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Energy Homeostasis and Obesity
Current Concepts

J.S. Bajaj

Emeritus Professor, National Academy of Medical Sciences
Honorary President, International Diabetes Federation
Professor and Head (Retd.), Department of Medicine, AIIMS

Energy Homeostasis : Obesity is a complex metabolic disorder resulting from dysregulation of energy homeostasis which essentially reflects an abnormality in the balance between energy intake and energy expenditure. Such dysregulation may have genetic and /or life style as well as behavioural origins, involving the quality and quantity of food intake on the one hand, and sedentary life style on the other. As obesity is commonly associated with insulin resistance and hyperinsulinemia, such a constellation increases the risk of glucose intolerance, type 2 diabetes mellitus (T2DM), dyslipidemia and cardiovascular disease (CVD). The all-inclusive term, metabolic syndrome, has been suggested for this constellation.

The Regulation of Energy Balance : There is a general consensus that a physiological system maintains homeostasis of energy stores in response to varying availability of food and changing demands of energy expenditure. The energy demands of the body under resting basal, active and stressful conditions are adequately and appropriately responded to in a short period of a few minutes, by glucose which constitutes a most dependable energy supply source on a short term basis, although in the long term, body adipose tissue responds to the needs for the maintenance of energy balance. Based on our collaborative studies nearly three decades ago, Bajaj et al. (1) proposed the existence of an entero-hypothalamo-insular axis. Subsequently Bajaj (2) summarized the evidence for its metabolic role delineating neuroendocrine mechanisms involved in the regulation of energy balance. In this publication, it was specifically observed : 'the rate of glucose utilisation seems to be the set point in the regulation of entero-hypothalamo-insular axis. However, this may be so for the maintenance of energy balance on a short term basis. Adipose tissue functions as the major source of energy fuel; during starvation, glycogen stores in the human body may sustain life for less than 24 hours while energy stored as triglycerides can maintain supplies to vital organs for 30-60

days. It is therefore possible that control of triglyceride storage may be of considerable influence as a long range regulator of entero-hypothalamo-insular axis'. The subject has been recently reviewed (3) and the validity of the hypothesis proposed earlier has been established.

Hypothalamic Regulation of Energy Homeostasis : The hypothalamus is the principal brain region that acts as a key determinant in the integrated control of feeding, energy homeostasis, and regulation of body weight. Hypothalamus senses neural, endocrine, and metabolic signals, integrates these inputs, and engages distant effector pathways, resulting in behavioural, autonomic, and endocrine responses (4). The hypothalamic control and regulatory mechanism is mediated through a complex array of neuroendocrinal signaling pathways involving synthesis and release of several neurotransmitters and neuropeptides. These include monoamine neurotransmitters, such as 5-hydroxytryptamine, norepinephrine, as well as orexigenic neuropeptides (neuropeptide Y, orexins A and B) and anorectic peptides (cocaine-and amphetamine-regulated transcript: CART; pro-opiomelanocortin : POMC and related peptides i.e. αMSH) (5). A brief overview of the action of these peptides is provided in the following paragraphs.

The positional cloning of ob gene in 1994 (6), and the subsequent discovery that the encoded protein, named leptin, functions as an adipocyte derived signal for the regulation of feeding behaviour, set the direction and pace of research during the last decade. Ob receptor gene was cloned a year later, in 1995 (7), and leptin receptors were demonstrated in the arcuate nucleus of hypothalamus (8). The arcuate nucleus is located in the mediobasal hypothalamus adjacent to the floor of the third ventricle. It contains neurons that respond to afferent signals, predominately hormonal, which reflect and relate to the *size* and *state* of adipose tissue stores. Although leptin is secreted primarily from adipocytes and insulin is released from the endocrine panacreas, both circulate at levels proportionate to body fat mass and exert relatively long-lived inhibitory effects on food intake via actions on their receptors in the arcuate nucleus. These actions are mediated through a set of neurons in the arcuate nucleus which coexpress neuropeptide Y (NPY) and agouti-related peptide (AgRP). These two peptides are potent stimuli of food intake; moreover, these peptides also reduce energy expenditure and thus promote weight gain. In contrast, the arcuate nucleus also contains neurons that synthesise αMSH (Melanocyte stimulating hormone) that exerts a powerful anorectic effect. αMSH is synthesized from its precursor proopiomelanocortin (POMC). Many POMC neurons also coexpress another peptide called CART (cocaine and

