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REGULATION OF ENERGY EXPENDITURE

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Updated: April 24, 2002

Humans gain or lose weight when a mismatch exists between energy intake and energy expenditure (Figure 1). Due to the potentially important role of energy expenditure in controlling body weight, there has been much interest in processes which contribute to and regulate total body energy expenditure. This interest takes the form of three general questions. 1) Is obesity caused by deficiencies in energy expenditure, and if so, what mechanisms are nonfunctional in obese individuals? 2) How is energy expenditure regulated and what molecular mechanisms are responsible for this regulation? 3) Can energy expenditure be increased by pharmacologic agents and can this be used as a treatment for obesity? Towards addressing these questions, this chapter will explore the role of reduced energy expenditure in causing obesity and the molecular mechanisms which are thought to regulate energy expenditure.

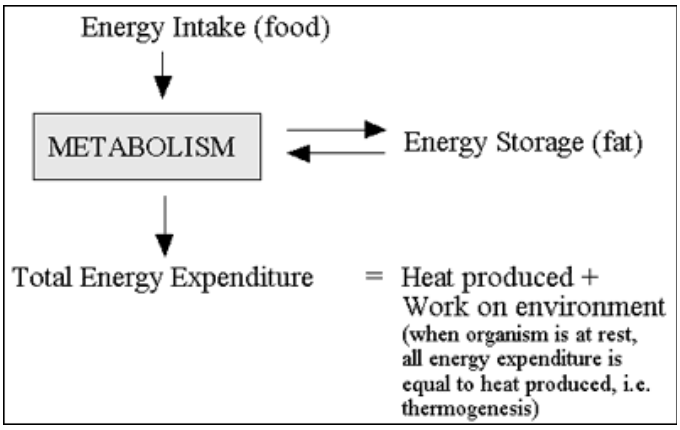

Figure 1.Fat stores represent the net balance between energy intake and energy expenditure. This figure was adapted from reference (29).

ROLE OF REDUCED ENERGY EXPENDITURE IN PROMOTING OBESITY

Animal Studies

Abundant evidence indicates that many rodent models of obesity have reduced energy expenditure and that this contributes importantly to the development of obesity. Perhaps the most compelling evidence comes from mice lacking leptin (ob/ob mice), the adipocyte-derived hormone, or mice lacking the receptor for leptin (db/db mice) (1, 2). These mice have both increased food intake and decreased energy expenditure. When the increase in food intake is prevented by providing only the amount of food eaten by wild-type controls (i.e. pair-feeding), obesity still develops (3). This dramatic finding demonstrates, unequivocally, that mice lacking leptin, or its receptor, have decreased energy expenditure and that this contributes to their obesity.

Human Studies

The role of reduced energy expenditure in promoting human obesity is much less clear. Difficulties in resolving this issue in humans are due, in part, to the heterogeneity of human subjects with respect to height and body composition, making it difficult to compare rates of energy expenditure between individuals, and added difficulties in performing carefully controlled experiments in human subjects. This later point is exemplified by the difficulty in obtaining accurate records of food intake.

A number of tools are used to assess energy expenditure in humans. The most common approach is to quantify rates of oxygen consumption and carbon dioxide production (indirect calorimetry) (4). This method requires that subjects be confined to a metabolic chamber. Another frequently used approach is the doubly labeled water method (5), which has the advantage of providing assessments of 24 hour energy expenditure in freely moving subjects. Through the use of such methodologies it has been shown that obese individuals, on a per person basis, have increased energy expenditure. The increase in energy expenditure is largely attributable to the increase in lean body mass which invariably parallels the expansion of fat mass (4). If rates of energy expenditure are normalized to lean body mass, in general, lean and obese subjects have similar rates of energy expenditure. From such findings, some have proposed that obese individuals have no deficits in energy expenditure. However, as will be discussed below, such a conclusion is likely to represent an oversimplification of a homeostatic process which is dynamic and complex.

While obese individuals, once obese, have normal rates of energy expenditure, it is hypothesized that these individuals have defective regulation of energy expenditure and that reduced energy expenditure, prior to the development of obesity, promoted their weight gain. Support for this view comes from a prospective study in which it was found that low energy expenditure, normalized for lean body mass, predicted future weight gain (6). To explain this observation, it has been hypothesized that each individual has a “fat mass set-point”, and that changes in fat mass above or below this set-point activates processes which function to return fat mass to the individual’s set-point. As fat mass is increased, homeostatic controls are activated which serve to resist further weight gain. These homeostatic controls are hypothesized to involve an increase in energy expenditure. Ultimately, an individual who is destined to become obese, arrives at his or her “obese” set-point and, at this set-point, has “apparently” normal energy expenditure.

