Expenditure and Storage of Energy in Man

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Introduction

Problems of energy balance underly some of our most serious health problems (1). Recently Modan and others (2) emphasized in this journal the link between obesity, hypertension, and glucose intolerance. Some of the common types of hyperlipidemia also are linked to diabetes and obesity (3), and all three are associated with the prevalence of vascular disease. One common denominator is the insulin resistance that develops as weight is gained or glucose tolerance deteriorates. In developing countries overnutrition is a problem as well as undernutrition. As western ways are introduced into these countries, hypertension, type II diabetes, and cardiovascular disease are increasing (4).

The field of energy balance has recently been active. Two areas of current interest will be considered in this perspective. The first is the popular question of whether there may be facultative thermogenesis, adaptive or protective responses to overeating. In 1902 Rubner (5) wrote: "The stream of food increases, but it does not determine the size of the consumption. . . . At first the organism builds reserves, then it deposits additional substrate and finally, with increasing heat production, it gets rid of the ample food intake, at least in part." Was he right? Are the obese and/or glucose intolerant limited in this respect?

The second is the degree to which composition of diet, rather than total caloric intake, may affect the final energy balance. Are we paying a penalty for living more off the fat of the land than the carbohydrate?

We will try to put into perspective these two apparently opposing mechanisms, the first of which would minimize and the second promote storage of energy, and consider how each may be modified by familial, genetic, and environmental factors. We will also consider why time and money for research in this area have so often yielded contradictory conclusions and which newer techniques and practices may help resolve the remaining questions.

The elements of energy expenditure defined

Atwater and Benedict (6) first used their studies employing respiratory and calorimetric chambers to demonstrate that the laws of conservation of energy could be applied to man and that there is a close correlation between direct and indirect calorimetry.

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One can divide energy expenditure into three major components. These are resting metabolic rate (RMR),[1] thermic effect of exercise (TEE), and thermic effect of food (TEF), also referred to as dietary-induced thermogenesis (DIT). Each has an obligatory component, and each may be increased or decreased in an apparently facultative manner in response to environmental change. In reality the three components of energy expenditure are not entirely discrete but are useful divisions when attempting to investigate factors that might regulate or control them.

The RMR is energy expended while resting in a neutral environment 8–12 h after meals or physical activity. It includes the costs of maintaining the integrated systems of the body and the homeothermic temperature at rest. It comprises 60–75% of the total and includes the energetics of the chemical reactions themselves, plus those due to interactions of thyroid hormones and the sympathetic nervous system.

The second largest component is the TEE, which includes the additional energy above basal, both during and after physical activity. In those not earning their livelihood by physical labor this amounts to ~ 20% of the total. Unlike the other compartment, it is greatly expandable by activities such as running a marathon.

The TEF includes the cost of its absorption, metabolism and storage within the body, and is the expenditure above RMR. Absorption and transport of foods requires a relatively small proportion of the total 24-h energy expenditure (7). A much larger proportion the synthesis of protein, fat, and carbohydrate is required for both the constant renewal of body tissues and storage. These together comprise the obligatory components of thermogenesis and amount to 60–70% of total TEF.

The term facultative thermogenesis refers to a response to change in environment where heat production is varied independently of the obligatory demands and provides no net synthesis or mechanical work. Facultative thermogenesis involves either uncoupled or loosely regulated coupling of oxidation to phosphorylation, thus reducing the efficiency of this process. It also may include the use of ATP to drive roundabout pathways of metabolism rather than more direct ones, thus reducing the net efficiency of coupled metabolism. The most clearcut example is the response of small animals to exposure to cold, in which the efficiency of mitochondrial coupling of oxidation to ATP production is reduced. Beta-1 sympathetic stimulation stimulates lipolysis, which in turn signals purine nucleotide binding to a protein, termed thermogenin, which uncouples proton conductance through the mitochondrial membrane from ADP to ATP (8). T3, formed locally by the beta-1 stimulation, acts synergistically.