amphetamine related transcript). Both αMSH and CART reduce food intake. α-MSH is an agonist for melanocortin 4 receptor (MC4R) which also appears to be involved in the regulation of appetite and body weight mediated through 5-hydroxytryptamine receptors (5HT2C), activation of which causes weight loss and deletion of which causes adult-onset obesity in mice (9).

Insulin and leptin signals, which are enhanced in a state of excess adipose tissue mass i.e. obesity, are inhibitory to NPY and AgRP neurons and facilitatory to POMC and CART neurons, the net effect of these hormones results in inhibition of feeding behaviour (10). It is through this reciprocal regulation of anabolic and catabolic neuronal circuits that insulin and leptin mediate their effects on energy balance. It was hypothesized that a decrease in plasma levels of insulin and leptin which follows a reduction in body fat mass, results in the activation of NPY/AgRP and inhibition of POMC neurons. While this may be partially true, the search for orexigenic peptides initiating feeding behaviour was intensified during the last decade but has met with remarkable success only recently. The recent discoveries, in this context, include the orexins in the lateral hypothalamus, the gastrointestinal hormone, Ghrelin in the gastric mucosa, and the Endocannabinoid system.

Orexins and Ghrelin : Two groups of investigators independently described peptides expressed exclusively in the LHA, zona incerta and PFA. While deLecea et al. named these peptides as hypocretins 1 and 2 (11), Sakurai et. al. designated them as Orexins A and B (12)(*G. orexis*, appetite). Orexin A consists of 33 aminoacids (Mol. wt. 3562 Da). Orexin A cDNA was made by reverse transcriptase treatment of rat brain mRNA followed by PCR. The structure of prepro-orexin gene has been determined, and it has been located on chromosome 17q21. Both the 33 aminoacid Orexin A and 28-aminoacid Orexin B (Mol. wt. 2937 Da) are encoded by a single mRNA transcript. Also identified were two Orexin receptors. Orexin A receptor (OX_1R) showed a 26% identity with NPY. While Orexin A was a specific high affinity agonist for OX_1R stimulation, a second receptor OX_2R with 64% identity to OX_1R and high affinity binding of Orexin B was sequenced. OX_2R also binds Orexin A (13). Impressed by the 'striking localisation' of Orexin containing neurons in the lateral hypothalamus and some of its adjacent areas, and none in the VMH-Arcuate-PVN area, Sakurai et. al. investigated the effect of Orexin administration through preimplanted indwelling catheters (12). Within one hour of intracerebroventricular administration of Orexin A bolus, food consumption was stimulated in a dose-dependent manner. These actions of Orexin were similar to, although of lesser order of magnitude, than those observed following NPY administration.

Ghrelin, an acylated peptide secreted by cells in the gastric mucosa, stimulates

food intake. Ghrelin activates previously identified orphan receptor (growth hormone secretagogue receptor) in the hypothalamus leading to release of growth hormone from the pituitary (14). It is now being increasingly realized that this peptide may be more important for initiating feeding behaviour through actions in the arcuate nucleus. Indeed, ghrelin is somewhat unique amongst gut hormones in *stimulating* food intake, and its chronic administration in rats causes obesity (15). Plasma levels of ghrelin rise significantly before meals, and fall following the intake of food (16). Colocalisation of ghrelin receptor and NPY mRNA in a group of neurons in acruate nucleus has been demonstrated (17). Thus ghrelin exerts its orexigenic effects by binding to its receptor on NPY/AgRP neurons, thereby activating this circuit.

