ERRATUM

Bacigalupe, L. D. and Bozinovic, F. (2002). Design, limitations and sustained metabolic rate: lessons from small mammals. *J. Exp. Biol.* **205**, 2963-2970.

In both the on-line and print versions of this paper, the authors' address was printed incorrectly. The correct address is:

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The first sentence of the third paragraph of the section 'The central limitation hypothesis', p. 2964, should read:

Perhaps the main idea that has led to the proposal that energy budgets are centrally limited is the observed body mass-independent linkage between resting and sustained metabolic rates (RMR and SusMR, or Field Metabolic Rate) (Drent and Daan, 1980; Kirkwood, 1983; Weiner, 1989; Speakman, 2000).

The fourth sentence of the second paragraph of the section 'The optimal design debate: Symmomorphis', p. 2965, should read:

In particular, Garland (1998) and Gordon (1998) point out reasons for refuting symmorphosis: (i) organisms must perform different functions simultaneously, which probably creates constraints that prevent them from reaching an optimal solution for all processes; (ii) biological materials have limitations related to their own histories; (iii) in general, environments are always changing, and natural selection often cannot follow the rhythm of change; and finally (iv) genetic drift can be an important factor in some populations.

We apologise for any inconvenience these errors may have caused.

The Journal of Experimental Biology 205, 2963–2970 (2002)
Printed in Great Britain © The Company of Biologists Limited
JEB4432

Review

Design, limitations and sustained metabolic rate: lessons from small mammals

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Accepted 18 July 2002

Summary

Physiological limitations affect an organism's capacity to acquire and expend energy over long periods of activity. These limitations could be related to the central machinery used for acquiring, processing and allocating energy, or by the energy-consuming machinery. Another possibility is that the capacities of central and peripheral organs and tissues are co-adjusted, implying an optimized design. Given the important consequences that rates of energy expenditure have on many ecological aspects of animal life, we need to understand which factors impose ceilings on sustained metabolic rate. Ceilings on sustainable energy expenditure represent the limit below which all the activities performed by an individual must occur. There have been many studies of design constraints on energy budgets, but the different procedures used to identify the type of physiological limitation do not necessarily resolve which factors actually impose metabolic ceilings in small mammals, which precludes a clear understanding of the ecological and evolutionary consequences of design constraints on energy budgets. We propose that the following steps are necessary to identify the physiological limits on sustained metabolic rate: (1) combining peak energy demands to differentiate a central limitation from a peripheral limitation; (2) pushing the animals to their physiological limits (e.g. asymptotic food intake); (3) testing for a central excess capacity (if the limit is set peripherally), or a peripheral excess capacity (if there is a central limitation); (4) utilizing different levels of energy demand to test for symmorphosis.

Key words: sustained metabolic rate, energy budget, physiological limit, central limitation, peripheral limitation, symmorphosis.

Introduction

A major goal of physiological and evolutionary ecology is to understand the intrinsic and the extrinsic factors that impose limitations on an animal's energy budget (McNab, 2002). It is well known that there is a negative relationship between the rate of energy expenditure and the duration of an activity performed by an organism (Weiner, 1989; Peterson et al., 1990; Speakman, 2000). On the one hand, burst metabolic rates of activity or thermoregulation, performed over short periods (i.e. minutes or hours), cannot be sustained indefinitely because organisms are not in energy balance during the exertion (Hammond and Diamond, 1997). In fact, an important part of energy expenditure is fueled by the body's reserves, which are depleted while the activity is maintained. On the other hand, during longer activity periods (i.e. days or weeks), energy expenditure must be fueled by concurrent energy intake, known as the sustained metabolic rate (SusMR), defined as '*time-averaged energy budget that an animal maintains over times sufficiently long that body mass remains constant because time-averaged energy intake equals time-averaged energy expenditure*' (Hammond and Diamond, 1997).

