca^{2+}-pumping smooth endoplasmic reticulum Ca^{2+} ATPase[1].

The problem with most of these suggestions is that the evidence is largely based on increases in gene expression. A mere increase in the level of an enzyme, however, would not be sufficient to lead to an increase in heat production. For instance, increased Na^+-K^+ ATPase amounts would lead to increased ATP degradation—thermogenesis—only if the Na^+ leak through the plasma membrane was simultaneously increased. Correspondingly, adding thyroid hormone directly to cells has not resulted in an increased heat production, indicating that the effect of the thyroid hormone may not be direct.

In this issue of *Nature Medicine*, Lopez *et al.*[2] advocate an unexpected mechanism that could initiate a paradigm shift in this metabolic area. Using rats, the authors uncover how increased amounts of thyroid hormone affect the hypothalamus—the part of the brain that controls basic physiological functions, such as eating. The study links this stimulation of the hypothalamus to an activation of brown adipose tissue, which leads to increased energy expenditure and weight loss, hallmarks of individuals suffering from hyperthyroidism. Targeting the hypothalamus may then represent a new strategy to treat diseases with altered body energy balance.

According to the study by Lopez *et al.*[2], the search for a direct peripheral molecular mechanism for the thermogenic effect of thyroid hormones was doomed to be fruitless because 'peripheral' thyroid thermogenesis of that type may not exist. The whole-body metabolic effects observed in individuals with hyperthyroidism then no longer imply that thyroid hormones directly govern the metabolism of each cell in the body, as was earlier believed.

In general, thyroid hormone research has recently seen a shift from explaining thyroid hormone effects as being the result of peripheral mechanisms to understanding these effects as being the result of central mechanisms, such as effects on glucose production in the liver being mediated by the sympathetic nervous system[3].

Concerning thyroid thermogenesis, evidence has implied that this phenomenon is not a direct peripheral effect leading to hyperthermia, but a centrally mediated effect, in that thyroid hormone upregulates the body temperature set by the brain (in reality thyroid hormone induces a 'fever'). An early and rarely cited study[4] may be analyzed[5] to support this theory. In this theory, the increase in metabolism—occurring in any cell in the body and not demanding any specified peripheral molecular mechanism—would mainly be a general effect where a centrally controlled increased body temperature of about 1 °C would thermodynamically correspond to an increase in metabolic rate of about 10%.

In line with this 'centralization' of thyroid hormone effects, Lopez *et al.*[2] show that the metabolic effects of treating rats with thyroid hormone by injecting it in the periphery can be fully mimicked by injecting it in much smaller amounts directly into the brain. They found local molecular effects of such injections in the hypothalamus, and, by using a series of specific inhibitors locally applied in the brain and locally expressed regulatory genes, they showed that these effects of thyroid hormones in the brain were sufficient to induce the whole-body

