Nutrition Discussion Forum

The role of dietary nucleotides in nutrition – Reply from Sánchez-Pozo

In a recent letter, Sarwar (1997) examined the issue of the benefits provided by dietary nucleotides. In his discussion, Dr Sarwar raised a question regarding the scientific soundness of our study (López-Navarro *et al.* 1996).

He is correct in stating that the nucleotide-free and the nucleotide-supplemented diets were not isonitrogenous. Under ideal conditions a perfect N balance between diets would be desirable. In our studies the diets were designed to be nutritionally adequate. Our intent was to contrast the results of a nutritionally adequate diet with the same diet supplemented with nucleotides. Under these circumstances the nucleotide supplement represented only 0·25 % of the total mass of the diet, of which N comprised approximately 0·05 %. Our results showed that the impact of nucleotides was proportionally far greater than the difference in N content. We feel therefore that our results have a real application.

We firmly agree with Dr Sarwar that the exact composition and the chemical structure of the supplementary purines and pyrimidines should be specified instead of using the vague term 'nucleotides'. Nomenclature is important because the different nucleotides, nucleosides and nucleobases vary in their metabolism and action.

We thank Dr Sarwar for his observations, which underscore the value of scientific dialogue. The controversy about nucleotides indicates a growing interest in this subject, which we believe will ultimately lead to a better understanding of nutrition.

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Partitioning between protein and fat during starvation and refeeding: is the assumption of intra-individual constancy of P-ratio valid?

It is now 20 years since Payne & Dugdale (1977) published their computer model of weight regulation, which embodies the elegant concept that the partitioning between lean and fat tissue compartments is an individual characteristic. Another equally important feature of this model is that within a given adult (non-growing) individual, the proportion of protein to fat withdrawn to meet an energy deficit must be equal to the proportion of protein to fat deposited during refeeding, and this proportion, termed the P-ratio, is numerically defined as the fraction of energy either mobilized or deposited as protein respectively. In other words, the P-ratio is set as a fixed characteristic (i.e. a constant) for a given adult individual during energy deficit and energy surplus, in order to prevent drifts in body composition with each succession of (simulated) weight loss and recovery.

To gain insights into the biological significance of these concepts in body composition regulation, Henry *et al.* (1997) tested these hypotheses regarding inter-individual variability and intra-individual constancy of energy partitioning by assessing the P-ratio of weanling and adult rats during fasting (P-fast) and refeeding (P-refed). They concluded that:

(a) their results in weanling and in adult rats, showing positive correlations between P-fast and P-refed, support the suggestion of Payne and Dugdale that particular P-ratio values are characteristic of individuals, and
(b) their results for adult rats, showing that the P-fast and P-refed animals were not significantly different, are in agreement with the proposition of the Payne–Dugdale model concerning the intra-individual constancy of P-ratio.

Whereas their first conclusion about P-ratio as a characteristic of the individual is fully justified, their second conclusion that P-ratio is a constant is not substantiated by the data in the rat nor in human subjects. Our point of contention is elaborated below.

First, it is clear that when examining the inter-relationship between P-fast and P-refed, the effect of growth per se is a confounding variable in testing the hypothesis of intra-individual constancy in energy-partitioning between protein and fat. This is well demonstrated in their recent work in Sprague–Dawley rats, and summarized in Table 1. It can be seen that despite the fact that the P-fast values in the

weanling rats and adult rats are quite similar (0·16 and 0·14, respectively), the P-refed values are markedly different, being several-fold greater in the rapidly-growing weanling rats (0·42) than in the slow-growing adult rats (0·13). Based upon this dependency of P-refed values upon the growth velocity, it is therefore reasonable to come to the conclusion that if growth rate in the slow-growing adult rats is taken into account, then such 'growth-adjusted-P-refed' values will be substantially lower than their P-fast values.

Second, an alternative way to 'adjust' for the effect of growth per se in examining P-refed would be to compare refed rats with *ad libitum*-fed controls growing at similar rates. From our own data in post-weanling Sprague–Dawley rats growing at a moderate rate, and subjected to refeeding after food restriction, it can be shown (Table 1) that their mean P-refed value of 0·30 is in fact much lower than the P-ratio found in *ad libitum*-fed controls growing at the same rate, whether in weight-matched controls (P-ratio = 0·45) or age-matched controls (P-ratio = 0·50). These data in the rat showing a lower P-refed value relative to the P-ratio during unperturbed growth are consistent with the common observation in children recovering from malnutrition that the gain in fat mass is disproportionately greater than the lean tissue deposition (Ashworth, 1969; Reeds *et al.* 1978; MacLean & Graham, 1980).

Third, an 'absolute' adjustment for the effect of growth per se in the interpretation of data on P-refed can in fact be achieved by studying the P-ratio in the non-growing adult individual: e.g. in adult humans. Such data exist, thanks to the Minnesota Experiment of Keys *et al.* (1950), in which changes in body composition were assessed in thirty-two men who underwent experimental starvation and subsequent refeeding. As shown in Table 1, the P-ratio during refeeding is clearly lower than that during starvation among the Minnesota men: an almost 2-fold difference is observed when the P-ratios are presented as mean values (0·11 *v.* 0·21) or as modal values when P-refed is 0·08 *v.* P-starvation of 0·15.

Taken together, the above analysis of data showing that (a) P-refed is clearly lower than P-starvation in the adult human, (b) the 'growth-adjusted-P-refed' is likely to be lower than P-fast in the adult rat, and that (c) in rapidly-growing rats the P-refed is lower compared with P-ratio of *ad libitum*-fed controls, all converge to question seriously a main stipulation of the Payne–Dugdale model of weight regulation, about the validity of assuming intra-individual constancy in the partitioning between protein and fat.