Rates of energy expenditure sustained over longer periods are limited to a lower level than rates of expenditure over shorter periods. In fact, SusMR are almost fivefold lower than short-term (burst) expenditures, and they rarely exceed the resting levels by sevenfold, in contrast to burst rates, which can reach values 36 times above resting levels (Bozinovic, 1992; Bundle et al., 1999). For small mammals in particular, asymptotic ceilings on SusMR could limit individual reproductive effort (since number and quality of offspring depends on milk production and quality; Knight et al., 1986; Rogowitz and McClure, 1995; Rogowitz, 1996, 1998), activity (i.e. foraging, escape from predators), thermoregulatory capabilities and survival to long-term cold exposure (Konarzewski and Diamond, 1994; McDevitt and Speakman, 1994a), as well as geographic distributions and breeding ranges. This is because ceilings on sustainable energy expenditure represent the upper limit for all energy consuming activities performed by an individual. Given the ecological and evolutionary consequences that sustained energy budgets have on many aspects of animal life, it is important to determine which factors impose ceilings on SusMR.

It has frequently been suggested that energy acquisition,

transformation, absorption, allocation and expenditure are intrinsically limited, and that these intrinsic design constraints act before potential extrinsic limitations such as food availability (Karasov, 1986; Wieser, 1991; Stearns, 1992; Weiner, 1992; Speakman, 2000). Drent and Daan (1980) suggested that a 'prudent parent' should not allocate more than four times its basal level of energy expenditure to reproduction. Since this seminal work there have been several studies of the design constraints on energy budgets (e.g. Weiner, 1992; Speakman, 2000). There are three principal hypotheses to explain the physiological limitation on energy budgets. (1) The 'central limitation hypothesis', where the shared central machinery limits SusMR; (2) the 'peripheral limitation hypothesis', where the energy-consuming machinery limits the SusMR; (3) symmorphosis (*sensu* Taylor and Weibel, 1981), where the capacity of the central machinery closely matches that of the peripheral tissues.

It should be noted that firstly, we are considering physiological constraints and not restrictions imposed by the environmental food supply (see Speakman, 2000). Secondly, recognized authors in the field have already extensively reviewed the hypotheses proposed (Peterson et al., 1990; Weiner, 1992; Hammond and Diamond, 1997; Speakman, 2000), but we contend that particular assumptions, as well as various empirical procedures used to identify the type of physiological limitation, have not been completely correct. Consequently, it is not entirely clear which factors impose metabolic ceilings in small mammals, precluding a clear understanding of the ecological and evolutionary consequences of design constraints on energy budgets. Thirdly, we will only discuss limits on SusMR, not on sustained metabolic scope (SusMS) (i.e. potential trade-off aspects of intake with future life history traits) (for a review, see Speakman, 2000).

The central limitation hypothesis

The central limitation hypothesis (Kirkwood, 1983; Weiner, 1989, 1992; Peterson et al., 1990; Koteja, 1996b) proposes that sustained metabolic rates are limited by the central machinery involved in acquisition, processing and allocation of energy, resources and waste products. Thus, metabolic limits are independent of the way in which energy is expended, so the same metabolic ceiling will be reached regardless of the mode of energy expenditure, and peripheral organs always possess an excess capacity.

Although there are different basic processes of central limitation (Speakman, 2000), most authors have suggested that the capacity of energy assimilation is the principal limit for sustainable energy budgets (Weiner, 1992). For small mammals, one way to confirm the presence of metabolic ceilings, and at the same time to determine if they are centrally limited, is provided by laboratory studies in which animals, fed *ad libitum*, are forced to reach their maximal SusMRs under different modes of energy expenditure (e.g. lactation, thermoregulation, activity). This procedure tests whether the metabolic ceilings for each activity reach the same value, as predicted by this hypothesis.