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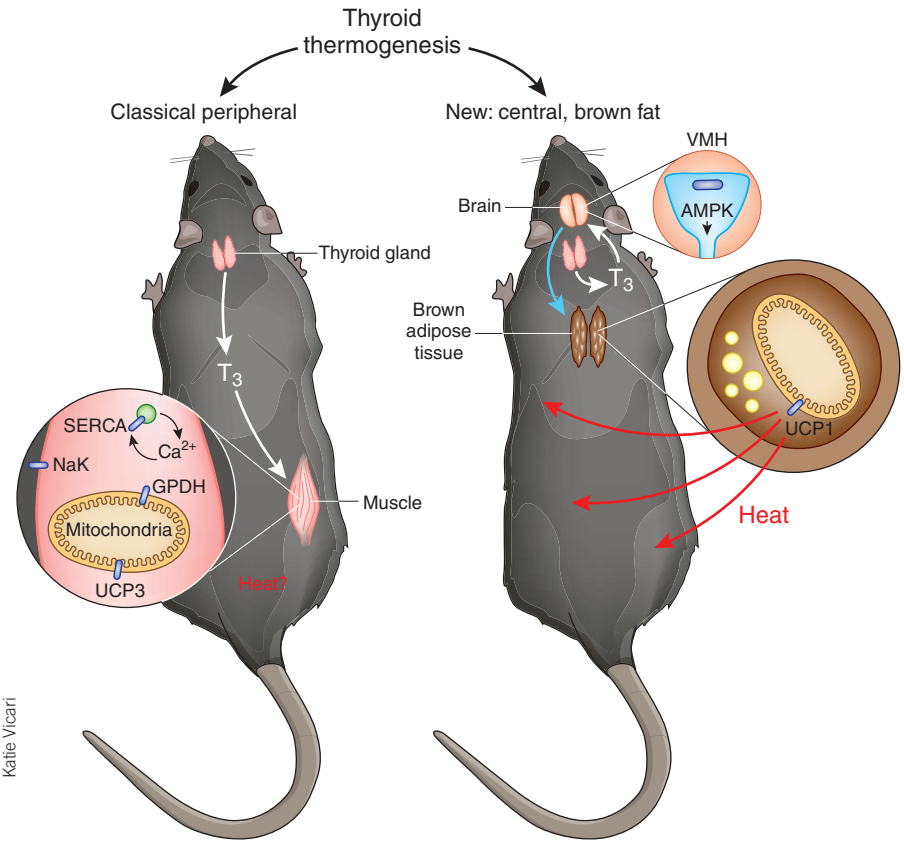

Katie Vicari

Figure 1 The views of thyroid thermogenesis. Thyroid hormone is released as T_4 from the thyroid gland and then converted to T_3 in various tissues. In the classical peripheral view of thyroid thermogenesis (left), T_3 directly interacts with cells in the periphery, primarily muscle and liver, and stimulates certain enzymes through an unknown mechanism—Na^+-K^+ ATPase (NaK), glycerol phosphate dehydrogenase (GDPH) and UCP3—in such a way that heat is produced. Increased Ca^{2+} cycling mediated by smooth endoplasmic reticulum Ca^{2+} ATPase (SERCA) in the endoplasmic reticulum of muscle cells has also been suggested. Using rats, Lopez *et al.*[2] introduce a new and specific concept where T_3 stimulates the VMH through inhibition of the AMP kinase (AMPK) (right). This pathway specifically leads to stimulation of the sympathetic nerves (blue) that innervate the brown adipose tissue and activate the brown fat–specific UCP1 in the mitochondria[7]. The ensuing heat production is observable as an increase in whole-animal metabolic rate—thyroid thermogenesis—and the combustion of energy in the brown adipose tissue leads to body weight loss.

effects on brown adipose tissue activation and body weight loss.

Lopez *et al.*[2] thus formulate the centralization of thyroid hormone action into a specific pathway involving the hypothalamus and its control of brown adipose tissue activity. Interestingly, with these techniques, the authors located the central effect of thyroid hormone to the ventromedial hypothalamus (VMH). This is a classical site for metabolic control, also known as 'the satiety center', but the role of the VMH in metabolic control was more or less dismissed in the 1970s. This change in perception, however, may have been premature[6]. Particularly, a very large amount of data implies a central role for the VMH in the regulation of brown adipose tissue activity[7].

The study by Lopez *et al.*[2] adds to this classical brown adipose tissue pathway by pointing to the VMH as the controlling area in the brain also for thyroid hormone effects on brown adipose tissue. In their model, the thyroid hormone is produced peripherally, but only when it reaches the VMH can it lead to intracellular events that activate brown adipose tissue, resulting in increased body energy expenditure—that is, thyroid thermogenesis (**Fig. 1**).