A number of studies support the view that individuals have a fat mass set-point, that this set-point differs from individual to individual, and that perturbations in fat mass above or below this set-point activates counteracting changes in energy expenditure. One of the most dramatic demonstrations of this phenomenon, as well as the strong effect of genetic background in causing variation in this response, comes from a now classic study where a number of identical twins were overfed a fixed amount of calories for an extended period (7). The effects of increased caloric intake on body weight gain were assessed and compared between twin pairs and within twin pairs. Between twin pairs, there was much variability in the amount of weight gained following equal increases in caloric intake (Figure 2). Within twin pairs, there was very little variability. Thus, the ability to resist weight gain following increased caloric intake is variable and is highly influence by genetic makeup. Since the increase in caloric intake was fixed in this study and equal amongst individuals, the observed resistance to increased weight gain must be accounted for by increased energy expenditure. This resistance to diet-induced obesity was explored further by another group where energy expenditure was directly assessed (8). It was found that variation in diet-induced weight gain was accounted for by variation in the ability of diet to increase energy expenditure. It was further suggested that the variation was due to a component of energy expenditure termed nonexercise activity thermogenesis (NEAT), which is thought to consist of energy expended during fidgeting, maintenance of posture, and performance of other physical activities of daily life. However, because the existence of NEAT has only been inferred, and has not yet been directly measured in the context of diet-induced weight gain, there is uncertainty regarding its significance with respect to resisting diet-induced obesity.

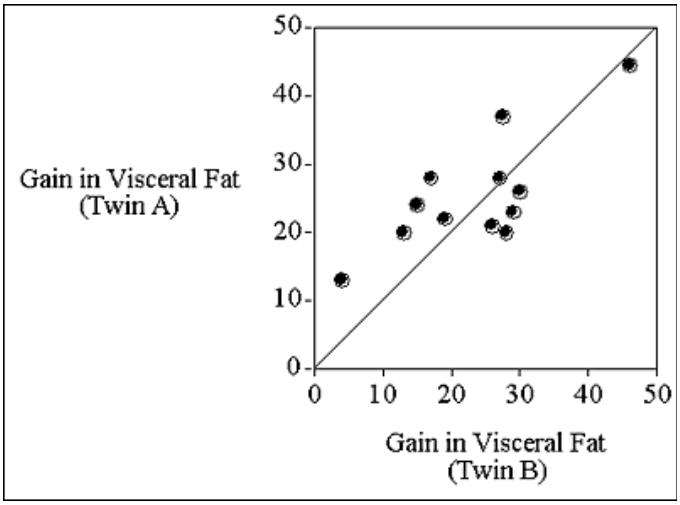

Figure 2.The effects of excess caloric intake on fat weight gain (7). Each point represents one pair of twins (A and B). The closer the points are to the diagonal line, the more similar the twins are to each other. The findings show the large variation between twin pairs and the little variation within twin pairs, demonstrating the strong influence of genes on resistance to diet-induced obesity. This slide was adapted from reference (7).

In another important study, energy expenditure was studied before and after experimentally imposed alterations in body weight (9). Caloric intake was increased or decreased in order to alter body weight up or down by 10%. Once the alteration was achieved, calories were provided such that body weight would be maintained at the new, steady state level. It was found that the increase in body weight caused an increase in energy expenditure above what would be observed for an individual of similar body composition who had never experienced such a weight gain. The converse was true for individuals with a 10% reduction in body weight. This study argues strongly for the existence of a “fat mass set point” and the involvement of altered energy expenditure as a means of defending that set point.

MOLECULAR MECHANISMS OF ENERGY EXPENDITURE AND ITS REGULATION

The proceeding discussion has reviewed the evidence that regulation of energy expenditure plays an important role in maintaining body weight. However, these studies, out of necessity, have largely treated energy expenditure as a “black-box”, disregarding its molecular basis. In order for the causes of obesity are to be identified and for rational therapies are to be developed, it will be important to understand the molecular basis for energy expenditure and its regulation.