1. *Abbreviations used in this paper:* RMR, resting metabolic rate; SNS, sympathetic nervous system.

Mechanisms which tend to protect against undesirable storage of fat

In both the animal and plant kingdoms there are striking examples of variations in rate or thermogenesis useful for special purposes (5). The bombardier beetle confounds his enemies with a blast of noxious steam generated by activation of a peroxidase in his nether regions. The fattened woodchuck slows his metabolic rate preparatory for hibernation as winter approaches. A rat presented with an abundance of low-protein-content junk food expends much of the non-protein caloric excess as heat. The bumble bee prepares for flight on a cool morning by increasing shuttling between fructose phosphates in his wing muscles. The skunk cabbage wafts its odor through the forest by superheating its perfume generators. Therefore it would not be surprising in man if the fire of life were capable of adaptive variation. Pathologic examples of rampant thermogenesis in man include the mitochondrial uncoupling of ATP production in Luft's syndrome.

20 yr ago when our lean volunteers in the Vermont study of experimental obesity earnestly undertook the job of eating a great excess of calories, there was marked individual variation in the ability to gain (9, 10). All readily returned to their usual weight when overfeeding was stopped. One who took longer to reach his lean weight was later found to have a family history of type II diabetes. This called our attention to the need to study energy expenditure as it applies to weight gain and loss in man and the potential sources of individual differences.

Sites of facultative thermogenesis in man

In man we have evidence for a number of possible sources of facultative thermogenesis. Much of our metabolic energy, perhaps a very large part (5), is still devoted to keeping the primordial brine out of our cells. This is dependent on the functional state of the cell membrane and the activity of (Na^+-K^+)-ATPase. This in turn is dependent on thyroid hormones and the supply of phosphatidyl-inositol (11, 12) and/or other factors. Insulin has a direct effect on ionic pumping (13), as may norepinephrine (8). Additional energy is presumably required for the very considerable shuttling of calcium across cell membranes. The role of brown fat is less prominent in man than in small animals, and some deny any role in the adult. It does serve to buffer the entry of the newborn baby into a cold world, but it has been assumed that little survives into adulthood. This may be true of coddled modern man, but Huttunen et al. (14) found in Finland that outdoor workers, in contrast to office workers, have increased brown fat demonstrable by its enzymatic pattern. Astrup et al. (15) identified major quantities in perirenal fat that are responsive to infused ephedrine.

The question of whether muscle is a major site of facultative thermogenesis has been reopened with new data from Astrup's laboratory (6), suggesting that skeletal muscle could account for as much as 50% of facultative thermogenesis. Fagher and coworkers (16) have found in man evidence of stimulation by the sympathetic nervous system (SNS)[1] of thermogenesis in muscle by direct microcalorimetry. Muscle is one major site of what has been called "futile cycling" of substrates, which is far from futile, as Newsholme (17) has emphasized. He suggests that such cycles, with enzymes controlling both directions of cycling, provide sensitive regulators of the flow of body fuels, and key an animal or man for a burst of exertion by revving up the metabolic processes. Epinephrine, norepinephrine, glucagon, adrenal corticoids, and possibly prostaglandins released locally in muscle from sympathetic stimulation may stimulate the cycling. Shulman et al. (18) recently demonstrated by means of a turnover technique involving stable isotopic tracers that the so-called futile cycling of glucose to glucose-6 and fructose-1 to fructose-1,6 phosphate are affected by thyroid hormones. One might speculate that this substrate cycling may only later in the course of evolution have come to serve as a primary source of heat production and a means of moderating weight gain.

