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How can energy balance be achieved by free-living human subjects?

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Human subjects in modern affluent environments ingest nutrients in a variety of complex situations which contain stimuli that can have major effects on intake. These include the immediate environmental, temporal, social and psychological stimuli and the properties of the foods. The immediate environment contains signals, both learned and unlearned that influence the amounts of nutrients ingested and food choices. These signals include location, companions, weather conditions, noise and even the distance of items from the subject (Engel & Hirsch, 1991). Temporal stimuli include stimuli associated with the hour of the day, the day of the week, the week of the month and the month of the year. The social context includes the influence of the presence of other people and culturally defined roles and expectations. Psychological stimuli include learning, subjective state and eating restraint. Finally, the foods and drinks themselves may vary markedly in their palatability, nutritional adequacy and energy content. Yet, in the face of all these potent influences on immediate-short-term intake, somehow intake is matched to expenditure. This results in, and is evidenced by, a stability in body weight. How this is accomplished is not known, and uncovering the mechanisms by which intake and expenditure are matched is a major challenge for students of nutrition and ingestive behaviour.

In order to study how these various stimuli affect intake in normal human subjects free-living in their natural environments we have employed the diet-diary technique (de Castro, 1987a,b, 1991 a,b,c, 1993a,b,c,d, 1994a,b,c; de Castro & Kreitzman, 1985; de Castro et al. 1986, 1990; de Castro & Elmore, 1988; de Castro & de Castro, 1989; de Castro & Brewer, 1992). In the usual protocol, the subjects record their intake for seven consecutive days during their everyday lives in a pocket-sized diary. They record in as detailed a manner as possible every item that they either eat or drink, the time they eat it, the amount they eat, how the food was prepared, and the social and environmental context (for detailed description and discussion of the diet-diary technique and its validity and reliability, see de Castro, 1994b). The records are analysed by computer, and overall and meal intakes recorded and studied. A variety of procedures are employed to encourage the accurate maintenance of records and ultimately the validity of measurement. These include rewards linked to accuracy of recording, corroboration by other individuals and most recently by having subjects photograph the meals. Including these pictures along with the diaries increases the estimates of intake by over 8% (Fig. 1).
IMMEDIATE ENVIRONMENT

The diet-diary data have been analysed to investigate the effects on intake of where and with whom the individual is eating. The analysis revealed that meals eaten out in restaurants were 38% larger than meals eaten at home and 44% larger than meals eaten in other locations (de Castro et al. 1990). The analysis also revealed that the amount ingested in the meal is influenced by the nature of the individuals who eat with the subject (de Castro, 1994c). In particular, while males appear to be relatively unaffected by the gender of their eating companions, females eat 13% more when eating with a male than when eating with another female. In addition, meals eaten with companions with whom the subject is very familiar or comfortable, spouse, family, or friend, are 22, 23 and 14% larger respectively than meals ingested with other companion types. On the other hand, meals eaten with co-workers are 16% smaller than meals ingested with other companion types. These results suggest that environmental factors have a substantial impact on the amount eaten.

TEMPORAL FACTORS

There are a large number of temporal variables that affect intake. Within the day, the longer the interval before a meal the more will be eaten in that meal (de Castro et al. 1986). In addition, there are circadian (Bernstein et al. 1981), weekly, lunar and even circannual (Rosenwasser et al. 1981) rhythms that affect intake. We have been able to demonstrate that these rhythms even affect the eating behaviour of free-living human subjects (de Castro, 1987a).

Circadian rhythms of intake are present even in free-living human subjects. The amount ingested in meals increases over the course of the day while the interval until the
next meal, the aftermeal interval, decreases (de Castro, 1987a). As a result, as the day progresses the individual eats more but waits for a shorter period of time before eating again. Hence, there would appear to be a decrease in the satiation produced by eating later in the day. This is evidenced by a 62% decrease in the satiety ratio (following interval divided by meal size) in the evening than in the morning. These circadian changes make the individual particularly vulnerable for overeating in the evening.

We have also documented a 7 d rhythm of intake (de Castro, 1991b). Intake increases by 8% (605 kJ/d) on Friday, Saturday, and Sunday relative to the other days of the week. This effect is probably socio-cultural (de Castro, 1991b,c). In addition we have recently been able to detect a small but statistically significant lunar rhythm in the energy and alcohol contents of meals over the lunar cycle (de Castro & Pearcey, 1995). Meal size increases by 8% while alcohol decreases by 26% at the time of the full moon relative to the new moon. The nocturnal illumination level was not critical since the effect was present both during the daytime and the evening.