The main evidence for the proposal that energy budgets are centrally limited is that the observed body-mass-independent linkage between resting and sustained metabolic rates (RMR and SusMR, or Field Metabolic Rate) are not linked to body mass (Drent and Daan, 1980; Kirkwood, 1983; Weiner, 1989; Speakman, 2000). It is argued that animals with higher sustained energy expenditures support their demand by increasing food consumption which, at the same time, increases the mass of the central organs (i.e. liver, kidneys, heart, lungs and small intestine). Given the high specific metabolism of these organs and their direct contribution to the RMR (Schmidt-Nielsen, 1995), then RMR and SusMR should increase jointly. There is abundant evidence of a phenotypic linkage between both traits, but the data are controversial (Koteja, 1987, 1991; Nagy, 1987; Daan et al., 1990; Peterson et al., 1990; Bryant and Tatner, 1991; Lindström and Kvist, 1995; Ricklefs et al., 1996; Hammond and Diamond, 1997; Speakman, 2000). Furthermore, there is abundant evidence of phenotypic flexibility in central organ mass, and the conclusions from these observations are more generally agreed (Bozinovic et al., 1990; Daan et al., 1990; Hammond and Diamond, 1992; Hammond et al., 1994; Konarzeswski and Diamond, 1994, 1995; Speakman and McQueenie, 1996; Derting and Austin, 1998; Konarzweski et al., 2000). It means that a high energy budget depends on expensive metabolic machinery (Diamond, 1993), which could explain why SusMR do not exceed RMR values by more than sevenfold (Hammond and Diamond, 1992).

Many studies have assessed the possible link between SusMR and RMR, and demonstrated the important consequences of it (Speakman, 2000). The existence of such a link would provide a theoretical framework for understanding variations in RMR among species, and also evidence to support the 'energy assimilation model' for the evolution of endothermy (Koteja, 2000), although it would not disprove the aerobic capacity model (Crompton et al., 1978; Bennet and Ruben, 1979; Bozinovic, 1992; Hayes and Garland, 1995; Ruben, 1995). In addition, if RMR and SusMR are indeed linked, one could argue that high RMR would allow high SusMR, which could explain differences observed in activity patterns and life history traits (McNab, 1980; Hayssen, 1984; Thompson and Nicoll, 1986; Derting and McClure, 1989; Harvey et al., 1991; Hayes et al., 1992; Thompson, 1992; Koteja and Weiner, 1993; Johnson et al., 2001a).

Finally, the central processing and transport organs may be able to supply energy and nutrients faster, the peripheral organs would not be able to convert this increased supply into work and heat at the same rate. SusMR would therefore be limited at the site of energy use (i.e. peripheral limitation).

The peripheral limitation hypothesis

Even though Weiner (1992) proposed that: '*alternative proposals of central physiological limits are rare*', the actual evidence seems to show that peripheral limitations are more the rule than the exception (see, for example, Hammond and

Diamond, 1997). Hence, comparison of SusMR values for different rodent species under conditions of lactation and cold exposure, challenges the central limitation hypothesis and its apparent generality, as noted by Weiner (Kenagy et al., 1989b; Hammond and Diamond, 1992, 1994; Hammond et al., 1994, 1996; Konarzewski and Diamond, 1994; Koteja et al., 1994; Koteja, 1996a; McDevitt and Speakman, 1994a,b; Speakman et al., 2001).

Peripheral organs and tissues may be limited by the rate at which ATP is generated and mobilized at these sites (Speakman, 2000). However, a very important exception in mammals is the heat generated by non-shivering thermogenesis in brown adipose tissue, which is one of the most important mechanisms for thermogenesis in small mammals in seasonal habitats (e.g. Heldmaier, 1993; Merritt et al., 2001). The peripheral limitation hypothesis predicts different metabolic ceilings for different modes of energy expenditure. This is because limits are set by the particular limitations of the tissues and organs using the energy, whereas central organs have an excess capacity (Hammond and Diamond, 1997). Thus, as for the central limitation hypothesis, a key approach to empirical evaluation of peripheral limitations on SusMR is provided by laboratory studies in which animals fed *ad libitum* are pushed to their maximal SusMRs under different modes of high energy expenditure (e.g. lactation, thermoregulation and activity).

