Symposium: Recent Discoveries in Genetic Influences on Obesity

Genetic Influences on the Response of Body Fat and Fat Distribution to Positive and Negative Energy Balances in Human Identical Twins[1,2]

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ABSTRACT This article summarizes a series of intervention studies conducted with pairs of young adult male identical twins and designed to determine whether there is any evidence for genotype × overfeeding or genotype × negative energy balance interaction effects in the changes in body weight, body composition, fat distribution, computerized tomography–assessed abdominal visceral fat, resting metabolic rate and thermic response to a standardized meal of mixed composition brought about by chronic exposure to appropriate experimental treatments. These studies demonstrated that individual differences in response to chronic alterations in energy balance are common. The comparison of the heterogeneity in response between the pairs of twins in contrast to the variance within pairs revealed that members of the same twin pair are significantly more alike than individuals who are not genetically related by descent. The intrapair resemblance in response was particularly strong for the changes in body mass, body composition, subcutaneous fat distribution and abdominal visceral fat. In contrast, the results of two long-term intervention studies showed that variations in resting metabolic rate following exposure to chronic overfeeding or negative energy balance induced by exercise were accounted for primarily by the changes in body mass. Finally, the thermic response to food was not modified by any of the experimental treatments. On the basis of these observations, we conclude that there are individuals at risk of gaining weight and body fat or who are resistant to weight loss. These differences in susceptibility to chronic overfeeding or in sensitivity to negative energy balance seem to be largely explained by genetic factors whose exact nature remains to be determined. J. Nutr. 127: 943S–947S, 1997.

KEY WORDS: • *overfeeding* • *negative energy balance* • *identical human twins* • *body mass* • *metabolic rate*

The determinants of the predisposition to gain body mass over time or to become obese are attenuated and perhaps totally obscured once weight gain has occurred or the individual is obese. Most of the research reported to date on the genetic basis of human obesity or energy balance determinants has been conducted with the tools of genetic epidemiology applied in the context of cross-sectional studies. These studies have helped to elucidate the role of inherited differences in a population perspective, but they are not able to clarify issues such as the role of genetic variation in response to changing energy balance conditions, various dietary practices or a sedentary mode of life. To deal with these topics, one must use a variety of experimental and molecular methods that have not been used extensively so far in humans.

There are considerable individual differences in response to various dietary manipulations, such as a diet rich in cholesterol or in saturated fats. The same phenomenon has been observed in response to chronic overfeeding. For instance, the Vermont overfeeding study showed that body weight gain varied among subjects and that it was below the expected weight gain based upon the ingested energy surplus (Sims et al. 1968). Experimental overfeeding, dietary energy restriction or exercise-induced negative energy balance studies conducted with human subjects can provide useful information on the heterogeneity of the response to controlled changes in energy balance conditions.

We have proposed that such experiments would yield better and more powerful data if they were performed with sets of monozygotic (MZ) twins (Bouchard et al. 1990a). We have elected to use this model in our experimental genetic studies of the causes and outcomes of variations in body fat and regional fat distribution in response to chronic alterations in energy balance. A unique aim of this research was to determine whether there was any evidence for a genotype × environment interaction (G × E) effect in the response to the treatment. Environment is defined here as the overfeeding or negative energy balance treatment. A G × E effect refers to a phenotype for which the response to an environmental challenge is significantly influenced by the genotype (Eaves 1976, Plomin et al. 1977). To date, we have completed four of these studies, two overfeeding experiments and two negative energy balance

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studies, with the collaboration of a total of 31 pairs of male MZ twins, ranging in age from 17 to 29 y.

GENE AND ENVIRONMENT INTERACTIONS

A G × E effect arises when the response of a phenotype to environmental changes depends on the genotype of the individual. Although it is well known that there are interindividual differences in the responses to various dietary interventions, very few attempts have been made to test whether these differences are genotype dependent. Most of the genetic epidemiology studies of human obesity have assumed the absence of G × E effects simply because of the difficulty in handling such interaction effects in quantitative genetic models. However, methods from both genetic epidemiology (unmeasured genotype approach) and molecular epidemiology (measured genotype approach) can be used to detect G × E effects. These methods are reviewed in an upcoming publication (Bouchard et al. 1997).