Categories of Energy Expenditure

Energy expenditure has many components and these components can be separated into a number of different categories. Over the years, many schemes have been employed to categorize energy expenditure. Each has its advantages and disadvantages but, unfortunately, none provide great insight into the molecular regulation of energy expenditure. Perhaps the simplest scheme (Figure 3) divides energy expenditure into three categories: 1) physical activity, 2) obligatory energy expenditure (i.e. that required for performance of cellular and organ functions), and 3) adaptive thermogenesis (i.e. that which occurs following increases in food intake, termed diet-induced thermogenesis, and decreases in environmental temperature, termed cold-induced thermogenesis). However, as is evident from the above discussion of NEAT (nonexercise activity thermogenesis), which may represent a diet-induced increase in activity-related energy expenditure, the distinctions between these three categories is unclear, and probably of limited utility.

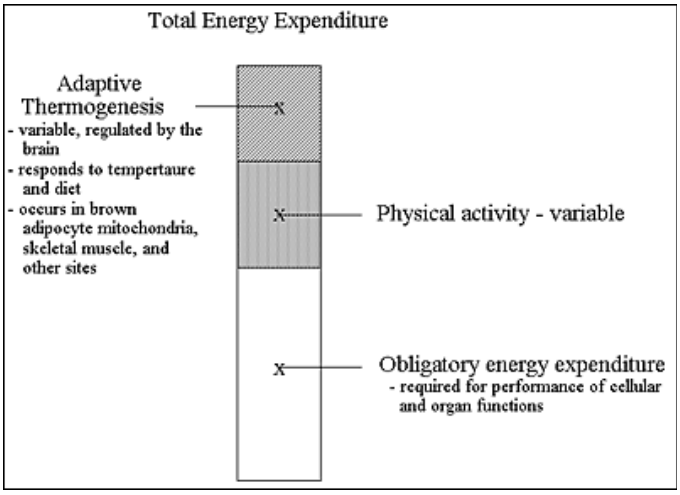

Figure 3.Categories of total body energy expenditure. This figure was adapted from reference (29).

Origin of Energy Expenditure – a Thermodynamic Perspective

Energy enters an organism as food and exits the organism as heat and as work on the environment. Energy is released from food as it is combusted to carbon dioxide and water. The organism controls this combustion such that energy can be channeled to perform work within the cell. This is accomplished by enzymatically controlled fuel metabolism and mitochondrial oxidative phosphorylation, step-by-step processes in which a portion of the energy content of food is converted to ATP (see Figure 4). Energy stored in the form of ATP is then used to perform biological work within the cell. While much of the energy content of food is converted to ATP, a significant portion is lost as heat. This is due to the fact

that in order for reactions to go forward, they need to be thermodynamically favorable (i.e. going from a state of higher energy to a state of lower energy) and, as a result, the conversion of fuel to ATP results in significant amounts of energy being released in the form of heat. Similarly, energy is also lost in the form of heat as ATP is used to perform biological work within the cell.