Hormones and facultative thermogenesis. Most but not all of the thermogenic mechanisms are subject to primary control by the SNS, whereas various hormones also influence energy expenditure. The finding that the beta-blocker propranolol considerably reduces the thermogenic response to infused glucose and insulin supports a role for the SNS (19, 20). Conventional wisdom suggests that hormones accomplish this by regulating the flux of substrates, the immediate factors in generating the ATP used in energy expending processes. This raises the question of whether hormones act directly or indirectly to stimulate or inhibit energy expenditure. Some, such as the rapidly responsive peptide hormones, act indirectly by regulating substrate fluxes and therefore substrate availability for ATP generation, whereas others may act directly to stimulate ATP utilization and therefore energy expenditure. All presumably act either synergistically or antagonistically in an integrated manner in their effects on thermogenesis.

Insulin is important in allowing glucose uptake into cells for oxidation as well as storage of glycogen, the one process generating and the other utilizing ATP. Insulin, however, is the major gatekeeper controlling whether fat is available from the fat stores when carbohydrate sources are limited. It has been suggested that insulin-mediated glucose metabolism in insulin-sensitive areas of the hypothalamus activates SNS outflow to stimulate the SNS-sensitive thermogenic processes, and that fatty acids may have a similar role. Both insulin and SNS activity in the periphery may alter the path of influx of sodium into cells and therefore stimulate the use of ATP to extrude the sodium from cells (14, 21). The relative roles of insulin and the SNS have recently been put into perspective by Tappy et al. (22). They compared the thermogenic response to ingestion of fructose, which only minimally stimulates insulin secretion, with that of glucose. Carbohydrate oxidation and the decrement in lipid oxidation were significantly greater with fructose than with glucose in spite of the minimal insulin response to the former. Reproducing the insulin response to fructose during a clamp study failed to give a comparable thermogenic response. However, propranolol beta-blockade reduced the thermic response to oral fructose, thus questioning the importance of a direct role of insulin in the thermogenic process.

The relative roles of insulin-stimulated glucose uptake and storage as glycogen (obligatory thermogenesis) and potentiation of SNS activity (facultative thermogenesis) during infusions of glucose and insulin has been neatly studied in normal subjects by Christin et al. (19). Insulin and glucose uptake were independently varied by using somatostatin to block endogenous insulin secretion. Insulin was found to play a permissive role whereas activation of the SNS was clearly more important in the facultative component of these studies. One must admit that these studies are all unphysiologic, because they involve infusion of glucose, insulin, and other hormones at unphysiologic concentrations and may involve SNS stimulation from the stress of the procedure itself. Calles (23) has recently demonstrated the importance of the early phase of insulin release in normal sub-

jects. When this was suppressed by somatostatin during administration of glucose, both an impaired glucose tolerance and a reduced thermogenic response were found. Cerasi and Luft (24) originally suggested that the loss of first-phase insulin release in response to glucose was a primary defect in diabetes, but later studies have shown that this defect in diabetes can be reversed by dietary treatment (25). At no time in the Vermont study of experimental obesity was an impairment of first-phase insulin release observed in association with the insulin resistance of overfeeding (10, 11). Further studies will be required to determine whether the delayed insulin response may be related to a blunted thermogenic response in those genetically at risk.

Relatively little is known of the thyroid hormones' thermogenic mechanisms of action, although the study of the longer half-life hydrophobic hormones, having predominantly a nuclear action, preceded by many years the study of the effect of the short half-life water-soluble hormones. As already noted, thyroid hormones affect the rate of futile substrate cycling (18). Recall that nutritionally directed changes in SNS activity and the peripheral metabolism of thyroid hormones are in the same direction. This raises the possibility, for which data is lacking, that SNS-related facultative thermogenesis may be synergistically modified by nutritionally induced alterations in thyroid hormone metabolism. Dietary composition, specifically the carbohydrate content of the diet, is important in these adaptations (26). One of us (27) has recently suggested that energy balance, rather than intake, may play the more important role in regulating changes in the peripheral metabolism of thyroid hormone. Observations of the changes induced by caloric deficit secondary to exercise at Laval University by Poehlman et al. (28) are also consistent with this. In contrast to the impact of energy balance on thyroid hormone metabolism, the level of caloric intake appears to modulate the rate of SNS activity. The adrenocorticoid and sex hormones both have effects on the deposition and distribution of body fat (5).