The Endocannabinoid System : To the previously defined components of central and peripheral control mechanism regulating energy homeostasis as described above, has been added one of the great discoveries of the current decade, namely the existence of an endocannabinoid system.

Cannabis sativa or *cannabis indica* grows wild in several parts of the world, and is found all over India. *Marijuana* cigarettes are prepared from the leaves and flowering tops of the plant while *Hashish* is prepared from concentrated plant resin. While smoking is the most common mode of use, the oral intake of *Bhang* as a concoction is practiced in several socio-religious groups, more so during festivals. As a part of folk-lore medicine, cannabis has been used over millennia for disorders as varied as joint pains and epileptic convulsions. It was only in 1964 that its active psychoactive constituent Δ^9-tetrahydrocannabinol (Δ^9-THC) was isolated, its structure identified, and partial synthesis accomplished (18). Δ^9-THC alongwith other naturally occurring and synthetic cannabinoids, bind with two separate G protein-coupled receptors. Cannabinoid receptor 1(CB_1) (19) is localized in the CNS including neurons in lateral hypothalamus, and in the periphery especially in the liver and adipose tissue. In contrast, cannabinoid receptor 2 (CB_2) is found primarily on cells of the immune system (20). A complex biochemical pathway for the synthesis, release, transport, and degradation of endocannabinoids alongwith their receptors CB_1 and CB_2, constitutes the new signaling system. Since the discovery of fatty acid amide, arachidonoy-lethanolamide, by Devane et al in 1992 (21), who also coined the term 'Anandamide' from the Sanskrit root 'Ananda', meaning 'internal bliss', more than 3500 scientific reports have been published exploring diverse aspects of endocannabinoid system. The subject has been recently reviewed (22).

Endocannabinoid system, particularly in the brain, is generally 'silent' under normal conditions. It becomes activated under certain circumstances of stress in the

body. For example, it helps the body to relax; it reduces pain and anxiety; it causes sedation, or a slowing down; it tends to eliminate the memory of unpleasant things in life. It works at several levels of the body to do this. Importantly, this system, when it gets turned on, is thought to increase appetite through a number of rewarding pathways. In other words, *'relax, rest, forget, protect, and eat'* constitute the 'panchsheel' of peaceful coexistence of internal and external environments. However, increased endocannabinoid system activity is associated with excessive food intake, obesity, and insulin resistance.

The neurometabolic effects of endocannabinoids can be better understood in the context of neuroregulation of feeding behaviour. Endocannabinoids stimulate anabolic circuits, namely the NPY and AgRP in the arcuate and their receptors in the lateral hypothalamus where melanin-concentrating hormone (MCH) and orexins are located. MCH binds to a receptor MCH-R1, which is a G-protein coupled receptor (GPCR). Deletion of MCH-R1 produces a lean mouse due largely to increased energy expenditure, rather than suppression of feeding (23). Likewise, a recent study demonstrates significant antiobesity effect of a MCH-R1 antagonist in diet-induced obesity in mice (24). In addition, chronic administration of the antagonist ameliorated obesity-induced hypercholesterolemia, hyperinsulinemia, hypertriglyceridemia and hyperlipidemia. This study focuses sharply on the role of MCH in lateral hypothalamus. Finally, neuroanatomical studies reveal a connection between arcuate neurons expressing POMC and AgRP/NPY, and MCH neurons in lateral hypothalamus (25).

Endocannabinoids, in addition to stimulating anabolic circuits, also inhibit catabolic circuits consisting of CART and α-MSH, and their projections into PVN. The net effect is excessive food intake, obesity, and insulin resistance. Finally, evidence has been generated during the last decade indicating this signaling system as a modulator of physiological functions not only in the central nervous system, but also in the autonomic nervous system, neuroendocrinal network, the immune system, the gastrointestinal tract, the reproductive system both in the male and the female, and in microcirculation. CB_1 knock-out mice are lean as compared to wild type littermates (26). Specific antagonists of CB_1 such as rimonabant decrease food intake, reduce peripheral and hepatic lipogenesis, and lead to weight reduction. Clinical trials with rimonabant in obesity, dyslipidemia and type 2 diabetes mellitus have shown promise both in terms of efficacy and safety. The clearance from drug regulatory agencies (i.e. FDA) is awaited (22).

