General Perspective

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The role of energy expenditure in ageing: “The rate of living theory”

Almost a century ago Rubner (1908) calculated the amount of food, expressed in calories, eaten over the life span by several domestic species of mammal (guinea pig, cat, dog, horse and cow). He was struck by the fact that these amounts were roughly proportional to the typical body mass of the species, so that the ratio, i.e., the caloric intake per lifetime per gram body tissue was little different between guinea pig and cow. These data led to the rate of living theory that was formulated in 1928 by Pearl based on Rubner’s work and on his own observations (Pearl, 1928). The theory postulates that the rate of senescence that ultimately leads to spontaneous death is positively associated with the rate of energy turnover of body tissue. In the remainder of the century an extensive debate followed and numerous studies were published inspired by this theory. Interspecific comparative studies in birds and mammals have now well established that metabolic rate is indeed inversely related to life span and that the Lifetime Energy Potential (LEP) is fairly constant between species within the order of birds and within mammals (Ku et al., 1993; Speakman, 2005a).

There are also experimental approaches. One line of evidence comes from experiments in exotherms. In fruitflies Drosophila melanogaster (Loeb and Northrop, 1917), houseflies Musca domestica (Ragland and Sohal, 1975) and the Nematode Caenorhabditis elegans (Van Voorhies and Ward, 1999) increasing ambient temperature (and thereby metabolic rate) results in reduced longevity. Increased activity results in decreased life span (Ragland and Sohal, 1975) and genetic factors related to longevity have been shown to decrease metabolic rate (Van Voorhies and Ward, 1999). Studies in endotherms (rodents) manipulating energy expenditure by voluntary exercise (Goodrick, 1980; Holloszy and Smith, 1987; Navarro et al., 2004), cold exposure (Johnson et al., 1963; Holloszy and Smith, 1986), caloric restriction (McCay et al., 1935; Yu et al., 1985) or evaluating interindividual variation in metabolic rate (Speakman et al., 2004) do not yield a uniform picture. The interpretation of these results is complicated because in most studies no attempt was made to accurately measure metabolic rate and/or body composition throughout the life span of the animals. Exercise studies have for instance been used to challenge the role that energy expenditure may play in the ageing process. Moderate exercise generally increases energy expenditure during the exercise bout, but does not decrease average life span: if anything, it increases the life span (in rats; Holloszy and Smith, 1987) and mice; (Navarro et al., 2004)). It may, however, well be that animals compensate for the extra expenditure in exercise by reduced energy metabolism during subsequent rest (Deerenberg et al., 1998). The necessary measurements to determine whether (mass-specific) daily energy expenditure (DEE) is indeed increased in exercising animals, have usually not been made.
Testing the rate of living theory: manipulations of energy expenditure

To investigate the relationship between energy expenditure and ageing within species, we manipulated energy expenditure in two strains of mice (Hsd:ICR and C57BL6J). In the former strain we manipulated energy expenditure by exploiting the spontaneous increase in exercise resulting from selective breeding, and in the latter we decreased ambient temperature (from 22°C to 10°C). In the exercise study (Chapter 6) we used mice selectively-bred for high voluntary wheel-running activity (S+) and compared them with their random-bred controls (C+). We also studied differences between activity-selected mice housed with (S+) or without (S-) a running wheel. As expected, S+ mice had the highest daily energy expenditure throughout life (increased by average 8 kJ d⁻¹), and C+ and S- mice had similar, low levels of DEE (average 59.0 and 61.2 kJ d⁻¹, respectively). According to the rate of living theory one would expect C+ and S- to have similar life spans, which should exceed that of S+ mice. We did find differences in life span, but unexpectedly S+ mice and S- mice both had life spans approximately 100 days shorter than C+ mice. To accurately compare experimental groups, we calculated life-time energy potential (LEP). Mass-specific LEP, no matter whether expressed per animal, per gram body mass, dry lean mass of the carcass or metabolic organ mass, was always higher in the S+ mice compared to the other groups.