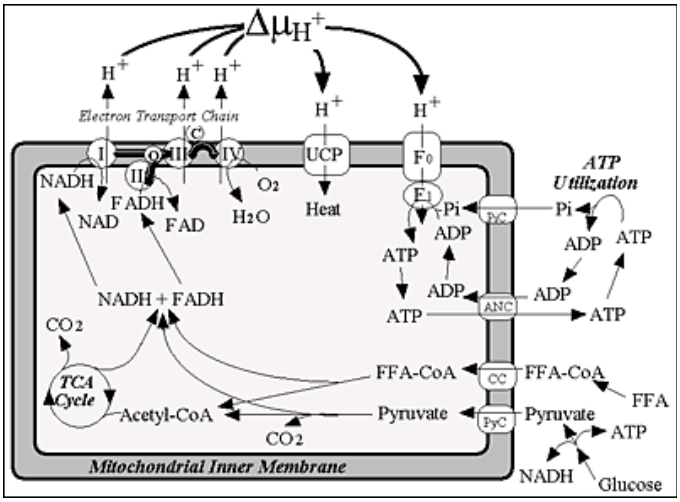

Figure 4.Step-by-step conversion of fuel into ATP and then ATP into biological work within the cell (30). Free fatty acids (FFAs) and glucose are oxidized generating NADH and FADH2 which donate electrons to the electron transport chain. Ubiquinone (Q) shuttles electrons from both complexes I and II to complex III while cytochrome C (C) shuttles electrons from complex III to complex IV. Molecular oxygen (O2) is the terminal electron acceptor. Protons are pumped out by complexes I, III and IV of the electron transport chain creating a proton electrochemical potential gradient (?uH+). Protons may reenter the mitochondrial matrix via the F0F1 ATPase, with energy being used to generate ATP from ADP and Pi. Protons may also reenter via an uncoupling protein (UCP), with energy being released in the form of heat. Proton rentry via ATP synthase depends upon the availability of ADP which is generated in the cytosol from reactions utilizing ATP. Abbreviations: ANC, adenine nucleotide carrier; CC, carnitine carrier; complex I, NADH-ubiquinone oxidoreductase; complex II, succinate:ubiquinone oxidoreductase; complex III, ubiquinone-cytochrome-c oxidoreductase; complex IV, cytochrome-c oxidase; PiC, phosphate carrier; PyC, pyruvate carrier. This figure was adapted from reference (29).

Reactions in Energy Metabolism are Coupled

Reactions in energy metabolism are tightly coupled, and this has great significance for the regulation of energy expenditure (10). This feature of energy metabolism is schematically shown in Figure 5. For a given molecule of fuel, a fixed amount of NADH and FADH is generated, which in turn results in a fixed number of protons being pumped out of the mitochondrial matrix by the electron transport chain. These protons re-enter the mitochondrial matrix via ATP synthase resulting in a fixed number of ATP molecules being created. Subsequently a fixed number of ATP molecules are then used to perform a fixed amount of biological work. For energy expenditure to be increased, one of two things must occur. Either an “uncoupling” of one of these steps in cellular metabolism must occur, or, alternatively, the consequences of biological work, for example, the pumping of ions across the plasma membrane, would need to be “undone” at a higher rate, say by an increase in the leak of ions back across the plasma membrane. This latter mechanism of increasing energy expenditure is often referred to as “futile cycling”. Thus, any molecular explanation for increased energy expenditure must involve either an “uncoupling” of one of the reactions of cellular metabolism or an increase in the activity of a “futile cycle”.

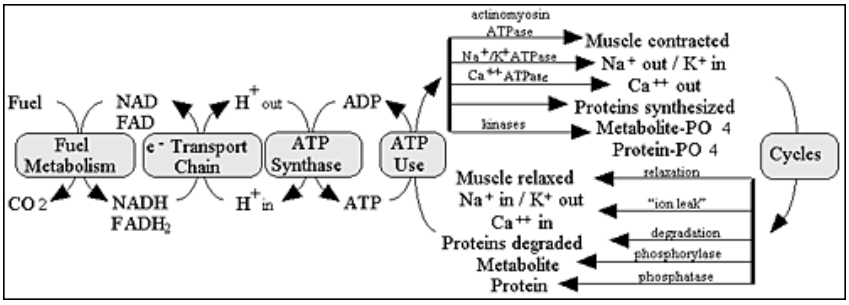

Figure 5.Coupling of reactions in energy metabolism and the operation of "futile cycles" (30). Metabolism of fuel generates a stoichiometric amount of NADH and FADH2. Oxidation of NADH and FADH2 results in 10 and 6 protons, respectively, being pumped out of the mitochondrial matrix. Three protons enter via ATP synthase in order to synthesize one molecule of ATP from ADP and Pi. One additional proton enters the matrix as it is co-transported with Pi via the phosphate carrier. ATP is then utilized to perform a fixed amount of work. The major consumers of ATP are shown above. Muscle relaxation, ion leaks, protein degradation and dephosphorylation create the possibility for "futile cycles". See Rolfe and Brown (10) for a complete analysis of the concept of coupling with respect to reactions in energy metabolism. This figure was adapted from reference (29).

Uncoupling Protein-1 (UCP1): The prototypical uncoupler

UCP1 is the only protein to date which has unequivocally been shown to increase energy expenditure by uncoupling a step in cellular metabolism (11). UCP1 is a mitochondrial inner membrane protein which leaks protons across the mitochondrial inner membrane (Figure 4, see above). The energy which had been stored in the mitochondrial proton electrochemical gradient is released in the form of heat and is not used to synthesize ATP. Hence, there is an "uncoupling" in the relationship between protons entering the mitochondrial matrix and synthesis of ATP. UCP1 is expressed exclusively in brown adipose tissue, a tissue that is abundant in small rodents. The primary function of brown adipose tissue is to generate heat in response to cold exposure. The critical role of UCP1 is evident from gene knockout mice which lack this protein (12). These animals are markedly impaired in their ability to maintain normal body temperature during cold exposure. Humans possess brown adipocytes which express UCP1, however, these cells are thought to be rare in adults, leading to the view that UCP1 is unlikely to be an important contributor to whole body energy expenditure in humans.