It has been proposed that different patterns of energy expenditure among species (i.e. central *versus* peripheral, and within this latter category, differences in levels and modes of energy expenditure) could be related to each species' life-history strategy (Koteja and Weiner, 1993; Koteja, 1995, 1996a; Hammond and Diamond, 1997). Accordingly, there is an implicit assumption that SusMR are adaptive. However, at present it is difficult to confirm this assertion (but see Koteja et al., 2000).

It is possible that organisms do not have excess capacities, and the capacity of central organs to supply energy has evolved to match expenditure capacity in peripheral tissues. This hypothesis, with no limiting step on SusMR, but with optimal organism design, is called symmorphosis (*sensu* Taylor and Weibel, 1981).

The optimal design debate: symmorphosis

Taylor and Weibel (1981) proposed the principle of symmorphosis, based on '*the firm belief that animals are built reasonably*'. Basically, this principle states that no extra structure is formed and maintained unless it is required to satisfy an organism's functional needs (Taylor and Weibel, 1981). In fact, symmorphosis is defined as '*a state of structural design commensurate to functional needs.....,whereby the formation of structural elements is regulated to satisfy but not exceed the requirements of the functional system*' (Taylor and Weibel, 1981). Although this principle was first proposed in a study of the relationship between structure and function in the mammalian respiratory system, it has since been established as a general hypothesis of economic design (Weibel et al., 1998; Weibel, 2000). Optimal design means an almost perfect match between structure and function (Weibel et al., 1991; Weibel, 1998). As a result, the structural trait becomes the factor that sets the limit of functional performance (Weibel, 1998, 2000). An important prediction of this principle is that if functional needs change, then structural components must change accordingly. This is because building and maintenance of structures over what is actually needed is costly (DeWitt et al., 1998).

Optimization models in biology make assumptions about (i) constraints acting on phenotypes, (ii) the optimization function and (iii) heredity (Maynard Smith, 1978), so is it possible for natural selection to lead to symmorphosis? In other words, is it possible for natural selection to produce an optimal design? Answers to both of these questions have been as controversial as the optimization models (e.g. Gould and Lewontin, 1979; Garland and Huey, 1987; Dudley and Gans, 1991; Garland, 1998; Gordon, 1998). In particular, Garland (1998) and Gordon (1998) point out reasons for refuting symmorphosis: (i) organisms must perform different functions simultaneously, which probably creates constraints that prevent them from reaching an optimal solution for all processes; (ii) biological materials have limitations related to their own histories; (iii) in general terms, environments are always changing, and natural selection often cannot follow the rhythm of change; and finally (iv) genetic drift can be an important factor in some populations. Nevertheless, even if animals are not optimally designed, Garland (1998) pointed out that optimization models can be useful tools for understanding the evolution of physiological systems. In this sense, they can indicate the 'best' design that an organism could achieve, and therefore the concept is useful as a reference for understanding the reasons for departure from optimality. To summarize, the main reason why symmorphosis would not be widespread is that particular structures, and even systems, are often used in different functions, making it unlikely that optimization could be achieved for each one (Lindstedt and Jones, 1987).

How can we test for symmorphosis? In accordance with Taylor and Weibel (1981) and Weibel and collaborators (1998), the limit of the functional process must be determined. Furthermore, it must be established whether this limit is related to the organism's design. A clear description of how to do this is given by Weibel (2000). In brief, the first step is a quantitative physiological study in which, with different levels of demand, functional performance is pushed to its maximum (i.e. its limit). The next step would be a morphometric study of design properties related to functional capacities, followed by evaluation of any agreement between the functional performance and the morphometric parameters. The original approach by Taylor and Weibel (1981) was between species, using adaptive variation (i.e. animals with the same body size adapted for different levels of functional performance) and allometric variation (i.e. animals of different body mass, in which scaling of morphometric structures should be similar to functional requirements), but the concept of symmorphosis