Controversy regarding facultative thermogenesis in normal man

Response to cold. The quintessential example of facultative thermogenesis in small animals is nonshivering thermogenesis in response to cold. The degree to which man retains the ability to adapt to cold by this or other mechanisms remains controversial. Joy et al. (29) found increased thermogenic responses to infused norepinephrine in military recruits acclimatized to cold for four weeks. In another military study, Skreslet and Aarefjord (30) found much of the acclimatization to cold was accomplished by increased thermal insulation by body fat, although there was also increase in plasma norepinephrine. Further carefully controlled studies of well-characterized subjects that include turnover rates of norepinephrine are in order.

Response to overfeeding in animals. A second example of facultative thermogenesis in small animals is the increased thermogenesis, again predominantly in brown fat (31), induced by overfeeding the so-called cafeteria diet. Cunningham et al. have shown in cafeteria-fed rats with glucose intolerance that, like their human counterparts, this protective adaptation is impaired. When fed a diet low in protein, there is a marked increase in thermogenesis (5). This apparently has survival value in enabling an animal to eat an excess of the equivalent of junk food without becoming obese.

Response to overfeeding in man. There has been conflicting evidence and much controversy (5) about whether there is a facultative increase in thermogenesis in man in response to caloric excess. Garrow (5) has critically summarized the widely varying results in 16 studies through 1977 of the response of normal man to overfeeding. He concluded that only with an intake > 20 Mcal was a measurable facultative increase in thermogenesis apparent. 10 additional studies were carried out through 1985 of which half supported a facultative component (5). Only three were ≥ 3 wk in duration and exceeded Garrow's suggested threshold.

The results of the early German studies of Neumann and others (5) and our Vermont study are often misquoted as indicating a failure to gain weight with increased intake, rather than a tendency for weight to plateau. Forbes (5) has emphasized that there is a linear relationship between total excess intake and the weight finally achieved. In mature animals and man, weight gain approaches an asymptote. With the limited data available and two-dimensional analysis, not including duration of overfeeding, it remains difficult to estimate whether there is plateauing of weight above that to be expected from an increase in lean body mass, the obligatory cost of metabolizing the increased substrate, and the increased cost of moving a larger body.

Recently Forbes et al. (32) studied 13 assorted men and women who were overfed 19–38 Mcal over 3 wk. Basal metabolic rate and body composition by ^{40}K were measured. Based on their results and comparable data from the literature, the cost of weight gain was 8.05 kcal (33.7 kJ)/g, close to the theoretical cost based on the composition of the gain. However, there was marked individual variation in the response, that could not be explained by any of the variables measured.

Another area of controversy and conflicting experimental results involves the role of catecholamines in the thermic response to a meal. Again, 19 studies have yielded variable conclusions (5). These studies have been limited by reliance on plasma concentrations of catecholamines, which are a poor index of activity. Schwartz et al. (33) have recently reported a close correlation between energy expenditure following a single meal and the appearance rate of norepinephrine, with no change in clearance. O'Dea et al. (34) had reported similar findings following 3 wk of moderate overfeeding in normals. Beta-blockade with propranolol reduces the thermic response to infused glucose (5). Danforth et al. (unpublished data) found a close correlation between the increased clearance and appearance rates of norepinephrine and both metabolic rate and glucose disposal rate during the euglycemic hyperinsulinemic clamp procedure.

Dallaso and James (35) suggested that the size of the depot available for fat storage may be a factor modifying the thermic response to food. Unless extreme obesity develops the adipocyte number remains constant in the adult years (5) and may provide such a limiting factor. The finding by Robbins et al. (36) in our laboratory that modest overfeeding produced a marked hypermetabolism in a young girl with lipoatrophy, and therefore limited storage space for fat, is consistent with this.