Finally, we have been able to document a circannual (yearly) rhythm (de Castro, 1991a). We observed that there was a 12% increase in total energy intake and meal size in the autumn relative to the other seasons. This was especially true for the intake of carbohydrates and was associated with an increase in the rate of eating. In addition there was a greater estimated amount of food in the stomach at the end of meals in the autumn. But, the subjects rated themselves hungrier. In the autumn the correlation between the amount eaten in the meal and self-rated hunger at the end of the meal was absent, while it was strong and negative in the winter and spring. The results suggest that seasonal rhythms may act by suppressing satiety mechanisms.

SOCIAL FACTORS

Social influences on people's intake were investigated by separating and characterizing meals eaten alone v. those eaten with other people present. Social meals were found to be on average 44% larger than meals eaten alone and contained larger amounts of all the macronutrients and alcohol (de Castro & de Castro, 1989). The effect goes beyond the mere presence or absence of eating companions (Fig. 2). Indeed, the number of other people present has a significant positive relationship with the amount eaten in the meal; the more people present the larger the meal (de Castro & Brewer, 1992).

This social facilitation of intake occurred for both snacks and meals, regardless of the presence of alcohol, and regardless of when or where the meal was eaten (de Castro et al. 1990). Finally, manipulation of the number of other people present at meals has demonstrated that social facilitation is causally related to intake (Redd & de Castro, 1992; Clendenen et al. 1994). These studies clearly demonstrate that social factors have a major impact on short-term intake.

PSYCHOLOGICAL FACTORS

Psychological factors can have a major effect on intake. The subjective state of the subject and eating restraint have a significant influence on intake (Wardle & Beales, 1987; de Castro & Elmore, 1988; Wardle et al. 1992; de Castro, 1995). The subjective state of hunger before a meal has a large positive relationship with intake, while to a lesser extent thirst, elation and anxiety are also positively related. Taken together these factors account for 35% of the variance in meal size ($R^2$).
In addition, the individual’s attempts to restrain intake can have a marked effect on the amount ingested. We found that eating restraint was associated with lower and less variable intakes (de Castro, 1995). High-restraint subjects ingest significantly less than their low-restraint counterparts with females ingesting 16% less and males ingesting 12% less. Bulimia, a pathological form of intake restraint, results in an even more profound effect on nutrient intake (Elmore & de Castro, 1990, 1991). Using diet-diaries we observed that untreated bulimic women purged all but 33% of the energy that they consumed, ingesting on average only 2816 kJ/d which were unpurged.

THE REGULATION OF FOOD INTAKE

The findings, so far reviewed, suggest that there are potent factors in the environment that can profoundly affect the amounts of foods ingested. This suggests that regulation of intake must occur in the face of these huge environmental influences. That food intake is indeed regulated and balanced with expenditure by a physiological system is apparent first from the relative stability of adult body weight and second from the findings of significant heritabilities of body weight and food intake.

Food intake and body size heritability

We investigated the role of inheritance in determining the body size and intake of adults by comparing the intakes of identical and fraternal twin pairs. We paid 109 identical and eighty-six fraternal adult twin pairs to maintain 7 d food-intake diaries (de Castro, 1993a). Both classical analysis of heritability and linear structural modelling revealed significant additive genetic influences on body size, height and weight, and body fatness (BMI). That body size is primarily determined by the genes has been previously described by a number
Fig. 3. Linear structural model and the most parsimonious model fitting the twin data for height, weight, and total daily energy intakes (—). (- - -), Non-significant paths. For all remaining variables, removing any one leads to a statistically significant reduction in the model's account of the observations. The reduced (parsimonious) model had a goodness of fit index of 0.924 ($\chi^2(58) = 60.42, P = 0.39$) which does not significantly deviate from an adequate fit. Values shown represent path coefficients.

of other investigators using both twin (Feinleib et al. 1977; Bray, 1981; Stunkard et al. 1986a, 1990) and adoption (Stunkard et al. 1986b; Price et al. 1987; Sorenson et al. 1989) study methodologies. However, we demonstrated that not only body size but also the amount of food energy ingested daily, as well as its macronutrient, alcohol and water content, were significantly affected by inheritance (de Castro, 1993a).

Applying linear structural modelling analysis to these twin data revealed that 65% of the variance in daily energy intake could be attributed to heredity. In addition, heredity accounted for 44% of the variance in meal frequency and 65% of the variance in average meal size. In contrast, the analyses indicated that the shared, familial, environments in which the twins were raised had no significant impact on the levels or pattern of intake in adulthood. We were further able to demonstrate that the heritability of overall daily intake was independent of body size, and that the heritability of meal size was independent of daily intake. This was demonstrated by employing linear structural modelling using the identical and fraternal twin body sizes, overall intakes and meal intake (de Castro, 1993b).