Control of Energy Expenditure : Rapid advances during the current decade have not only delineated the molecular mechanism underlying neural regulation of energy intake (feeding) as also of energy storage (adipogenesis and lipogenesis), but

have also provided new insights into the cellular mechanisms that control and regulate energy expenditure. Energy expenditure can be viewed as occurring in three major compartments. The first is the obligatory energy expended on basic cellular and physiologic functions that require ATP. The second is the energy spent in physical activity; some of this is noexercise activity thermogenesis (NEAT). It is due to physical activities other than volitional exercise. The third is referred to adaptive thermogenesis. The components of energy expenditure subject to ready alteration include physical activity and adaptive thermogenesis. It is the latter which has became the subject of current research, and subsequently of major scientific advance.

To facilitate easy understanding, it is useful to analyse energy expenditure from a thermodynamic perspective. Total energy expenditure reflects the conversion of oxygen and food (or fuels stored in the body as fat, glycogen and protein) into carbondioxide, water, heat, and work on the environment. Work performed on the environment plus heat produced during biological combustion of food or body fuels equals the amount of heat measured as calories. Adaptive thermogenesis, sometimes called facultative thermogenesis, is defined as heat production in response to environmental temperature or dietary intake.

Two main aspects of recent work on adaptive thermogenesis include the neural circuitory that activates thermogenesis and the peripheral tissues where oxidation of fuels takes place. As part of a unifying concept of energy homeostasis, it is not entirely unexpected that the hormonal signaling and neural pathways that control food intake are closely interlinked with these involved in energy expenditure. The role of insulin in diet-reduced thermogenesis is an illustrative example. Insulin injection into the ventromedial and paraventricular nucleus in the hypothalamus resulted in an increase in body temperature and energy expenditure, *in addition* to a reduction in food intake (27,28). In rats treated with diazoxide (a potent inhibitor of insulin secretion from pancreatic β-cells), there is a significant attenuation of the thermogenic response to a carbohydrate meal (29). Based on these studies, it can be surmised that insulin action in the hypothalamus not only reduces food intake, but also increases SNS (sympathetic nervous system) outflow to BAT (brown adipose tissue) to produce heat from fatty acid oxidation, thereby increasing energy expenditure.

These physiological and pharmacological observations may provide a rational basis for understanding obesity-associated hypertension, with hyperinsulinemia in obesity mediating the increase in SNS activity (30). Indeed, systemic insulin administration increases plasma catecholamine levels. An interesting corollary may be that elevated free fatty acids (FFA) commonly seen in

obesity may result from insulin activation of sympathetic nervous system. Such an increase in FFA may be causally linked with insulin resistance in the muscle. With this perspective, the association of obesity, insulin resistance, and type 2 diabetes mellitus, becomes readily understandable (30). However, more data need to be generated to substantiate such a linkage.

Besides insulin, leptin is the other major signal emanating from adipose tissue. Mutations in leptin, leptin receptor, melanocortin 4-receptor (MC4-R) and MCH all strongly influence both food intake and energy expenditure. Exogenous administration of leptin in ob/ob mice not only reduces food intake, but also increases energy expenditure. The mitochondrion ATP metabolism and thermogenesis mediated through one or more uncoupling proteins (UCP) not only in the adipose tissue but also in the skeletal muscle, constitute other focal points of recent investigations.