Based upon our reanalysis of data from the Minnesota Experiment in adult humans, however, we believe that this concept can remain valid only if a clear distinction is made between (a) the control of energy-partitioning between protein and fat, which refers to a control system that dictates the partitioning characteristic of the individual, and (b) the P-ratio which, defined as the fraction of energy mobilized or deposited as protein, refers to the integrated outcome of several control systems (the control of energy-partitioning being only one) that operate to regulate body composition. Bearing this distinction in mind, we were able to show, by applying both statistical and numerical approaches to the Minnesota data, that the lower P-ratio during refeeding than during starvation was not due to a shift in energy-partitioning characteristic of the individuals, but was due to excess fat being laid down (Dulloo *et al.* 1996). There is now evidence to suggest that this excess fat results from the operation of another control system that suppresses thermogenesis for as long as the fat stores remain substantially depleted (as is often the case in the early phase of weight recovery), with the energy thus conserved being directed specifically towards the rapid replenishment of the fat stores (Dulloo, 1997*a,b*). In other words, the value of the P-refed is determined by the integration of two autoregulatory control systems: one operating through the control of energy-partitioning between protein and fat, and the other operating through a control system with a feedback loop linking the state of depletion of the fat stores to mechanisms that suppress thermogenesis.

Payne & Dugdale (1977) have emphasized that if their computer model is run with P-refed set at a value lower than P-starvation, then the body fat percentage will rise with each episode of weight loss followed by recovery. In the real world, this predicted consequence of a lower P-refed value does occur after severe fat depletion, as evidenced by the data from the Minnesota Experiment showing that when body fat was 100% recovered, lean tissue recovery was less than 50% completed: a situation that eventually led to the phenomenon of poststarvation fat overshooting (Dulloo *et al.* 1997), and which Keys *et al.* (1950) referred to as 'poststarvation obesity'.

Table 1. P-ratio* during weight loss and weight recovery in response to food deprivation in rat and man

	Weanling rats (1) (rapid growth)	Adult rats (1) (slow growth)	Post-weanling rats (2) (moderate growth)	Minnesota men (3) (non-growing)
Fasting–starvation P-ratio	0·16[a]	0·14		0·21[a]
Refeeding P-ratio	0·42[b]	0·13	0·30[a]	0·11[b]
Ad-lib.-fed controls P-ratio:				
Weight-matched			0·45[b]	
Age-matched			0·51[b]	

References: (1) Henry *et al.* 1997; (2) Dulloo & Girardier (1990); (3) Dulloo *et al.* 1996.
[a,b] Within each column, values with unlike superscripts are significantly different ($P < 0·001$).
* P-ratio is defined at the fraction of energy mobilized or deposited as protein. All P-ratio values are mean values.

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Energy partitioning between body storage of protein and fat during starvation and refeeding: sources of intra-individual variation of P-ratios – Reply by Henry et al.

We read with great interest the comments made by Dulloo (1998) in response to our paper (Henry *et al.* 1997). We are entirely in agreement that the partitioning of stored energy between lean and fat tissue compartments is an individual characteristic. This characteristic is probably genetically determined and serves, as Dulloo *et al.* (1996) have described, as a metabolic 'memory' for a preferred set of body tissue proportions.

However, we beg to differ with the inferences Dulloo has drawn from a comparison between the results from our experiments and those of his own. We suspect that divergence of views is as much the result of semantic problems as of any fundamental conceptual conflict. We, therefore, will try once more to express our position as carefully as possible.

1. In the computer model of adult body weight regulation, Payne & Dugdale (1977) made three assumptions. One of those was that for a given individual there would always be close equality between the proportion of energy withdrawn from tissues in the form of protein (P_{fast}), during negative energy balance and the corresponding proportion deposited during positive balance (P_{refed}). It is important to recognize that the long-term stability of the model depends on this condition being applied to the calculation of day-to-day changes in energy balance. These changes are then summed over time, and then used to predict longer-term weight variations. The key assumption, therefore, is the short-term equality of P_{fast} and P_{refed}. For practical reasons, however, actual measurements of P-ratios usually have to be made over time periods substantially longer than one day.
2. It is likely that changes in the absolute values of P_{fast} and P_{refed} will occur in response to changes of energetic efficiency and body composition throughout growth, reproductive activity and senescence. Indeed, Dugdale & Payne (1975) had already pointed out that the partition of stored energy in individual human infants swings rapidly and repeatedly over a manifold range, during the first months of life. Needless to say that other environmental and metabolic insults are also likely to bring changes in the absolute values of P_{fast} and P_{refed}.
3. The objectives of our recent work have therefore been: (i) to quantify some of the changes in P-ratios throughout growth and development; (ii) to assess the degree to which P_{fast} and P_{refed} remained equal despite those changes (by measuring them sequentially in the same individuals, using periods of time kept as short as possible); (iii) to test the hypothesis that despite changes in the average ratios of groups of individuals intra-individual differences are still maintained. We reported only partial success: P-ratios measured during fasting in older but still slowly-growing rats were not significantly different from those measured during subsequent refeeding. Intra-individual differences were sustained throughout the measurements, in both weanling and the older animals. However, we found a major inequality between P_{fast} and P_{refed} in the weanling animals. The average ratio measured during a 3 d fast at the mid-point-age of 31 d was followed by a value three-fold higher, during subsequent refeeding, at a mid-point-age of 41 d. This effect is the reverse of the differences shown in Dulloo's Table 1 (p. 108). There the values for P_{refed} derived from cross-sectional measurements on