These models indicated that 96% of the variance in height and 85% of the variance in weight were due to heredity (Fig. 3). In addition, from 25 to 50% of the variance in daily food intake is due to genetic influences independent of the body size, gender, and age of the individual. Furthermore, another model indicated that from 18 to 42% of the variance in average meal size is due to genetic influences independent of the overall daily intake,
gender, and age of the individual. These results of the twin study strongly suggest that body size, overall intake and meal intake have interacting influences on one another, but, in addition, there are separate genetic influences on each of these factors that operate independently.

Prior intake effects on subsequent intake

In the short term, food intake appears to be affected by a large number of salient non-regulatory influences, suggesting that short-term intake cannot be tightly regulated. However, the heritability data suggest that body size and overall and meal intakes are regulated at a level established by the genes. Since heredity operates by encoding physical structure, the heritability results suggest that there are underlying physiological processes which regulate intake in the face of the large short-term fluctuations. The next question is: what is the nature of these processes?

A clue as to their nature can be found in data we have obtained on the effects of intake and expenditure over one day on intake on subsequent days. Accounting for the variance in daily intake has been difficult. Although across subjects daily intakes are positively correlated (Hankin et al. 1967; Hartman et al. 1990), within subjects autocorrelations have been found to be small and predominantly positive (Morgan et al. 1987; Tarasuk & Beaton, 1991). ‘The finding challenges the belief that some short-term homeostatic mechanism exists that causes high energy intakes to be followed by low ones’ (Tarasuc & Beaton, 1991). Hence, there is currently little understanding relating to how intake affects subsequent intake to produce energy balance.

To investigate further whether evidence of regulation can be identified in the effects of daily intake on intake during subsequent days we re-analysed the 7 d diet-diary reports of intake that had been collected over the last decade (de Castro, 1987a,b, 1991a,b,c, 1993a,b,c,d, 1994a,b,c; de Castro & Kreitzman, 1985; de Castro et al. 1986, 1990; de Castro & Elmore, 1988; de Castro & de Castro, 1989; de Castro & Brewer, 1992). Daily intake effects on subsequent days intake were investigated with an autocorrelational analysis including univariate and linear structural modelling approaches.

Fig. 4. Mean z transformed autocorrelations between total energy intake in 1 d and the total intake on each of the previous 4 d. Mean value was significantly different from the zero (t test): *P < 0.05.
The mean autocorrelations between the total amount of food energy ingested in one day and the amounts ingested on each of the four preceding days are presented in Fig. 4. These data indicate that the amount ingested on 1 d has a small negative feedback on intake on the subsequent day, but a much larger effect 2 d later. This continues into the third day, but vanishes by the fourth day.

Autocorrelation assumes that the error terms are uncorrelated and can overestimate the proportion of variance accounted for. Simplex autoregressive LISREL models (Boomsma et al. 1989; Neale & Cardon, 1992) do not make such an assumption and so were applied to investigate the factors controlling the day-to-day regulation of food intake. These models use data across subjects. Hence, to correct for individual differences, the proportion of the average daily intakes ingested on each day, rather than the absolute amounts, were employed in the analyses. The results of this analysis (Fig. 5) parallel the autocorrelations. Only two of the six paths indicating the effect of intake of one day on the next day's intake were significant. On the other hand, all the paths from intake occurring 2 or 3 d before were significant. Hence, it would appear that intake during one day has its maximum negative effect on eating 2 to 3 d later.

Fig. 6(a,b,c) presents the mean autocorrelations between the total amount of carbohydrate, fat and protein respectively ingested in one day and the amounts ingested of each of the macronutrients on each of the four preceding days. Interestingly, there are macronutrient-specific effects. Carbohydrate intake has larger negative correlations than either fat or protein with the amount of carbohydrate ingested. Fat intake had larger negative correlations than either carbohydrate or protein with amount of fat ingested. Similarly, protein intake had larger negative correlations than either carbohydrate or fat with the amount of protein ingested. Hence, the negative effect of ingestion of a macronutrient appears to be greatest on the subsequent intake of that particular macronutrient 2 d later.

The relationship between daily average meal sizes and frequencies on subsequent days were analysed with an elaborate simplex autoregressive linear structural model (Fig. 7). This analysis indicates that average meal size and frequency exert a significant influence on one another within a single day. For all 7 d, average-meal-size effects on frequency had larger negative path coefficients than meal-frequency effects on size. On the other hand, there was very little influence of average meal size on meal frequency, or meal frequency on average meal size, 1, 2, or 3 d later. Only two of the thirty paths were significant. In contrast, the effects of average meal size on average meal sizes on subsequent days, and the effects of meal frequency on meal frequencies on subsequent days, followed a similar pattern to that seen with overall daily intake, with the most powerful negative feedback influences apparent on average meal sizes and frequencies occurring 2 and 3 d later. This suggests that the delayed negative feedback which adjusts overall daily intake results from two separate negative feedback mechanisms affecting subsequent meal sizes and frequencies respectively.