There are, however, several issues that call for further experimental studies. The authors do not clarify the first steps of how thyroid hormones act in the VMH, although their data show that decreased AMP kinase activity, increased acetyl CoA carboxylase activity and increased activity of fatty acid synthase along with concomitant lipogenesis are successively involved. In this, they refer to other lines of investigation pointing to lipid synthesis in the hypothalamus as a monitor and controller of the energetic state of the body. How these alterations in lipid metabolism in the VMH are translated into the observed increased activity of the nerves innervating brown adipose tissue has not been determined, neither in the present study[2] nor in the previous investigations concerning hypothalamic lipid synthesis regulating whole-body metabolism.

Direct effects on brown adipose tissue thermogenesis were not presented in the report, but acute sympathetic nerve activity to the brown adipose tissue and alterations in UCP1 mRNA levels, a good surrogate for persistent alterations in nerve activity, were examined. Also, even though rats in the study lost body weight in spite of eating the same amount of food—a reasonable indication for an increase in metabolism—there were no direct data showing alterations in the metabolic rate of the rats.

More specifically, the essentiality of brown adipose tissue for thyroid thermogenesis was not formally shown. Such a demonstration would involve examining the existence or absence of thyroid thermogenesis in mice lacking the means for brown fat thermogenesis, such as mice lacking the brown fat–specific UCP1 (ref. 8). On the basis of studies of these mice, an increasing number of metabolic states in which brown adipose tissue activity is essential have been identified. These states, at least in our view, include classical nonshivering thermogenesis, diet-induced thermogenesis and leptin-induced thermogenesis[9]. On the basis of the new findings by Lopez *et al.*[2], thyroid thermogenesis may be added to this list, once studies in mouse models of brown tissue functionality are completed.

This study arrives at a time at which, in the last three years[10], there has been a total shift in the appraisal of the role of brown adipose tissue in human metabolism. It is now generally accepted that a large fraction of all adult humans possess active brown adipose tissue; nearly all young adults have it[11,12]. This means that extrapolation of the data of Lopez *et al.*[2] to humans may be permissible. Many, but evidently not all, manifestations of hypothyroidism or hyperthyroidism in humans correspond to the expected metabolic effects resulting from a decrease or an increase, respectively, of brown adipose tissue activity. The weight gain and decreased cold tolerance observed in individuals with hypothyroidism, and the weight loss and sweating seen in individuals with hyperthyroidism, are the predictable effects of pathological alterations in brown adipose tissue activity caused by these altered thyroid states, according to the model proposed by the authors[2].

Not every adult human being has the same amount of brown adipose tissue, as, in general, it decreases in abundance with age—perhaps causing or aggravating obesity[11,12]. The clinical manifestations, therefore, of the hypo- or

hyperthyroid state may be modulated depending on whether the individual has brown adipose tissue. The new findings of this study may, therefore, explain the increased metabolism in hyperthyroid individuals and why it manifests differently depending on the person.

Whereas the new findings may not lead to any direct alterations in the clinical treatment of hyperthyroidism, they profoundly alter general concepts in metabolism. The study also points to the possibilities of modifying metabolic rates through manipulations of the hypothalamus, opening new avenues for altering metabolic efficiency by activating brown adipose tissue to prevent or ameliorate obesity.

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NET loss of air in cystic fibrosis

A Murat Kaynar & Steven D Shapiro

Thick, adherent mucus in the airway causes respiratory failure—the leading cause of death in cystic fibrosis. A new study now shows how the formation of neutrophil extracellular traps (NETs) in the airway, in an attempt to kill colonizing bacteria, results in chronic cell carnage that thickens the sputum, worsening lung function in individuals with cystic fibrosis (pages 1018–1023).

Cystic fibrosis is one of the most common inherited disorders in populations of European descent, occurring in about one in 3,000 births[1]. Autosomal recessive mutations in the cystic fibrotic transmembrane conductance regulator (CFTR) cause changes in epithelial cell Cl^- secretion and Na^+ absorption, resulting in a variety of abnormalities including abnormal sweating, pancreatic insufficiency, intestinal dysfunction and thick, tenacious mucus in the airways that is difficult to clear. In fact, individuals with cystic fibrosis struggle for hours each day, using mechanical vests, inhaled medications, chest percussion and other procedures to breathe more easily.