Futile Cycles

There has been much interest in the possible role of futile cycles in regulating energy expenditure. However, because the activity of futile cycles is difficult to study in the context of an intact organism, it has been difficult to assess their importance in regulating energy expenditure. One dramatic, pathologic example of a futile cycle increasing energy expenditure is the condition known as malignant hyperthermia, which in some cases is due to a mutation in the skeletal muscle ryanodine receptor (13), the calcium release channel of the sarcoplasmic reticulum. Abnormal calcium release, triggered by anaesthesia and/or stress, leads to increased pumping of calcium back into the sarcoplasmic reticulum, a process which consumes large amounts of ATP. The consumption of ATP, in turn, leads to an increase in all the steps of fuel combustion which precede the synthesis of ATP. The net result is a large increase in energy expenditure.

Abnormalities in futile cycles have not yet been linked to obesity. Cycles, which could in theory contribute importantly to whole body energy expenditure, because they involve reactions consuming

large quantities of ATP, include the leak of ions across membranes, which would lead to increased ion pumping, and the degradation of proteins which would lead to increased protein synthesis (10). Other futile cycles could also be important regulators of energy expenditure.

Energy Expenditure is Regulated by the Brain

The brain detects alterations in environmental temperature and diet and, through neural circuits, which are presently the subject of intense investigation, activates efferent pathways that control energy expenditure (see Figure 6). The pathway controlling diet-induced thermogenesis is likely to involve neurons in the arcuate nucleus of the hypothalamus that express proopiomelanocortin (POMC), which is processed in these neurons to a-melanotroph stimulating hormone (aMSH). The arcuate POMC neurons are activated by leptin and project directly to sympathetic preganglionic neurons in the intermedial lateral column of the spinal cord and to neurons in key central automomic control sites, such as the paraventricular nucleus, which control sympathetic outflow (14, 15). The melanocortin-4 receptor (MC4R) is the likely mediator of aMSH's effects on sympathetically-driven diet-induced thermogenesis. In support of this view, MC4R gene knockout mice are obese (16) and have impaired diet-induced thermogenesis (17).

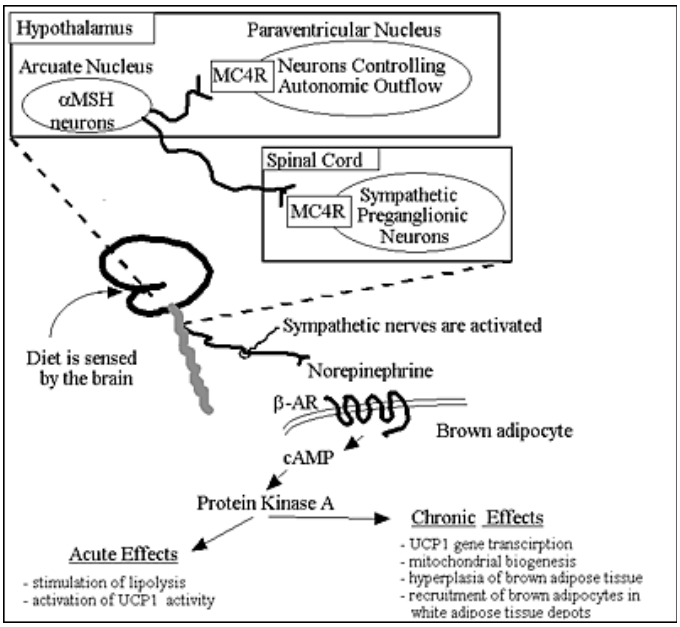

Figure 6.Central and efferent pathways regulating energy expenditure. Diet and cold is sensed by the brain. In the case of diet-induced thermogenesis, a strong case can be made for the role of aMSH neurons in the arcuate nucleus of the hypothalamus which project to neurons in the paraventricular nucleus of the hypothalamus controlling sympathetic outflow, as well as to sympathetic preganglionic neurons located in the intermedial lateral column of the spinal cord. As discussed in the text, MC4Rs are likely to play an important role. These pathways lead to increased activity of sympathetic nerves which release norepinephrine, activating bARs. This has acute and chronic effects on brown adipocytes which promote increased thermogenesis. This figure was adapted from reference (29).