**Effects of previous expenditure on subsequent intake and expenditure**

Another clue as to the nature of regulatory processes can be found in data we have obtained on relationships between expenditure and subsequent intake. Expenditure was estimated in free-living human subjects using continuous-activity monitoring employing triaxial accelerometers (Mini Motionlogger Actigraph, Ambulatory Monitoring Inc., Ardsley, NY, USA). Intake was assessed with 7 d diet diaries. The total daily activity was correlated with the total intake on the same day and for the subsequent 4 d (Fig. 8). None of these
correlations was significant, indicating that expenditure is not significantly related to intake either on the same day or on subsequent days. These results are similar to those observed by Edholm et al. (1970).

The total daily activity was autocorrelated with itself for the subsequent 4 d (Fig. 9). Activity was not related to activity on the next day, but was significantly negatively related to activity occurring 2 d later. This is a fascinating result, suggesting that expenditure may
not affect energy balance by influencing intake but rather by affecting subsequent expenditures with a lag of at least 2 d.

**DISCUSSION**

The results of these studies indicate that in the short term meal sizes and even daily intakes are markedly affected by a large array of salient but transient influences. These effects make it extremely difficult to observe any regulatory process operative on a meal-to-meal or even day-to-day basis. However, we were able to detect significant delayed negative-
feedback effects occurring over several days. These effects may provide clues as to the way that regulation of intake–expenditure balance may occur in free-living human subjects.

Although the delayed negative-feedback effects are statistically significant, the sizes of the effects are small. The largest univariate intake autocorrelation reported is $-0.19$ which accounts for less than 4% of the variance in daily intake. Similarly, the largest univariate

Fig. 7. Simplex autoregressive linear structural model applied to the prediction of daily average meal sizes and frequencies (freq). (-- -- -- --), Non-significant paths. Removal of any path coefficient presented in the figure produces a significant degradation of the model's fit as assessed with a $\chi^2$ test ($P < 0.05$). ξ, Unaccounted variance. Values shown represent path coefficients.
Fig. 8. Mean correlation coefficients between daily activity and total energy intake on the same day and for each of the subsequent 4 d.

Fig. 9. Mean autocorrelation coefficients between daily activity and activity on each of the previous 4 d. Mean value was significantly different from zero (t test): *$P < 0.05$. 
activity autocorrelation is $-0.25$ which accounts for only 6% of the variance in daily activity. In the linear structural modelling analyses, the largest amount of variance accounted for was 16.1% total or macronutrient intake for any day, and 30.1% and 22% for meal frequency and size respectively. Furthermore, the sizes of most of the effects were much smaller than these. Hence, previous intake only accounts for a small proportion of the variance in the daily or meal intake of nutrients.

To some extent the small sizes of the effects are not surprising given that these data were acquired from human subjects in their natural environments in which complex arrays of stimuli affect the individual and create variance in behaviour. That single variables, e.g. negative feedback, only account for a small proportion of the variance may simply be a reflection of the fact that there are large numbers of variables operative in these environments. However, the question remains as to how negative feedback could promote regulation given that it only accounts for a small amount of the variance in intake.

A key to understanding how this may occur is in the realization that although other variables may be producing much larger effects, these influences are transient (de Castro, 1996). They have large but short-term effects on momentary intake, which average over time and result in no net effect on intake. On the other hand, previous intake and expenditure appear to feed back after a delay of at least 1 d and usually longer. The delayed intake effect thus persists and can be envisioned as setting a bias that promotes an adjusted overall level of intake. The bias may not be apparent as its influence is overshadowed by more potent stimuli. However, its presence is persistent and continues to shift intake, producing a cumulative net alteration of intake and over time leading to regulation of intake.

Genetic influences may operate similarly by setting the overall bias of the system and only becoming apparent over prolonged periods of time. This is apparent in that the degree of genetic influence obtained in the twin studies was related to the integrated amount of time involved in the determination of the level of the variable, with body weight having the greatest effect, weekly intake the next, and daily and meal intakes the least (de Castro, 1993a,b,c).

The physiological mechanisms that may underlie these phenomena are a mystery. The long delay involved eliminates gastrointestinal, plasma and hepatic factors as intermediaries and makes a learning explanation unlikely. Rather, it suggests that feedback from a long-term energy storage depot (fat) may be involved. However, a fascinating outcome of these studies is the specificity of the delayed negative feedback. Macronutrients primarily affect the subsequent intake of the same macronutrient. Meal size affects only subsequent meal sizes, while meal frequency affects only subsequent meal frequencies and activity affects only subsequent activity. Such specificity suggests that the mechanism is not simple and cannot be unitary. Rather it suggests that there is a large array of negative feedback loops each of which operates specifically on only a single aspect of intake and expenditure. There may not be any such thing as energy balance regulation. What may appear to be regulation may simply be the sum total of the operation of multiple independent negative feedback loops.

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