Response to exercise. Devlin and Horton (37) have found that exercise increases resting metabolic rate (RMR) and the thermogenic response to various stimuli for at least 12–18 h after high intensity exercise. The thermic effect of infused glucose and insulin is also increased. Beilinski et al. (38) measured energy expenditure for a 42-h period in the respiratory chamber at Lausanne. 18 h after a 3-h period of exercise at 50% VO_2 max, the RMR was still 4.7% elevated. A meal given 4 h after the exercise induced a greater fraction of lipid oxidation, and the effect persisted into the following day. In normal women, Segal and Gutin

(39) found that when a meal precedes a bout of exercise, the energy expenditure due to the exercise is increased by as much as 11%. This may reflect increased substrate cycling or increase in sympathetic response. They also found the reverse effect, a potentiation of the thermic effect of a meal by previous exercise. Zahorska-Markiewicz (40) reported findings similar to those of Segal and Gutin, but recent studies by Belko et al. (41) failed to confirm them.

Controversy regarding possibly defective facultative thermogenesis in the obese

Again, there is controversy and much disagreement in experimental results as to whether the obese are handicapped by a diminished thermogenic response to excess caloric intake. Of 24 studies in the last 15 years, only half give evidence of a thermic response differing from that of the lean (5). A recent study by Golay et al. (42) suggests that there is a lack of facultative thermogenic response to infusion of glucose and insulin in the diabetic obese.

Due to their increased lean body mass and cost of moving the added weight, the total energy expenditure of the obese is usually increased above the normal. Nair and coworkers in Garrow's laboratory (43) currently report that whereas subjects with impaired glucose tolerance have a blunted thermic response to a meal, their RMR per lean body mass estimated by total body potassium, is higher than that of normals, and their total energy expenditure during a meal is increased. Thus, one cannot say that reduced total energy expenditure is a usual cause of obesity. However, any difference in response of the lean and the obese at their habitual weight to a caloric excess or deficit can be important in development and accentuation of obesity.

Unfortunately, we still do not know whether the defects in metabolism and energy expenditure are primary or secondary in the various subtypes of the obese and in spontaneously diabetic obese. Neither do we know the primary defect or defects which are inherited so strongly in non–insulin-dependent diabetes. These clearly remain among the most important questions regarding obesity and energy expenditure.

Ravussin et al. (44), when working in our laboratory, found that the thermogenic response to infused glucose and insulin is reduced in the obese and more so in those with non–insulin-dependent diabetes. The response correlated with the rate of nonoxidative glucose disposal. In later studies in Lausanne, Ravussin et al. (20) demonstrated that, if adequate insulin is available to overcome the insulin resistance and produce the same rate of glucose disposal in both the obese and the obese diabetic as in the lean, the thermogenic response and obligatory cost were the same. In addition, the SNS component of the facultative increment in thermogenesis above the obligatory cost, as indicated by blockade with propranolol, was the same in all subjects in whom comparable glucose storage was induced. But unfortunately the obese or non–insulin-dependent diabetic patient usually does not have such compensation for his or her insulin resistance. Bogardus et al. (45) have demonstrated in patients with non–insulin-dependent diabetes that compensation may, however, be at least partially restored if dietary modification and increased physical activity reduce insulin resistance (45).

Recent studies by Bazelmans and others (46) that have included measurement of the turnover rate of norepinephrine in response to feeding have indicated a blunted SNS and thermogenic response in the obese, not noted in earlier studies in which catechol concentrations alone were measured (33).

The potentiation of the thermogenic response to exercise following a meal seen in normals is blunted or absent in obese subjects (39).

Thus, the bulk of evidence indicates a prolongation of the obligatory thermogenic response to overfeeding and a blunting of the facultative thermogenic response to feeding in at least some subtypes of the obese. The SNS response apparently is more reduced, probably because of reduced stimulation of the hypothalamic centers. Many factors may be responsible (5), but limitation of space prevents elaboration here.