In the temperature experiment (Chapter 8) we also reared three experimental groups. One group was housed at 22°C (warm, WW) throughout life, another at 10°C (cold, CC) and a third group was housed at 10°C until 15 months of age and at 22°C thereafter (coldwarm, CW). The cold mice spent approximately 50% more energy than warm mice. The CW mice had an increased daily energy expenditure early in life, but not later in life compared to warm mice. The rate of living theory implies a cumulative effect of energy turnover on life span. We should thus expect the effects of the manipulation restricted to early life and leaving conditions late in life unchanged, to have an impact on life span as well. Strikingly, cold mice had a similar life span to warm mice throughout life, and CW mice had a ~80 days shorter median life span. LEP expressed either per gram body mass, dry lean mass or organ mass was highly increased in CC mice compared to WW and CW mice in the cold experiment.

In conclusion, these two experiments manipulating energy expenditure neither showed the expected inverse relationship between metabolic rate and life span based on the rate of living theory, nor did they show a constant LEP between the groups. We emphasize that the conclusion is based on relatively large sample sizes for longevity assessments (n=60), on full-day measurements of energy metabolism in the home cages, and on careful analysis based on age-specific assays of body mass and composition.

Caloric restriction experiments and the Lifetime Energy Potential

Caloric restriction (CR) is the only manipulation that increases both median and maximum life span in rodents, as first shown in rats by McCay et al. (1935).
Because CR decreases the rate of ageing, it constitutes an excellent approach to better understand the mechanisms underlying the ageing process. The fact that CR slows the rate of ageing suggests that a reduction in some aspects of energy metabolism should be related to the rate or ageing. This idea is supported by the suppression of growth and many findings of reductions in levels of biochemical parameters in CR animals, like serum glucose, insulin, growth hormone and glucocorticoids (Masoro, 2005). In addition, oxidative damage to proteins, lipids, and DNA is reduced in CR animals compared to Ad libitum fed controls of the same age (Gredilla et al., 2001; Lopez-Torres et al., 2002; Barja, 2002a). Several investigations have reported that these decreases in oxidative damage are related to a lowering of mitochondrial free radical generation rate in various tissues of the CR animals (Sohal et al., 1994; Gredilla et al., 2001; Lopez-Torres et al., 2002). Thus, similar to what has been described for long-lived animals in comparative studies (Perez-Campo et al., 1998; Barja, 2002b), a decrease in mitochondrial free radical generation has been suggested to be one of the main determinants of the extended life span observed in CR animals (Barja, 2004a).

The question whether these effects of CR can be attributed to a reduction in energy expenditure in calorically restricted animals has been the subject of considerable debate. Masoro, McCarter and co-workers showed that caloric intake per gram body mass is actually increased in mice subjected to CR (Masoro et al., 1982), although the total energy turnover rate decreases. There is no significant effect on 24h metabolic rate expressed per gram lean mass (McCarter et al., 1985; McCarter and McGee, 1989). Right after the start of caloric restriction, a slight decrease in 24-h metabolic rate (corrected for lean mass) was observed, but this difference disappeared after approximately 10 weeks (McCarter and McGee, 1989). Interpretation of the results is complicated because metabolic rate has to be corrected for body size in some way. Greenberg and Boozer (2000) have shown that the mass of the most metabolically active organs (heart, liver, kidney, brain) better explained differences in metabolic rate (see also Daan et al., 1990). Studies measuring organ-specific metabolic rates have shown that even though the internal organs comprise only ~5% of the total body mass, they are responsible for approximately 50% of the resting energy expenditure in both humans and rats (for summary of the results see Ramsey (Ramsey et al., 2000), Table 4). Changes in organ mass that are too small to result in a significant change in total or lean body mass can thus exert large effects on the energy expenditure of the animal, and expressing energy expenditure per gram “organ mass” would thus allow a more accurate comparison between experimental groups. A problem that remains when interpreting data normalized forlean organ masses is that is assumes that all components (heart, liver, kidney and brain) have similar rates of oxygen consumption. Although this assumption is probably incorrect, there is no way of further partitioning the total metabolic rate into organ-specific contributions.
Calculations based on organ metabolic rates