could be evaluated within a particular species, using a similar protocol. As mentioned above, in the context of physiological limitations on SusMR, the symmorphosis principle predicts a match between central and peripheral organs and tissues. To test for this match, SusMR should be determined under different levels of demand (e.g. SusMR at temperatures of –10°C, 0°C and 10°C during cold exposure). The next step is to evaluate the adjustment between the different SusMRs obtained, and the morphometric parameters of central and peripheral organs and tissues (e.g. the dry mass of these organs might be considered a good first approximation). Nevertheless, we must bear in mind that a better quantitative approach is neccesary to test for symmorphosis (Weibel, 2000).

Evidence in favor of symmorphosis (e.g. Taylor et al., 1996; Weibel et al., 1996; Suarez, 1998; Bundle et al., 1999; Chappel et al., 1999; Hammond et al., 2000; Weibel, 2000) is as abundant as the evidence against it (e.g. Garland and Huey, 1987; Diamond, 1992; Diamond and Hammond, 1992; Alexander, 1998; Ricklefs, 1998). At present the optimal design debate remains unresolved. Furthermore, even when evidence against symmorphosis is strong, it does not invalidate the usefulness of the concept (e.g. Diamond, 1992; Diamond and Hammond, 1992) and, as Diamond and Hammond (1992) stated: '*the concept is worth posing not because we believe it to be literally true, but because only by posing it as a testeable hypothesis of economic design can one hope to detect where it breaks down, and to identify the interesting reasons for its breakdown*'.

Sorting out the evidence

As mentioned above, SusMR refers to the energy expenditure that can be sustained over long periods of time by concurrent energy intake while animals are in mass balance. Consequently, food intake has been extensively used as a measure of SusMR. This does not present a problem when most food is metabolized, as occurs in cold acclimation (Konarzweski and Diamond, 1994; Koteja, 1996; McDevitt and Speakman, 1994a), and in these cases, limits on intake could be considered limits on expenditure. However, during lactation (a widely used stressor) not all ingested food is metabolized. In fact, an important part is exported as milk (i.e. it does not represent an expenditure *per se*) (Johnson et al., 2001b). In this case, the actual level of energy expenditure would be expected to be lower than expenditure estimated from food intake, as has been demonstrated in a few scant studies (Johnson et al., 2001b; Johnson and Speakman, 2001; Scantlebury et al., 2000). Then, even though food intake in animals subjected to various stressors may be different (i.e. possible peripheral limitation), the real expenditure may be equal (i.e. possible central limitation). Certainly, more work is needed to determine the extent to which these two estimates differ.

In the particular case of the central limitation hypothesis, Koteja (1996a,b) proposed that: '*the alimentary bottleneck hypothesis is supported by numerous observations and experiments demostrating that changes in current energy demand or food quality are associated with changes of gut size…*' (Gross et al., 1985; Bozinovic et al., 1990; Loeb et al., 1991; Toloza et al., 1991; Hammond and Diamond, 1992, 1994; Hammond et al., 1994; Konarzewski and Diamond, 1994). Nevertheless, this assertion does not validate the central limitation hypothesis. A change in morphology of the digestive tract with increasing energy demands, or a decrease in food quality, does not mean that the digestive tract is the limiting step to energetic expenditure. It simply shows that the digestive tract is plastic enough to change according to demand, and that there is a cost for supporting high performance levels when these levels are not required (DeWitt et al., 1998). So, a possible reason why these organs grow under high food intake or energy requirements is that they possess limited functional reserves under conditions of low demand (Hammond and Konarzweski, 1996; Hammond and Kristan, 2000). Similarly, if metabolic ceilings reach the same value under different modes of expenditure, most authors would agree that a central limitation exists. However, this procedure does not exclude the possibility of a peripheral limitation on SusMR because, by chance, different modes of energy expenditure might have equal values. A way of discriminating between both hypotheses is through a combination of peak energy demands. If central limitation really is the cause of the metabolic ceiling, one would expect a conflict in energy allocation when different high-energy-demanding activities are being performed simultaneously. Conversely, if limits on SusMR are set peripherally, no conflict in energy allocation would be expected since central organs possess an excess capacity.