Role of the Sympathetic Nervous System

The primary efferent pathway regulating energy expenditure is believed to be the sympathetic nervous system, which heavily innervates the thermogenic target tissue, brown adipose tissue (3). Indeed, animals treated with various blockers of the sympathetic nervous system, as well as mice lacking

norepinephrine and epinephrine due to knockout of the dopamine beta hydroxylase gene, have impaired brown fat function and are unable to maintain body temperature during cold exposure (18). In addition, administration of beta adrenergic receptor agonists leads to a marked increase in energy expenditure (3, 19). There are three b-adrenergic receptors (bARs) which could mediate sympathetically driven thermogenesis, however, the relative importance of each is presently unknown. One of these receptors, the b3-AR, merits further discussion. This sub-type is expressed nearly exclusively on white and brown adipocytes in rodents, and on brown adipocytes in humans (20). Selective ligands have been developed and these have marked anti-obesity actions in rodents (21). The development of agents with similar anti-obesity effects in humans has been problematic. This may be because humans, in contrast to rodents, have relatively fewer brown adipocytes and express b3-ARs on brown but not white adipocytes (20, 22).

Thermogenic Target Tissues of the Sympathetic Nervous System – Mice versus Humans

Brown adipose tissue, with its high expression of the mitochondrial uncoupling protein UCP1, is an important mediator of sympathetically-regulated thermogenesis in rodents. Given that humans have a relative lack of brown adipocytes, it has been suggested that other relevant thermogenic target tissues may also exist. At present, evidence indicates that skeletal muscle may play an important role. A significant portion of the variation in metabolic rate between humans can be accounted for by differences in skeletal muscle energy expenditure (23). Also, epinephrine infusion, which in humans causes a 25% increase in energy expenditure, increases forearm muscle oxygen consumption by as much as 90% (24). The molecular mediators of thermogenesis in skeletal muscle, however, are presently unknown.

Target Genes within Tissues Mediating Thermogenesis (UCP1 and PGC-1)

As discussed above, brown adipose tissue is an important mediator of sympathetically-driven thermogenesis. Thus, the proteins responsible for this activity in brown fat have been the subject of intensive investigation. The importance of UCP1 as a mitochondrial uncoupling protein have already been discussed. The molecular explanation for exclusive expression of UCP1 in brown adipocytes has been an important area of investigation (Figure 7). A 220 base-pair enhancer has been identified in the UCP1 promoter, located approximately 2.4 kb upstream of the mouse and rat UCP1 genes, which mediates brown fat specific expression and induction by bAR stimulation (25, 26). However, analysis of this complex enhancer element has failed to lead to the identification of a brown fat-specific transcription factor. Instead, this element has been shown to bind a number of nuclear hormone receptors including the thyroid hormone receptor, the retinoic acid receptor and the peroxisome proliferator-activated receptor-g (PPARg). Since PPARg is expressed in white and brown fat, and the thyroid hormone and retinoic acid receptors are widely expressed, the brown fat-specific activity of this enhancer has been enigmatic. This apparent paradox may, in part, be resolved by the recent discovery of the transcription coactivator, PPARg coactivator-1 (PGC-1) (27). PGC-1 binds to and increases the transcriptional activity of many transcription factors, including PPARg, the thyroid hormone receptor and the retinoic acid receptor, and is expressed at high levels in brown but not white adipocytes. Furthermore, PGC-1 expression in brown adipocytes is highly induced by increased activity of the sympathetic nervous system, an effect mediated by bARs. Thus, PGC-1 may explain the brown fat-specific expression of UCP1, and its induction by sympathetic stimulation.