Greenberg estimated energy expenditure based on organ metabolic rates, and found that metabolic rate per unit of organ mass was directly related to the rate of ageing in studies on CR animals, cold-exposed animals or exercising animals (Greenberg, 1999). In his calculations Greenberg used data from McCarter’s study on lifelong metabolic rate in Fischer 344 rats that were fed ad libitum or CR (60%). He found that the ratio of whole-body BMR to total body-part BMR was decreased in CR animals compared to ad libitum fed controls. He thus concluded that the rate of living theory was valid as long as one took organ tissue metabolism into account. We used data from the same study by McCarter (metabolic measurements, (McCarter and Palmer, 1992) and Yu (life span measurements, (Yu et al., 1982)) to calculate the LEP of the animals (see Table 10.1 for a summary of the data). LEP_{BM} was much greater (+48%) in CR animals than ad lib fed animals, with 478 and 324 kJ g^{-1} respectively. This difference became slightly less when we calculated LEP based on organ mass, but was still much greater (+34%) in CR animals (LEP_{OM} = 11240 vs. 8378 kJ g^{-1}, respectively). These findings show that although there was a negative relationship between overall energy expenditure and life span, life span is not quantitatively predictable from the hypothesis of a constant LEP as predicted by the rate of living theory, even when expressed per gram mass of the four metabolically expensive organs. This conclusion is the same as for our studies on the effects of high activity (chapter 6) and low temperature (chapter 8). Within species, the rate of living theory is thus not supported by the data in its strict formulation of constancy of lifetime mass-specific energy turnover.

Interspecific comparisons

We may now also have an other look at where the rate of living theory came from: the comparison of species. As mentioned in chapter 1 interspecific comparisons yield an inverse relationship between energy expenditure and longevity. However, body mass and lack of phylogenetic control are confounding factors in such analysis (Speakman, 2005b). Energy expenditure rate per gram scales to body mass with an exponent of approximately −0.3, and life span scales to body mass with the exponent of approximately +0.3 like durations of most biological periods (Daan & Aschoff 1982). The product of the two, being the mass-specific Lifetime Energy Potential, thereby has an exponent of 0, i.e. is mass independent.

This dependence is based on correlation. It does not prove that there is a causal relationship between energy turnover and lifespan. If there is a causal relationship, it does not prove that it should also cause the constancy of LEP. The association may come about only due to the causal association of both to body mass. For instance, the relationship between body mass and energy expenditure may be defined by physical constraints in relation to body size, i.e., larger animals must have lower metabolic rates per gram because of their surface area (where heat can be dissipated) is relatively smaller. Ecological constraints (e.g., predation risk) in relation to size may determine the association between body mass and life span.
Analysis of residuals (also correcting for phylogeny, see (Speakman, 2005b)) still yield a negative association between energy expenditure and life span. This shows that it is not a simple artefact emerging from their relationship to body mass (Speakman et al., 2002; Speakman, 2005a). Nonetheless, a large part of the variation around the regression line remains unexplained. For instance, birds expend typically in the order of 1.47 times as much energy as a mammal of similar size in BMR and 1.59 in DEE (Daan et al., 1991), but have greater longevity (Holmes et al., 2001; Brunet-Rossinni and Austad, 2004). Marsupials spend less energy than would be expected for a certain mass and have shorter life spans than other mammals (Austad and Fischer, 1991). These discrepancies cannot be explained by the rate of living theory, and may be related to physiological or ecological differences that have occurred during evolution between larger taxa.

The role of free radicals & defense against them in ageing: “The free radical theory of ageing”

Reactive Oxygen Species

We have shown that within species life span is not solely dependent on the rate of energy expenditure throughout life and that Lifetime Energy Potential is not necessarily a constant, even if expressed in the proper way, as the total turnover per gram of the high-energy turnover organs (Chapter 6 and 8). This does not imply that metabolic rate is not involved in ageing, and that increased energy turnover does not speed up the ageing process. The free radical theory of Denham Harman (Harman, 1956) proposed that ageing was caused by the accumulation of oxidative damage by oxygen free radicals (reactive oxygen species, ROS) produced during aerobic metabolism.