With the exception of a few studies (Hammond et al., 1994; Derting and Austin, 1998; Hammond and Kristan, 2000; Johnson and Speakman, 2001), this topic (i.e. design constraints and conflict among demands) has not been explicitly approached, even though it plays a key role in determining, at least theoretically, the signs and magnitudes of genetic correlations among high-energy-demanding activities and, consequently, their response to natural selection (Stearns et al., 1991; Stearns, 1992). In particular, the response of any two genetically correlated traits to natural selection is dependent on the sign of the correlation (Stearns et al., 1991). If the correlation is negative, a positive response to selection in one trait would generate a negative response in the other. Thus a central limitation on SusMR could generate a negative correlation among different activities using energy in parallel, with the consequences manifest in the response to natural selection. Furthermore, in many aspects of ecology and evolutionary biology (e.g. mechanistic aspects of life history evolution) (Stearns, 1992), an implicit central limitation, in the form of the Principle of Allocation (Cody, 1966), is always assumed. Nevertheless, the presence of peripheral limitations could challenge this view and force it to change or to be restricted to particular situations (i.e. when central limits are in fact operating).

Even though methodology that combines energy demands might distinguish between central and peripheral limitations, it does not exclude the possibility that structure and function adjust to the new conditions (i.e. symmorphosis). The central and peripheral limitation hypotheses assume that organisms