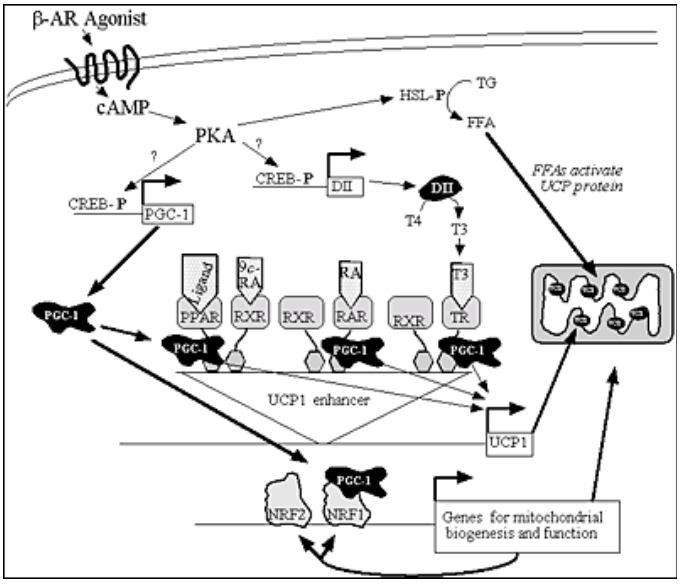

Figure 7.Pathway for bAR-mediated activation of thermogenesis in brown adipocytes (30). a-adrenergic receptor (a-AR) agonists stimulate generation of cAMP which in turn activates protein kinase A (PKA). PKA phosphorylates CREB (cAMP regulatory element binding protein) which leads to increased gene transcription. It is hypothesized that activated CREB directly induces expression of PGC-1 and the type II thyroxine deiodinase (DII). PGC-1 coactivates transcription factors assembled on the UCP1 enhancer, thus increasing UCP1 gene expression. In addition, DII increases synthesis of triiodothyronine (T3), the ligand for the thyroid hormone receptor, further increasing UCP1 gene expression. PKA also activates hormone sensitive lipase (HSL), increasing the concentration of free fatty acids (FFAs) which in turn activate UCP1 protein activity. PGC-1 also coactivates the transcription factor, NRF-1 (nuclear respiratory factor-1), which leads to an increase in genes required for mitochondrial biogenesis, including NRF-1 and NRF-2. This results in marked stimulation of mitochondrial biogenesis. Abbreviations: PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; RAR, retinoic acid receptor; 9c-RA; 9-cis-retinoic acid; RA, retinoic acid; TG, triglyceride This figure was adapted from reference (29).

In addition to expressing UCP1, brown fat has other specialized characteristics which contribute to its thermogenic property. One important, distinguishing feature of the brown adipocyte is its abundant mitochondria. Thus, thermogenesis in brown fat depends upon a large number of uncoupled mitochondria. The abundance of mitochondria in brown fat, similar to brown fat-specific expression of UCP1, is also very likely to be mediated by PGC-1 (Figure 7). PGC-1 binds to and increases the transcriptional activity of a number of transcription factors involved in the complex program of mitochondrial biogenesis (28).

SUMMARY

Much data suggests that abnormalities in energy expenditure contribute to the development of obesity. However, at present, there is little knowledge regarding the molecular mechanisms which control energy expenditure in humans. Because of this, it has not been possible to find genetic causes of decreased energy expenditure or to develop therapies designed to specifically target energy expenditure in obese humans. More work, integrating knowledge form the genome project along with genetic engineering in mice, where candidate genes can be manipulated and effects whole body energy expenditure evaluated, are required in order to identify the molecular mechanisms responsible for

regulating energy expenditure. Given the mature status of current genome studies as well as genetic engineering techniques, it is anticipated that much will be learned in the not too distant future.

References

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature. 372:425-432. 1994.

2. Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI, Friedman JM. Abnormal splicing of the leptin receptor in diabetic mice. Nature. 379:632-635. 1996.

3. Himms-Hagen J. Brown adipose tissue thermogenesis and obesity. Prog Lipid Res. 28:67-115. 1989.

4. Ravussin E, Lillioja S, Anderson TE, Christin L, Bogardus C. Determinants of 24-hour energy expenditure in man. Methods and results using a respiratory chamber. J Clin Invest. 78:1568-1578. 1986.

5. Schoeller DA, van Santen E. Measurement of energy expenditure in humans by doubly labeled water method. J Appl Physiol. 53:955-959. 1982.

6. Ravussin E, Lillioja S, Knowler WC, Christin L, Freymond D, Abbott WG, Boyce V, Howard BV, Bogardus C. Reduced rate of energy expenditure as a risk factor for body-weight gain. N Engl J Med. 318:467-472. 1988.

7. Bouchard C, Tremblay A, Despres JP, Nadeau A, Lupien PJ, Theriault G, Dussault J, Moorjani S, Pinault S, Fournier G. The response to long-term overfeeding in identical twins. N Engl J Med. 322:1477-1482. 1990.

8. Levine JA, Eberhardt NL, Jensen MD. Role of nonexercise activity thermogenesis in resistance to fat gain in humans. Science. 283:212-214. 1999.

9. Leibel RL, Rosenbaum M, Hirsch J. Changes in energy expenditure resulting from altered body weight. N Engl J Med. 332:621-628. 1995.