In cystic fibrosis, the entire airway, composed of the trachea, bronchi and bronchioles, is draped with abnormal and adherent mucus, making it difficult to clear by the 'mucociliary elevator'—the first line of innate host defense. This is also an attractive breeding ground for infectious bacteria, particularly *Pseudomonas aeruginosa*. Neutrophils, the major acute innate specialized phagocyte, attack the bacteria, but also they die trying to clear the infection. This leaves the airway littered with matrix-destructive proteinases—enzymes used by neutrophils to degrade pathogens—and DNA from neutrophils and other dead cells, which, in turn, makes the mucus even thicker, prone to limiting airflow into the lungs and causing breathlessness.

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A clear understanding of why there is so much DNA and debris in the airway is still elusive. Apoptosis of neutrophils followed by macrophage clearance is normally a very efficient process and should prevent excess debris and DNA. We also do not know why bacteria thrive in this environment. More important, why can't we eliminate this viscous cycle of infection, inflammation and destruction and clogging of the airway in people with cystic fibrosis?

In this issue of *Nature Medicine*, Marcos *et al.*[2] provide new insights into the genesis of the thick DNA-rich sputum and its implication in the pathology of cystic fibrosis. The authors show that NETs, which are the last effort of the neutrophil to trap pathogens, accumulate in the airways, worsening lung function in individuals with cystic fibrosis. Moreover, activation of the neutrophil G protein–coupled membrane chemokine receptor CXCR2 led to the formation of the NETs, and its blockage resulted in improved lung function in a mouse model of cystic fibrosis (**Fig. 1**). This study provides new hints on how to alleviate airflow obstruction in the clinic.

Neutrophils, which are immune cells armed with defense weapons in the bone marrow, are deployed into the bloodstream. In cystic fibrosis, numerous neutrophils invade the airway, a field of unresolved conflict, representing an unusual instance of perpetual and acute inflammation[3]. Activated neutrophils phagocytose bacteria, delivering them to their lysosomal machinery, which uses reactive oxygen species and degrading proteinases for bacterial killing. Before the intracellular killing machinery winds down and the neutrophil prepares for death, it has one more trick up its sleeve—'NETosis'.

NET formation, or NETosis[4,5], begins in the nucleus, where chromatin mixes with histones, alkaline proteins that normally act as spools upon which DNA is wound and packaged and also seem to be antimicrobial. Next, nuclear and granular membranes dissolve, allowing additional antimicrobial proteins to attach to the DNA backbone, including crucial components of primary granules—myeloperoxidase, which generates reactive oxygen species, and neutrophil elastase, a serine proteinase with multiple means of killing bacteria and degrading host proteins. Finally, the cell membrane is disrupted, allowing the NET to extrude into the extracellular space—the neutrophil, now dead, has secreted a web of nuclear and mitochondrial DNA and chromatin sprinkled with antimicrobial factors that continue to capture and kill bacteria at a distance while confining the powerful NET proteins.

A variety of inflammatory stimuli that activate neutrophils, including the chemokine interleukin-8, bacteria and bacterial products such as lipopolysaccharide, were believed to stimulate neutrophil receptors—predominantly CXCR1—and sequentially set off both intracellular killing and NETosis[5] through an NADPH oxidase–dependent mechanism. CXCR1 ligation leads to activation of NADPH oxidase, which is an enzyme that catalyzes the formation of reactive oxygen species. These reactive oxygen species both mediate killing within phagolysosomes and cause membrane disruption and mixing of nuclear and granule NET components during NETosis[4,5].

In cystic fibrosis, however, proteinases shed CXCR1, making CXCR2 the dominant