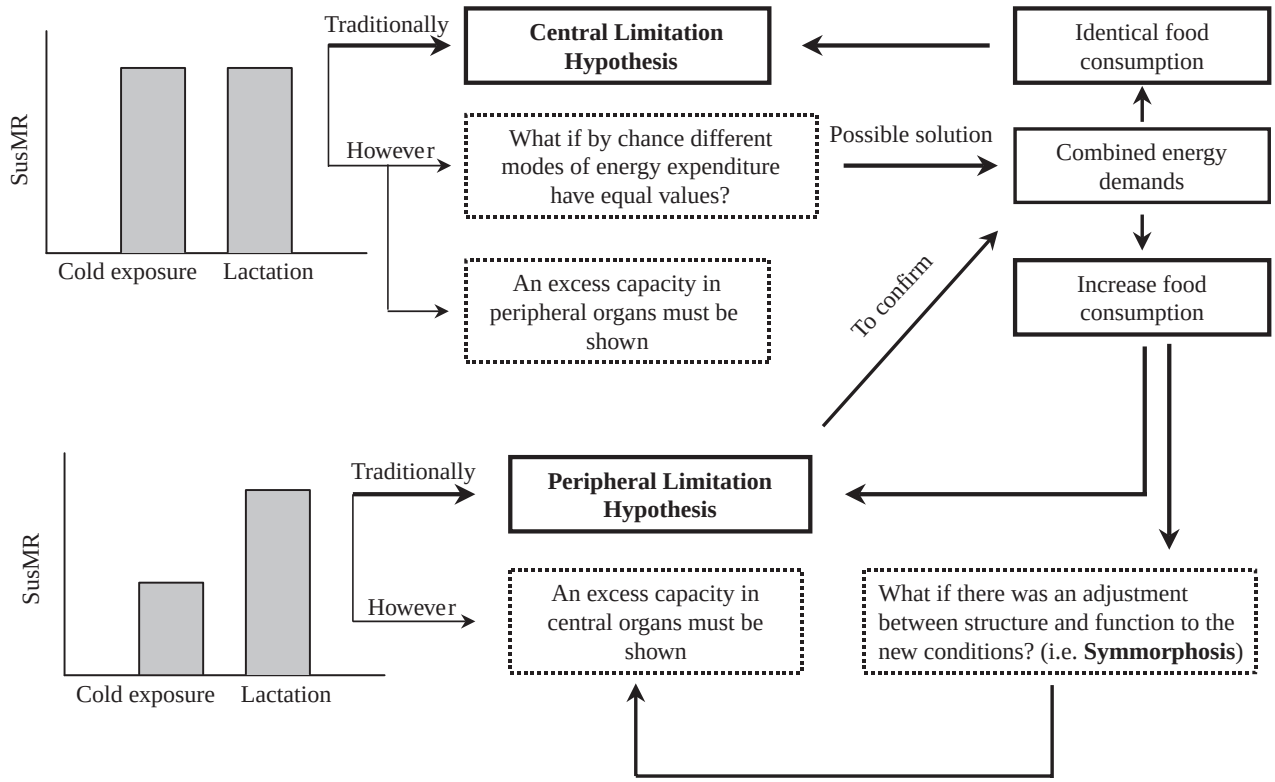

Fig. 1. Summary of the empirical procedure used to test for physiological limits on SusMR and some possible sources of error. SusMR values are representative estimates of the sustained activities (i.e. lactation or cold exposure). In the lower graph either of the sustained activities could have the greater value.

have evolved with certain limiting steps in energy expenditure, while other steps have kept unused reserve capacities. In this sense, it is not enough to demonstrate the site of limitation; one must also demonstrate the existence of excess capacity in the central machinery (if the limitation is set peripherally), or excess capacity of peripheral organs (if the limitation is central) (Fig. 1). This has only been tested on a few occasions, however, and always using laboratory species (Toloza et al., 1991; Diamond and Hammond, 1992; Hammond and Diamond, 1992; Hammond et al., 1994; Konarzewski and Diamond, 1994), so we are not able to draw firm conclusions as to whether limits are set centrally or peripherally.

Considerations of optimal design necessitate caution in interpreting changes (morphological or physiological) associated with phenotypic plasticity. At first glance, it may seem that such changes are a consequence of symmorphosis or optimal design (e.g. Lindstedt and Jones, 1987; Weibel, 1998); however, more detailed inspection may eliminate optimal design (Toloza et al., 1991; Diamond and Hammond, 1992). For example, food intake by lactating females increases almost linearly with the total mass a mother must support (mass of mother and young), plus time of lactation, because each young requires more milk as it grows (e.g. Hammond and Diamond, 1992). Although there is an increase in food intake following parturition, however, the digestive efficiency of *Mus musculus* does not change either with number of young or the duration of lactation (Hammond and Diamond, 1992). How can digestive efficiency be maintained under these high-energy-demanding conditions? One possibility is that the small intestine has excess capacity, and efficiency can therefore be maintained in spite of the increase in food intake. In this case, according to the symmorphosis principle, design is not considered to be optimized due to this *excess* capacity. Another possibility is that the small intestine grows rapidly enough during lactation to match the increasing food intake. Here, optimal design is implicated, because there is an adjustment between structure and function. In general, both kinds of changes are happening (Toloza et al., 1991; Diamond and Hammond, 1992; Hammond and Diamond, 1992). As shown, a morphological change in accordance with changes in functional needs seems to be the result of an optimal design; however, a detailed analysis could show another point of view, that is, excess capacities should indicate a *suboptimal* design.