Additional factors of overriding importance may be operative in some subtypes of the obese as well (5). The degree of spontaneous physical activity is emerging as a potentially important factor in energy balance. Widdowson first emphasized the difference in the amount of unconscious or spontaneous movements and activities between individuals, and Bullen and Jean Mayer noted the apparent inactivity of obese girls as compared to lean during periods of recreation (5). Rose and Williams (5) reported in 1961 that the single measure discriminatory between lean large and small eaters was the speed with which they carried out an errand. The improvements in respiratory chambers have enabled measurement of spontaneous physical activity as well as 24-h energy expenditure, resting, and sleep metabolic rates. Using the new respiratory chamber at the National Institutes of Health Clinical Research Section in Phoenix, Ravussin et al. (47) have discovered a wide individual range of spontaneous activity for which they have used Widdowson's term "fidgeting". With the addition of sensitive wrist motion sensors and radar or infrared sensors of overall body movement, it is possible to quantitate physical activity in excess of that required for breathing. They find that this may vary between individuals from 100 to as much as 900 kcal per day. There is suggestive evidence that this variation may be a familial characteristic.

Factors promoting storage of energy as fat

Effect of the CHO/FAT ratio in diet. It is now apparent, at least in the short term, that an increase in quantity of fat in the diet is a more critical factor than a comparable increase in that of carbohydrate in determining whether the stores of triglyceride in adipose tissue will be increased (48). It is well known that when dietary carbohydrate is converted to fat by de novo synthesis the obligatory cost is 23% of original calories from carbohydrate, whereas the cost of carbohydrate storage as glycogen is only 7%. The cost of deposition in adipose tissue of dietary fatty acids as triglyceride, in contrast, is only 3% (49).

Recent work at the Institute of Physiology of the University of Lausanne has brought out several important considerations regarding the fate of ingested fat vs. that of carbohydrate. There is marked limitation of lipogenesis from carbohydrate in man. Acheson, Flatt, and Jequier (50) found by indirect calorimetry that the net synthesis of fat from a 500-g carbohydrate meal over a 10-h period was only 9 g, and fat balance was actually negative. They also measured the effect of antecedent diets designed to reduce, maintain, or increase stores of glycogen on net lipogenesis and energy expenditure. Carbohydrate conversion to fat over a period of 24 h following the 500-g meal was highest when the glycogen stores were increased but still was limited to 9 g, and again the fat balance was negative. The thermic effect of the meal was also greater following the high carbohydrate diet designed to increase glycogen stores.

One might expect that giving fat supplements with meals would affect the oxidation of carbohydrate and protein. However,

the same group (51) found that over a 9-h period the same amounts of carbohydrate, protein and fat were oxidized, regardless of whether a 50-g fat supplement was included. In a group given no fat supplement, the fat stores were actually depleted. These studies suggest that fat balance is related to the amount of fat in the diet, whereas that of carbohydrate and protein is closely regulated. The body tends to maintain carbohydrate and protein balances, whereas at least short term fat balance is directly influenced by fat intake.

Our experience with a later group of subjects in the Vermont study of experimental obesity is consistent with these findings. A group for whom dietary fat alone was increased above maintenance requirements gained weight more readily, at least initially, than earlier groups given excess of mixed diets (10). The experiment was, however, limited by the fact that the level of anxiety, caffeine intake, and smoking were not controlled. In addition, as a result of long-term overfeeding, as indicated above, other mechanisms may come in to play which ultimately limit fat storage. A number of our subjects had great difficulty gaining in spite of an increase in fat intake and a reduction of carbohydrate intake.

The facts regarding the importance of the composition of the diet are sobering when one considers that the average content of dietary fat in the United States was 27% in 1910 as opposed to 44% in 1984 (48). Aside from the atherogenic properties of certain fats, they lend support to the recommendations for a reduction of dietary fat and indicate the need for further research in this area.