Here, we will focus exclusively on data acquired with the experimental MZ design. The procedure requires that both members of several pairs of MZ twins be subjected exactly to the same experimental treatment (Bouchard et al. 1990a). If there are individual differences in response to the treatment, within-pair and between-pair variances can be obtained. Full control over all aspects of the treatment is critical to the success of such an experiment. The procedure is quite similar to that used in animal genetics when testing for a G × E effect by comparing various strains of a given species exposed to a given treatment. The main difference is that by using MZ twins, we have only two subjects per genotype. However, the number of genotypes (MZ pairs) can be relatively large depending on the requirements of the research.

Data gathered before and after treatment on both members of several twin pairs can be analyzed by a two-way ANOVA for repeated measures on one factor (the treatment effect). Twins are nested in pairs. *F* ratios for the treatment effect and for the genotype × treatment interaction effect can be obtained. The intraclass correlation computed with the absolute or relative response to the treatment can also be computed from the within- and between-pair means of squares, without or with adjustment for the pretreatment level (Bouchard et al. 1990a).

A larger between-pair variance than within-pair variance suggests that the response to the experimental treatment is more heterogeneous in subjects who are genetically different. If a pair of MZ twins is assumed to represent a given genotype (for all relevant loci), then one can illustrate the within- and between-genotype differences under the scenario of no G × E effect (**Fig. 1,** *left panel*) and compare that with the scenario of a significant genotype × diet interaction effect (Fig. 1, *right panel*). In a series of experiments conducted with MZ twin pairs over the last 10 y in our laboratory, we have shown that the experimental model helps explain the role of the genotype × treatment interactions for such variables as chronic overfeeding, regular exercise, and regular exercise with negative energy balance. In general, these studies have revealed that G × E effects were ubiquitous.

There are, however, important limitations with this design. For instance, even though it is possible, at least from a theoretical point of view, to exert satisfactory experimental control and reach full standardization over energy or nutrient intake in overfeeding or underfeeding studies, it is not possible to fully standardize energy expenditure. Clamping of energy expenditure at the same level for all subjects of a given group cannot be achieved because of individual differences in resting metabolic rate, thermic response to food, fidgeting or variations in body mass that effect the energy cost of weight maintenance.

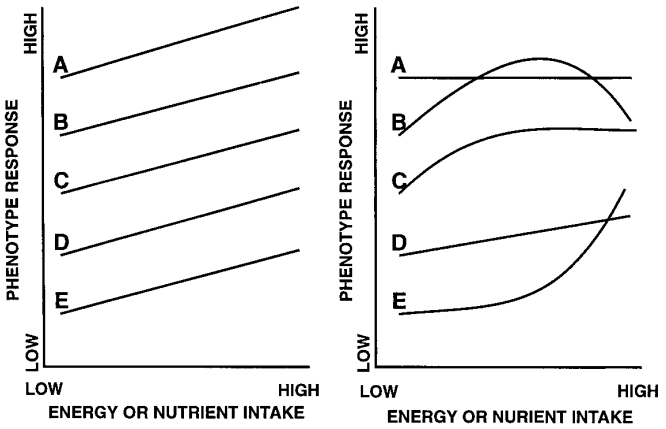

FIGURE 1 Phenotype response with chronic exposure to different levels of energy or nutrient intake conditions. In the *left panel,* the changes in the phenotype are not influenced by the genotype (A = B = C = D = E responses). In the *right panel,* the genotype determines the response to the dietary treatment, i.e., there is a genotype × diet interaction effect (A ≠ B ≠ C ≠ D ≠ E responses). Reproduced from Bouchard (1996).

The method can be extended to experimental studies with singletons and relevant molecular markers. Indeed, it is possible to investigate the response to chronic alterations in energy balance or nutrient intake in relation to genetic characteristics at given candidate genes and/or sets of molecular markers. This approach has the potential to identify the genes that are associated or linked (with siblings and dizygotic twins) with individual differences in the response to the treatment. For instance, such studies could yield important data on the genes responsible for the susceptibility to be in positive energy balance or gain weight over time as well as those associated with the resistance to lose weight.

THE POSITIVE ENERGY BALANCE EXPERIMENTS

It is generally recognized that there are some individuals prone to excessive accumulation of fat, for which losing weight represents a continuous battle, and that there are others who seem relatively well protected against it. We have tried to test whether such differences could be accounted for by inherited differences. In other words, we asked whether there were differences in the sensitivity of individuals to gain fat when chronically exposed to positive energy balance and whether such differences were dependent or independent of the genotype. If the answer to these questions was affirmative, then one would have to conclude that there was a significant genotype × energy balance interaction effect. The results from two experiments suggested that such an effect probably exists for body weight, body fat and fat distribution.