The presence of a link between RMR and SusMR is the principal idea behind the proposal of a central limitation on energy budgets (Speakman, 2000, and references therein). However, we contend that caution is needed when considering the argument that this link is determined by a central limitation. In mammals, the greatest increase in energetic demands occurs during lactation (Millar, 1978; Mattingly and McClure, 1985; Kenagy, 1987; Kenagy et al., 1989a,b, 1990), and also cold exposure (e.g. Konarzweski and Diamond, 1994; Merritt et al., 2001; Nespolo et al., 2001). Both activities result in an increase in food consumption (Hammond et al., 1996). This involves

processing (i.e. digestion, absorption and transport) of greater amounts of nutrients, which could produce hypertrophy of the central organs associated with these processes and a resultant increase in RMR. In this respect, SusMR and RMR may be correlated, but the type of limit on SusMR (i.e. central or peripheral) remains an open question. The observation of a link between both traits alone is not enough to confirm a central limitation, nor is the absence of a link enough to support the opposite conclusion (i.e. peripheral limitation). Thus there is a need for correlational studies, complemented by experiments. For example, values of RMR and SusMR in *Mus musculus* (Hammond and Diamond, 1997) using different modes of energy expenditure, provide evidence that there is an important correlation between the two rates, which would suggest a central limitation on SusMR. However, the combined works of Hammond and coworkers on the physiological limitations in white mice demonstrated that the limitation is not central (Hammond and Diamond, 1992, 1994; Hammond et al., 1994, 1996; Konarzewski and Diamond, 1994).

In summary, we feel that these hypotheses lack strong empirical data to demonstrate that central and peripheral physiological limitations hold true both in animals in the laboratory and in the wild (Fig. 1). We propose that the following steps are necessary to identify the intraspecific physiological limits on SusMR: (1) use of a combination of peak energy demands to differentiate between central limitation and peripheral limitation; (2) pushing animals to their physiological limits (e.g. asymptotic food intake), (3) testing for a central excess capacity if the limit is set peripherally, or a peripheral excess capacity if there is a central limitation, and (4) utilizing different levels of energy demand to test for symmorphosis. Finally, without more empirical evidence it is not possible to determine which design is most common in nature and why, nor can we identify the ecological and evolutionary consequences of each type of physiological limitation. In addition, studies that incorporate locomotory activity as a stressor are needed. Testing for symmorphosis with this stressor, could be done by comparing SusMR of non-selected *versus* selected lines for different levels of running activity.

Concluding remarks

Empirical data about physiological limitations on energy budgets are scant. Only a few studies have been explicitly designed to measure SusMR, and the sustained energy expenditure does not exceed seven times the resting expenditure (Speakman, 2000). This raises two important questions. (1) Why is energy expenditure during long periods only slightly elevated above resting requirements compared to energy expenditure during short periods? (2) Do organisms function at their physiological limits (Speakman, 2000)?

The answer to the first question has been associated with the potential decrease in fitness that a mammal might experience if it were to expend more energy than it routinely does (Murie and Dobson, 1987; Wolf and Schmidt-Hempel, 1989; Stearns, 1992; Martin and Palumbi, 1993; Daan et al., 1996; Finkel and Holbrook, 2000; Speakman, 2000). However, the evidence for this trade-off (i.e. energy expenditure *versus* fitness) is not conclusive (Tuomi et al., 1983; Hare and Murie, 1992; Speakman, 2000). As to the second question, organisms could function at or near their physiological limits, but are prevented from doing so because of energy limitations imposed by the environment (e.g. Stenseth et al., 1980; Speakman, 2000). At present there is insufficient evidence to offer definitive answers to these questions, and we cannot conclusively identify which physiological factors may impose limits on SusMR. Hence, there is a need for further studies aiming to unravel the nature of the physiological limit on SusMR (i.e. central, peripheral or symmorphosis) and the steps where this limit occurs.

We thank Joseph Merritt, Paula Neill, Roberto Nespolo and Enrico Rezende for a critical revision of the manuscript. L.D.B. wishes to acknowledge Patricia Nicola for comments and tremendous support during the preparation of this manuscript. Also, L.D.B. acknowledges a DIPUC doctoral fellowship. This work was funded by Fondo Nacional de Ciencia y Tecnología FONDAP grant no. 1501-0001, Programa 1.

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