Many other factors and mechanisms in the spontaneously obese beyond the scope of this perspective make them particularly vulnerable to the influence of the antecedent diet and the composition of meals (5).

Familial and genetic aspects of energy expenditure. Many years ago Boothby and Sandiford (52) found that the resting metabolic rate in man could vary by as much as +10%. This variability could account for large differences in long-term energy balance. A question of current interest is whether the RMR in relation to the respiring fat-free mass can also vary and on what basis. Only 50–80% of the variance of RMR can be accounted for by differences in body size, age, and sex. Using the ventilated hood technique at Phoenix, Bogardus et al. (53) have found in 130 adult Pima Indians with varying degrees of obesity and normal glucose tolerances that 11% of the variance in RMR was accounted for by family membership and was independent of differences in fat free mass, age, and sex. The correlation of RMR to fat-free mass was close ($r = 0.91$; $P < 0.0001$. The RMR in monozygotic twins (54), as well as the thermic response to feeding, is genotype dependent, both at baseline and after physical training (28). Retrospective studies of adopted twins in Denmark also suggest a strong genetic component (55).

Impediments to progress of clinical investigation related to energy balance

The present system of funding research in the United States tends to support relatively short-term studies that often emphasize the particular technology that is the specialty of the individual laboratory. More comprehensive and longer term studies are required to solve the important problems of energy balance and their relation to disease states.

The problem of heterogeneity of control and experimental subjects. There is often heterogeneity in research studies even of normal subjects and particularly of uncritically selected obese subjects (5). Frequently in clinical studies, those published in this journal not excepted, characterizations are limited to age, sex, and a few physical dimensions. However, supposedly normal control subjects may be "physiologically obese" whereas the physically active obese may be metabolically normal (56). A physically inactive, overweight subject who has a strong family history of non–insulin-dependent diabetes may respond differently to a particular stimulus than one with lifelong familial obesity who is physically active. The former is likely to have central distribution of body fat, which we now know to be associated with endocrine abnormalities and a risk factor for hypertension, diabetes, and hyperlipidemia (57). Even more heterogeneity may be encountered in experimental subjects. As a result of selection of inadequately characterized subjects, there may be conclusions discordant with those of other laboratories drawing on a different subpopulation of subjects. Also, as Callaway and Greenwood (58) have emphasized, significant potential findings may be "washed out" due to inclusion of subjects with divergent responses.

It is critical that there be an initial baseline period in which body weight is in stable equilibrium. Studies of too short duration may fail to reflect total thermogenic and metabolic responses. The degree to which a meal is appetizing or revolting affects the thermogenic response (59). The size and composition of a test meal are also important. The degree of insulin resistance, as noted, is an important variable to assess. In women, the stage of the menstrual cycle must be considered (5).

The thermogenic effects of smoking and caffeine have to be taken into consideration (5). Finally, the level of physical activity, antecedent diet, and prior gain or loss of weight are important variables.

Improved experimental techniques and the future needs for research

There are four areas of progress in the techniques for the study of energy balance in man: (*a*) improvements in estimation of insulin resistance and the flow of substrates within the body, (*b*) improved or more generally applicable methods for estimating body composition, (*c*) a method for estimating energy expenditure of a free-living subject over prolonged periods, and (*d*) finally, a method for standardizing the data base and increasing characterization of control and experimental subjects.