The short-term experiment. In a first study, we exposed six pairs of male MZ twins to a 4.18 MJ/d energy intake surplus for 22 consecutive days (Bouchard et al. 1988, Poehlman et al. 1986b). Individual differences in body weight, fat mass, subcutaneous fat, and site of fat deposition gains were observed with this short-term overfeeding protocol, but these differences were not randomly distributed. Indeed, significant intrapair resemblance was observed for the changes in most body composition and fat distribution variables despite the fact that the treatment was of short duration and that the changes induced

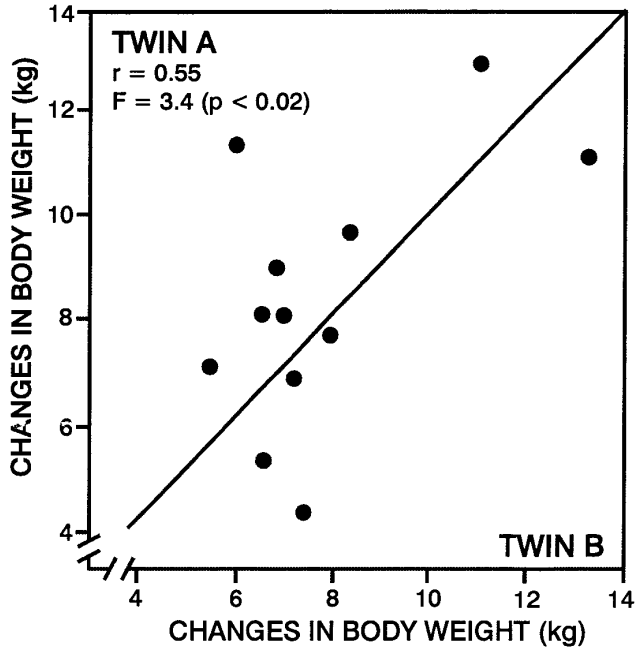

FIGURE 2 Changes in body weight in response to a 100-d overfeeding protocol in 12 pairs of young adult male identical twins. The within-pair resemblance in response is illustrated. Reproduced from Bouchard et al. (1990b) by permission of the Massachusetts Medical Society.

by the treatment were not large. The intrapair resemblance in the response to overfeeding, as assessed by the intraclass coefficient computed with the individual changes, reached 0.88 for total fat mass and 0.76 for fat-free mass. Subjects gained body weight and body fat, but there was a nonsignificant 7% increase in resting metabolic rate (Poehlman et al. 1986a).

Long-term overfeeding experiment. Twelve pairs of male MZ twins ate a 4.18 MJ/d energy surplus, 6 d a week, during a period of 100 d (Bouchard et al. 1990b). Significant increases in body weight and fat mass were observed after the period of overfeeding. Data showed that there were considerable interindividual differences in the adaptation to excess energy and that the variation observed was not randomly distributed, as indicated by the significant within-pair resemblance in response. For instance, there was at least three times more variance in response between pairs than within pairs for the gains in body weight (**Fig. 2**), fat mass and fat-free mass. These data, and those reported earlier for the response to short-term overfeeding, demonstrate that some individuals are more at risk than others to gain fat when energy intake surplus is clamped at the same level for everyone and when all subjects are confined to a sedentary lifestyle. The within-identical twin pair response to the standardized energy intake surplus suggests that the amount of fat stored is likely influenced by the genotype.

At the beginning of the overfeeding treatment, almost all the daily energy surplus was recovered as body energy gain, but the proportion decreased to 60% at the end of the 100-d protocol (Dériaz et al. 1993). The weight gain pattern followed an exponential with a half-duration of about 86 d. It has been estimated that the weight gain attained in the experiment reached about 55% of the anticipated maximal weight gain had the overfeeding protocol been continued indefinitely (Dériaz et al. 1993).

The mean body mass gain for the 24 subjects in the 100-d overfeeding experiment was 8.1 kg, of which 5.4 kg was fat mass increase and 2.7 kg was fat-free mass increase. Assuming that the energy content of body fat is ~38.91 MJ/kg and that of fat-free tissue is 4.27 MJ/kg, then ~63% of the excess energy intake was recovered on average as body mass changes. This proportion is of the same order as that reported by other investigators (Norgan and Durnin 1980, Ravussin et al. 1985), i.e., between 60% and 75% of total excess energy intake.