10. Rolfe DF, Brown GC. Cellular energy utilization and molecular origin of standard metabolic rate in mammals. Physiol Rev. 77:731-758. 1997.

11. Nicholls DG, Locke RM. Thermogenic mechanisms in brown fat. Physiol Rev. 64:1-64. 1984.

12. Enerback S, Jacobsson A, Simpson EM, Guerra C, Yamashita H, Harper ME, Kozak LP. Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. Nature. 387:90-94. 1997.

13. Denborough M. Malignant hyperthermia. Lancet. 352:1131-1136. 1998.

14. Elmquist JK, Elias CF, Saper CB. From lesions to leptin: hypothalamic control of food intake and body weight. Neuron. 22:221-232. 1999.

15. Elmquist JK. Hypothalamic pathways underlying the endocrine, autonomic, and behavioral effects of leptin. Int J Obes Relat Metab Disord. 25:S78-82. 2001.

16. Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, Gu W, Kesterson RA, Boston BA, Cone RD, Smith FJ, Campfield LA, Burn P, Lee F. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell. 88:131-141. 1997.

17. Butler AA, Marks DL, Fan W, Kuhn CM, Bartolome M, Cone RD. Melanocortin-4 receptor is required for acute homeostatic responses to increased dietary fat. Nat Neurosci. 4:605-611. 2001.

18. Thomas SA, Palmiter RD. Thermoregulatory and metabolic phenotypes of mice lacking

noradrenaline and adrenaline [see comments]. Nature. 387:94-97. 1997.

19. Himms-Hagen J, Cui J, Danforth E, Jr., Taatjes DJ, Lang SS, Waters BL, Claus TH. Effect of CL-316,243, a thermogenic beta 3-agonist, on energy balance and brown and white adipose tissues in rats. Am J Physiol. 266:R1371-1382. 1994.

20. Ito M, Grujic D, Abel ED, Vidal-Puig A, Susulic VS, Lawitts J, Harper ME, Himms-Hagen J, Strosberg AD, Lowell BB. Mice expressing human but not murine beta3-adrenergic receptors under the control of human gene regulatory elements. Diabetes. 47:1464-1471. 1998.

21. Arch JR, Ainsworth AT, Cawthorne MA, Piercy V, Sennitt MV, Thody VE, Wilson C, Wilson S. Atypical beta-adrenoceptor on brown adipocytes as target for anti- obesity drugs. Nature. 309:163-165. 1984.

22. Grujic D, Susulic VS, Harper ME, Himms-Hagen J, Cunningham BA, Corkey BE, Lowell BB. Beta3-adrenergic receptors on white and brown adipocytes mediate beta3-selective agonist-induced effects on energy expenditure, insulin secretion, and food intake. A study using transgenic and gene knockout mice. J Biol Chem. 272:17686-17693. 1997.

23. Zurlo F, Larson K, Bogardus C, Ravussin E. Skeletal muscle metabolism is a major determinant of resting energy expenditure. J Clin Invest. 86:1423-1427. 1990.

24. Simonsen L, Bulow J, Madsen J, Christensen NJ. Thermogenic response to epinephrine in the forearm and abdominal subcutaneous adipose tissue. Am J Physiol. 263:E850-855. 1992.

25. Cassard-Doulcier AM, Gelly C, Fox N, Schrementi J, Raimbault S, Klaus S, Forest C, Bouillaud F, Ricquier D. Tissue-specific and beta-adrenergic regulation of the mitochondrial uncoupling protein gene: control by cis-acting elements in the 5′- flanking region. Mol Endocrinol. 7:497-506. 1993.

26. Kozak UC, Kopecky J, Teisinger J, Enerback S, Boyer B, Kozak LP. An upstream enhancer regulating brown-fat-specific expression of the mitochondrial uncoupling protein gene. Mol Cell Biol. 14:59-67. 1994.

27. Puigserver P, Wu Z, Park CW, Graves R, Wright M, Spiegelman BM. A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. Cell. 92:829-839. 1998.

28. Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, Spiegelman BM. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. Cell. 98:115-124. 1999.

29. Lowell BB, S-Susulic V, Hamann A, Lawitts JA, Himms-Hagen J, Boyer BB, Kozak LP, Flier JS. Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. Nature. 366:740-742. 1993.

30. Lowell BB, Spiegelman BM. Towards a molecular understanding of adaptive thermogenesis. Nature. 404:652-660. 2000.