Methods for accurately measuring the flow and composition of gases and physical movement have made possible respiratory chambers such as that described by Ravussin et al. (47) in a recent issue of this journal. In indirect calorimetry, the ratio of expired carbon dioxide and oxygen together with the rate of nitrogen excretion is used to estimate the proportion of substrates metabolized within the body. This has been effectively combined with the glucose clamp procedure, in which the effect of variation in the concentration of insulin or glucose can be varied independently. Estimation of the facultative component of thermogenesis has depended upon subtraction from the measured energy expenditure, based on oxygen consumption, and estimated thermic cost of the measured glucose disposal by oxidative and nonoxidative pathways. However, the cost of the alternative pathways varies. The formation of glycogen via the triose pathway is more costly than the direct route (60). The degree of suppression of splanchnic glucose production and the proportion of glucose disposed of in liver and muscle also affect the obligatory cost. It is an intriguing question whether SNS activity or humoral agents may affect the pathway taken and hence the

thermogenic response by this means. Newer techniques are available to improve these estimates. A technique involving the isolation of isotopically labeled diflunisal glucuronide from urine is now available for noninvasively sampling the pool of UDP-glucuronate and hence glycogen synthesized in the liver (61). From this the minimum (normally 25–30%) following the indirect, energetically more costly pathway can be estimated. This technique, as well as that of Shuman et al. (18) recently employed to study the effect of thyroid hormones on futile cycling, should be useful in clarifying the response of normals and obese to overfeeding. Improvements are available in the measurement of splanchnic glucose production (62, 63). By combining the clamp procedure with measurements of glucose uptake across a muscle bed, Jackson and associates (64) have further defined the estimations of glucose disposal. Nuclear magnetic resonance spectroscopy provides an additional resource (65).

More than 30 years ago, Lifson (66) first used water doubly labeled with deuterium and ^{18}O to estimate total energy expenditure in animals. The technique is based on the observation that through the hydration of CO_2 oxygen is lost from the body both as H_2O and CO_2, whereas labeled hydrogen is lost only in water. Using an assumed or measured respiratory quotient, energy expenditure can then be estimated. Under ideal conditions, Schoeller and others (67) have now demonstrated that the method can provide estimates in man of more than acceptable precision and accuracy for periods of 2 wk, after which the isotope tracers must be enriched. Drawbacks are the expense of the isotopes and the need for a high degree of accuracy in the mass spectroscopic measurement of the deuterium and ^{18}O involved. There is also the possibility of serious error if the respiratory quotient over the period of calculation is incorrectly estimated.

All estimations of body composition, whether by body density or by isotopic methods, include assumptions that are not always reliable. These are, to name only a few, the water content of fat-free tissue, the constancy of bone density, intracellular potassium, and the reliability of estimation of residual air in the lung and gas in the gut. Newer techniques can serve specific purposes. By computed tomography the quantity and distribution of fat can be measured (68). Two newer methods, based on distortion by the fat-free mass of an electromagnetic field or its electroconductivity, have their own set of assumptions and therefore uncertainties. However they may have promise, particularly for epidemiologic studies (69).

Because we are dealing with the heterogeneous human animal species, a standardized data base is desirable to aid in characterizing both control and experimental subjects. This problem cuts across many disciplines and extends to clinical trials as well as discrete studies (70). Efforts at developing methods of characterization and ultimately of classification for use in the research, epidemiologic, and clinical fields have increased in recent years (58). The more general availability of personal computers and sophisticated software for handling data bases for clinical investigation gives promise of our increased ability to deal with heterogeneity in both control and experimental subjects (71). In addition, studies of pairs of monozygotic twins are particularly promising for clarifying the genetic aspects of this field while controling for the effects of heterogeneity.

Conclusion

We have described two counterbalancing mechanisms that affect energy balance in man. The one tends to limit storage of energy as fat by promoting the dissipation of nonessential dietary calories and tends to preserve mobility. It appears to be largely under control of the sympathetic nervous system. The other promotes storage of dietary fat in the form of fat, and tends to protect in time of famine. These two mechanisms operate in varying proportions in the heterogeneous animal that is man. The predominance of the second mechanism of fat storage and the insulin resistance that often accompanies it is associated with modern lifestyles and is closely related to the byproducts of obesity, hypertension, non–insulin-dependent diabetes, and hyperlipidemia. Continued research into the factors controling these two main mechanisms is therefore of prime importance.

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