If on average 63% of the extra energy consumed was accounted for by the changes in fat mass and fat-free mass, one was left with 121 MJ to be accounted for. We measured the energy content of the feces for several days before and after the overfeeding treatment in 16 of the 24 subjects. There was no significant change in the amount of energy that was not absorbed during digestion. Thus, the remaining energy must in all likelihood be associated with the estimated costs of protein (13.95 MJ) and fat (33.87 MJ) tissues gained and with increases in resting metabolic rate, thermic effect of food, standard postures, moving the body around and fidgeting. In fact, we were able to account for 91% of the 351 MJ energy surplus ingested by the 24 young adults in the study (Tremblay et al. 1992). One must also consider the possibility that increases in energy expenditure for postures, bodily activities and fidgeting were present not only as a result of increases in body mass but perhaps also because of a shift in the daily pattern of activities toward a more energy-demanding profile even though subjects were kept under sedentary conditions. The remaining unexplained energy expenditure (about 9%) was likely accounted for by errors of measurement, errors in the assumptions made to estimate overall energy balance at the onset of the study, and by small differences in the daily pattern of activities over the duration of the overfeeding protocol.

The long-term overfeeding study also revealed that there was six times more variance between pairs than within pairs for the changes in upper body fat and in computerized tomography–determined abdominal visceral fat when both were adjusted for the gain in total fat mass (**Fig. 3**) (Bouchard et al. 1990b). These observations indicate that some individuals are storing fat predominantly in selected fat depots primarily as a result of undetermined genetic characteristics. It also suggests that variations in regional fat distribution are more closely related to the genotype of the individuals than are variations in body mass and in overall body composition.

Resting metabolic rate in absolute terms increased by about 10% with overfeeding ($P < 0.05$). However, the increase was only marginal ($P = 0.06$) when expressed by unit of fat-free mass (Dériaz et al. 1992, Tremblay et al. 1992). The intrapair resemblance for the changes in resting metabolic rate brought about by overfeeding was significant ($P < 0.05$), but it became nonsignificant when the changes in body mass or body composition were taken into account. The thermic response to food, as assessed by indirect calorimetry for 4 h following the ingestion of a 4.18-MJ meal of mixed composition, did not increase with overfeeding when resting metabolic rate was subtracted from postprandial energy expenditure (Tremblay et al. 1992). In contrast, postprandial energy expenditure and the total energy cost of weight maintenance increased significantly ($P < 0.05$), but the increments were mostly due to the gain in body mass.

THE NEGATIVE ENERGY BALANCE EXPERIMENTS

The short-term experiment. A short-term negative energy balance experiment was also undertaken with six pairs of MZ twins. The energy deficit was achieved by exercise performed twice a day, for 22 consecutive days, for about 50 min per

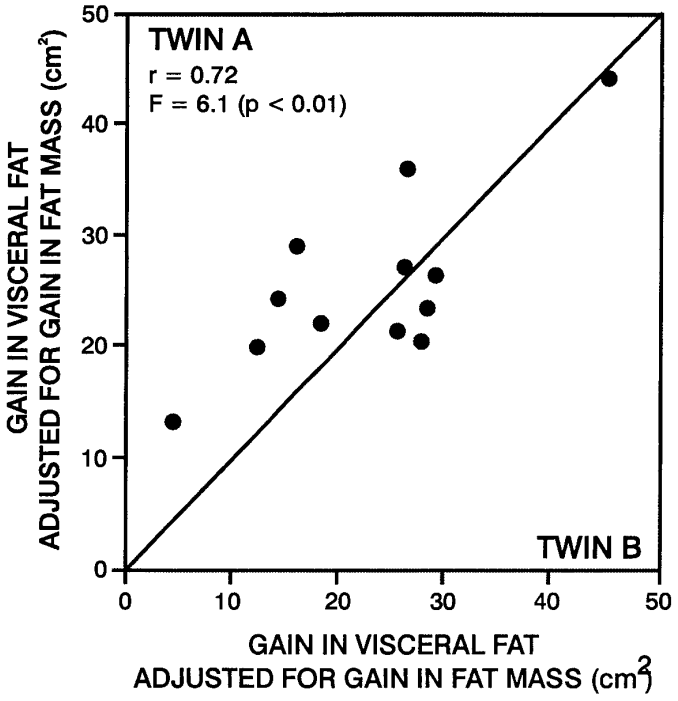

FIGURE 3 Changes in abdominal visceral fat in response to a 100-d overfeeding protocol in 12 pairs of young adult male identical twins. The within-pair resemblance in response is illustrated. Reproduced from Bouchard et al. (1990b) by permission of the Massachusetts Medical Society.