Free radicals influence cellular function because they can cause damage to macromolecules: DNA, lipids and proteins. With age this damage accumulates and eventually results in death (Harman, 1956). Numerous studies have investigated the involvement of free radicals in the ageing process, and is clear that they play an important role (Beckman and Ames, 1998; Sohal et al., 2002; Barja, 2002b). Theoretically a higher metabolism would result in a higher production of reactive oxygen species (ROS) and thus in more rapid ageing and a shorter life span. The relationship between metabolic rate and the production of ROS is not linear. The amount of ROS that is produced is thought to depend on the state of respiration in mitochondria (state 3 or 4), and also on the activity of uncoupling proteins (UCP) (Brand, 2000). When non-limiting amounts of ADP are available, mitochondria are in state 3 respiration. When ADP is absent there can be no ATP production and proton transduction mechanisms become backed up: state 4 respiration. During state 3 respiration there is an abundant flow of protons across the inner membrane, while during state 4 respiration no protons flow through complex IV, and free radical production is expected to be higher (Brand, 2000). During state 4 oxygen con-
consumption is reduced (leak) and in state 3 the demand for energy and O₂ consumption is the highest. UCP's uncouple oxidative phosphorylation from ATP generation and generate heat instead. When uncoupling occurs, the respiratory chain is speeded up, and less ROS are produced per unit O₂ consumed. That this can have a positive effect on life span has been shown in a study by Speakman and Selman (2004) where mice with more uncoupling (and higher metabolic rate) had longer life spans.

Once ROS are produced there are several protection mechanisms that reduce the damage they can cause. First, antioxidant enzymes can scavenge ROS. The main endogenous antioxidant is superoxide dismutase (SOD) which catalyzes the dismutation of superoxide (O₂⁻) into oxygen (O₂) and hydrogen peroxide (H₂O₂). Hydrogen peroxide is still a ROS and can fall apart in hydroxyl radicals (OH⁻). Catalase (CAT) and glutathione peroxidase (GPx) can prevent this by catalyzing the decomposition of hydrogen peroxide to the harmless water (H₂O) and oxygen. Antioxidant enzymes thus reduce the amount of ROS and the damage they can cause. Studies on mice with the genes for such enzymes knocked out have supported the important role of these enzymes. Mice lacking manganese superoxide dismutase die within 10 days (Li et al., 1995). When damage does occur, DNA repair and protein turnover can counteract the damage to macromolecules before it causes permanent loss of function. These defense mechanisms (uncoupling, antioxidant enzymes and repair) influence the relationship between metabolism and ageing (see Figure 1.1).

The fact that exercising mice generally spend more energy, but do not show a reduced (or even an increased) life span (Holloszy and Smith, 1987; Navarro et al., 2004), could be explained by changes in the systems described above. For instance, exercise moves mitochondrial respiration toward state 3, which may reduce the ROS produced. Also, voluntary exercise increases the production of antioxidant enzymes and cellular protection against cellular damage (Powers et al., 1999; Kakarla et al., 2005).

**Changes in defense systems and life span**

To see whether changes in these mechanisms could explain the differences we observed in the life span in our experimental groups we measured UCP mRNA expression in muscle, brown adipose tissue and white adipose tissue, SOD and GPx activity in heart and liver, SOD, CAT and GPx mRNA expression in liver and muscle, and protein turnover in liver and muscle (Chapter 7 and 9, for summary see Table 10.2). No significant differences in UCP expression were found between the groups in any of the tissues (data for exercise experiment not shown, for cold experiment see Figure 9.5, for summary see Table 10.1). In the cold experiment, UCP expression was slightly increased in all tissues and at both ages measured, indicative of an increase in uncoupling. Several other studies have reported increases in UCP mRNA expression in response to cold exposure (Carmona et al., 1998; Simonyan et al., 2001; von Praun et al., 2001), but others did not (Boss et al., 1998;
It remains unclear whether increases in mRNA UCP levels do result in an increase in mitochondrial uncoupling. Differences in uncoupling, antioxidant defense and/or protein synthesis could not explain the difference we observed in life span. No major differences were found between the groups in our exercise or cold experiment for either variable.