In the cold-induced thermogenesis in BAT in rodents, the primary molecule involved is UCP-1, a mitochondrial inner-membrane protein that uncouples proton entry from ATP synthesis (31). Two homologues of UCP-1 have been identified. These are UCP-2 (32) and UCP-3 (33). While all three uncoupling proteins are abundantly expressed in BAT, UCP-3 is also predominantly expressed in skeletal muscle whereas varying levels of UCP-2 are expressed in most tissues. These proteins do possibly play a major role in whole body energy expenditure outside of BAT; nevertheless, none of the UCP alone has been shown to exhibit an unequivoral anti-obesity effect. Moreover, in adult humans, the amount of BAT is minimal and UCP-1 expression is physiologically unmeaningful. Nevertheless, UCP-2 and UCP-3 were considered possible candidates in the human. Whether sympathetic drive plays a role has been investigated by developing 'betaless' (deletion of β_1, β_2 and β_3 receptors) mice which develop severe obesity even when food intake is identical with wild-type mice. Although direct evidence for such an effect in human obesity is lacking, studies on the expression of UCP-3 in different physiological situations in humans (and animals) tend to provide some support for its role in energy balance and lipid metabolism (34).

The recent discovery of PPARγ coactivator (PGC)-1 that can coactivate many transcription factors that participate in adaptive therogenesis, has provided a new insight linking adaptive thermogenesis with adipocyte biology (35). The cold-induced PGC-1 is the result of interaction of β_3 –adrenergic receptors and cAMP (36). When PGC-1 is expressed in white fat or skeletal muscle, a broad programme of thermogenesis is initiated which includes mitochondrial biogenesis, expression of UCP-1 in fat cells and UCP-2 in muscle, and an increase in cellular respiration. The ability of PGC-1 to promote mitochondrial biogenesis is due to its ability to turn on the expression of nuclear

respiratory factors NRF-1 and NRF-2, which in turn regulate mitochondrial transcription factor A (mt TFA) which directs the transcription and replication of mitochondrial genome (37). In addition to PPARγ, PGC-1 also binds to other nuclear receptors including those of retinoic acid and thyroid hormone, which in turn positively regulate expression of UCP-1.

UCP-2 and UCP-3 are also regulated by various nutritional perturbations in a tissue-selective manner. Present insights into the transcriptional regulation of UCP-2 and UCP-3 have come from *in vivo* administration to animals or *in vitro* treatment of cells with PPAR ligands such as thiozolidinediones. These studies have indicated that PPAR-α and PPAR-γ are positive regulators of UCP-2 and UCP-3, with specificity defined by the tissue or cell-type being examined. Given that fatty acids and/or their derivatives are ligands for these receptors, the PPARs may account for much of the nutritional regulation of UCP-2 and UCP-3.

Human obesity being a multifactorial syndrome, there may be a possibility that a combination of genetic defects may significantly enhance the susceptibility to obesity as compared to that due to independent gene defects. β_3 –adrenergic receptor (ADR β_3) is expressed in adipocytes and mediates the rate of lipolysis in response to catecholammies, whereas PPARγ_2 regulates adipogenesis. Although Trp64 Arg mutation of ADR β_3 has only a modest association with human obesity (38) . a recent study was undertaken to evaluate evidence for interaction between ADR β_3 Trp64 Arg mutation and PPARγ_2 Ala variants with respect of obesity in a cohort of 453 Mexican-American subjects. Interestingly, only those subjects who carried both the variants (32/453) exhibited increased obesity, in contrast to those with only PPARγ2 variant. The ADR β_3 variant was not significantly associated with any of the obesity-related traits (39). Further population studies aimed at investigating more than one gene variant shall be of interest.

Future Perspective : Obesity is emerging as a worldwide epidemic. Basic researches have yielded a rich harvest of knowledge enhancing our understanding of hormonal regulation of feeding behaviour and energy intake, the adipocyte biology underlying adipogenesis and adiposity, and the role of adaptive thermogenesis in the regulation of energy expenditure. New therapeutic strategies in the management of obesity are now emerging, targeting all these potential sites, through the use of modern tools of molecular pharmacology and pharmacogenomics. Nevertheless, life-style changes and positive behavioural alterations constitute the most safe and cost effective strategies to-date.

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