session, on the cycle ergometer. The exercise prescription was precisely controlled for each subject, during each exercise session, and was designed to induce an extra energy expenditure of 4.18 MJ/d over resting metabolic rate while baseline energy intake was maintained throughout (Poehlman et al. 1987). The changes in body composition were generally small and not related to the twin lines. The only exception was for the fat-free mass changes, which were more similar within pairs in comparison to the between-pair variance.

The long-term experiment. Seven pairs of young adult male identical twins completed a negative energy balance protocol during which they exercised on cycle ergometers twice a day, 9 out of 10 d, over a period of 93 d while consuming a constant daily energy and nutrient intake. The mean total energy deficit caused by exercise above the estimated energy cost of body weight maintenance reached, on average, 243 MJ. Baseline energy intake was estimated over a period of 17 d preceding the negative energy balance protocol. Mean body weight loss was 5.0 kg and was entirely accounted for by the loss of fat mass. Fat-free mass was unchanged. Mean body energy losses reached 192 MJ, which represented about 78% of the estimated energy deficit. Decreases in metabolic rates and in the energy expenditure of activity not associated with the cycle ergometer protocol must have occurred to explain the difference between the estimated energy deficit and the body energy losses. Subcutaneous fat loss was slightly more pronounced on the trunk than on the limbs, as estimated from skinfolds, circumferences and computed tomography. The reduction in abdominal visceral fat area was quite striking, from 81 to 52 cm^2. At the same submaximal power output level, subjects oxidized more lipids than carbohydrates after the program, as indicated by the changes in the respiratory exchange ratio.

Intrapair resemblance was observed for the changes in body

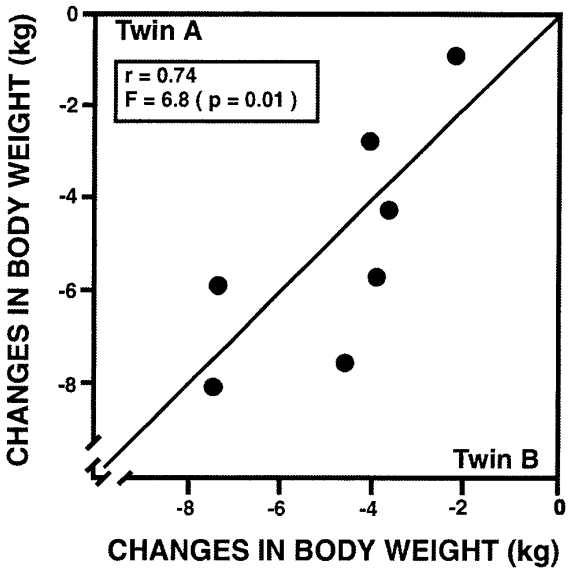

FIGURE 4 Changes in body weight with the negative energy balance protocol in seven pairs of young adult male identical twins. The within-pair resemblance in response is illustrated. Reproduced from Bouchard et al. (1994) with permission.

weight (**Fig. 4**), fat mass, percentage of fat, body energy content, sum of 10 skinfolds, abdominal visceral fat (**Fig. 5**) and respiratory exchange ratio during submaximal work. Even though there were large individual differences in response to the negative energy balance and exercise protocol, subjects with the same genotype were more alike in responses than subjects with different genotypes, particularly for body fat, body energy and abdominal visceral fat changes. High lipid oxidizers and low lipid oxidizers during submaximal exercise were also

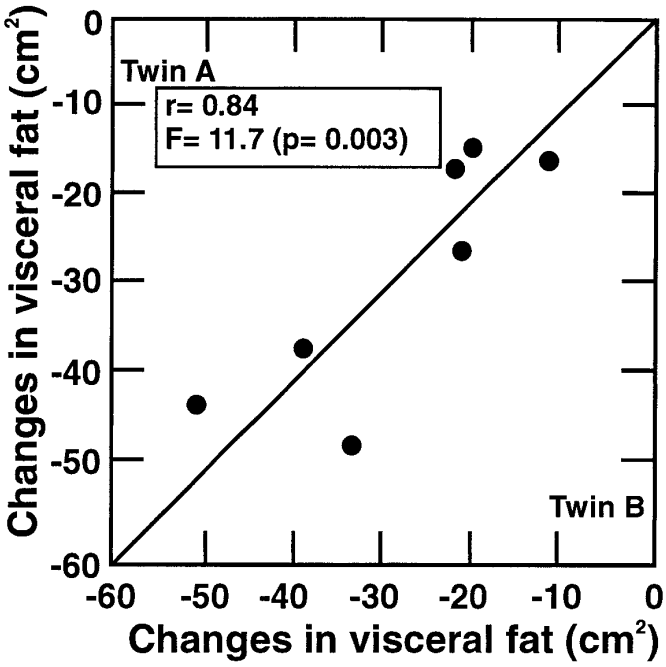

FIGURE 5 Changes in abdominal visceral fat with the negative energy balance protocol in seven pairs of young adult male identical twins. The within-pair resemblance in response is illustrated. Reproduced from Bouchard et al. (1994) with permission.

seen despite the fact that all subjects had experienced the same exercise and nutritional conditions for about 3 mo.

Resting metabolic rate decreased significantly with the negative energy balance protocol, but there was no change when the metabolic rate was adjusted for body mass and body composition (Tremblay et al. 1997). Similarly, the twin resemblance in the response of resting metabolic rate vanished when body mass and fat-free mass changes were taken into account. There was no significant change in the thermic response to a 4.18 MJ meal over a 4-h period. Finally, the energy cost of standardized treadmill walking exercise bouts decreased significantly with the negative energy balance protocol. Twin resemblance was consistently observed in these energy cost changes with and without controls over body mass fluctuations (Tremblay et al. 1997).

Thus, changes in body mass, body fat and body energy content were characterized by more heterogeneity between twin pairs than within pairs. These results are remarkably similar to those that we reported for body mass, body fat and body energy gains with 12 pairs of twins subjected to a 100-d overfeeding protocol.

IMPLICATIONS

The studies summarized in this article demonstrate that individual differences in response to chronic alterations in energy balance are ubiquitous. They are observed for a wide variety of phenotypes, including body weight, body fat content, subcutaneous fat distribution and amount of abdominal visceral fat. The comparison of the between-pair and within-pair heterogeneities reveals that members of the same twin pair (twin brothers) are generally more alike in their response to variation in energy balance than people who are not related by descent. These two series of observations support the notion that genotype $\times$ energy balance interaction effects are quite common.

In contrast, the results of the two long-term intervention studies reveal that fluctuations in resting metabolic rate were accounted for by changes in body mass and body composition. There was no twin resemblance in resting metabolic rate changes after the data had been adjusted for the concomitant morphological alterations. The thermic response to food was not changed significantly by any of the experimental treatments.

On the basis of the results summarized here, we believe that it would now be useful to undertake similar intervention studies with a large number of singletons or pairs of brothers so that the full range of the heterogeneity in response to chronic alterations in energy balance can be documented. Such studies would also provide the opportunity, if the sample sizes were large enough, to investigate candidate genes and other molecular markers in an attempt to define the molecular basis of the G $\times$ E effects. These studies have the potential to lead to the identification of genes associated with either a susceptibility or a resistance in the response to environmental pressures designed to cause weight loss or weight gain.

CONCLUSIONS

Some form of biological determinism can make a person more susceptible to evolve toward chronic positive or negative energy balance over prolonged periods of time. For a minority of individuals (e.g., the high gainers in our experimental overfeeding studies or the low losers in the negative energy balance experiments), the genotype seems to contribute in no minor way to the continuous battle they have to wage in order to maintain body mass within acceptable limits. It is remarkable to see that such genotypes were observed in the overfeeding studies reported here despite the fact that the MZ twin pairs were not themselves at risk for obesity or obesity-related complications based on their own body mass status and their family history.

The genes responsible for these individual differences in sensitivity to alterations in energy balance remain to be identified. It would not be surprising if these genes turn out to be numerous, considering the complexity of the biological systems involved in body weight homeostasis, nutrient partitioning, energy expenditure and regulation of energy balance. A variety of genetic and molecular research strategies will be needed to identify these genes and to delineate the nature and extent of the genetic polymorphisms involved.

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