The role of endocrine factors in ageing

The difference in life span we observed between control and activity-selected mice may be due to factors that have unintentionally been influenced during the selection procedure. This could potentially include any physiological or behavioural trait that has previously been shown to differ between the lines, such as for instance corticosterone levels (Girard and Garland, Jr., 2002; Malisch et al., 2006). In a study on rats, Cavigelli (2003) showed that neophobic rats (inactive in new environment) had increased basal levels of corticosterone throughout life, and that they had a 60% higher chance to die relative to neophilic animals (active in new environment) at all ages. The median life span was 100 days shorter for the neophobic rats. We measured basal corticosterone levels in our mice at various ages, but found no differences in basal corticosterone between the groups in the exercise (Chapter 3, Figure 3.2) experiment. A previous study did show an increase in basal corticosterone at 2 months of age, mainly in female activity-selected mice (Malisch et al., 2006). Based on our results we may presume that these differences become smaller with age (when differences in wheel-running activity also become smaller, see Chapter 4 and 6). Also, when measured in an elevated-plus maze, no differences in anxiety between control and activity-selected mice were observed (personal obser-

<table>
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<th>Ad lib</th>
<th>CR</th>
<th>Ref</th>
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<tr>
<td>DEE (kJ d⁻¹)</td>
<td>183</td>
<td>105</td>
<td>McCarter (1992)</td>
</tr>
<tr>
<td>90% survival (d)</td>
<td>800</td>
<td>1240</td>
<td>Yu (1985), from graph</td>
</tr>
<tr>
<td>Body mass (g)</td>
<td>451</td>
<td>272</td>
<td>McCarter (1992)</td>
</tr>
<tr>
<td>Lean mass (g)</td>
<td>355</td>
<td>232</td>
<td>McCarter (1992)</td>
</tr>
<tr>
<td>Organ mass (g)</td>
<td>17.4</td>
<td>11.6</td>
<td>Yu (1985), Greenberg (1999)</td>
</tr>
<tr>
<td>DEE₉₀ (kJ g⁻¹ d⁻¹)</td>
<td>0.40</td>
<td>0.39</td>
<td></td>
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<td>10.5</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>LEP₉₀ (kJ g⁻¹)</td>
<td>324</td>
<td>478</td>
<td></td>
</tr>
<tr>
<td>LEPOM (kJ g⁻¹)</td>
<td>8378</td>
<td>11240</td>
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McCarter (1992) measured body mass, lean mass and 24h metabolic rate in ad libitum fed and calorically restricted Fisher rats at 6, 12, 18 and 24 months of age. The graph shows the average value for these measurements. Data on 90% survival were obtained from the survival curve in Yu et al. (1985). Estimates of organ masses were taken from Greenberg (1999), based on measurements by Yu.

von Praun et al., 2001). It remains unclear whether increases in mRNA UCP levels do result in an increase in mitochondrial uncoupling. Differences in uncoupling, antioxidant defense and/or protein synthesis could not explain the difference we observed in life span. No major differences were found between the groups in our exercise or cold experiment for either variable.
vation, data not shown). Differences in corticosterone or response to a novel situation thus do not appear to have had a role in differences in life span between the control and selected groups.

In Chapters 4 and 5, we have shown that mice selected for high wheel-running activity show some interesting adaptations to their active phenotype. The most intriguing was the observation that plasma adiponectin levels are significantly increased in S mice compared to the levels found in their random-bred controls (Chapter 4, Figure 4.2). This increase was found in S mice irrespective of the availability of running wheels and occurred in all of the separate selection lines. This suggests that the increase in adiponectin is a trait genetically co-segregated with selection for increased wheel-running activity, instead of being mediated via increased physical activity per se. There are several reasons to believe that increased circulating adiponectin levels might contribute to the different phenotypes seen in S mice compared to C mice. For example, Fruebis et al. (Fruebis et al., 2001) found that chronic administration of gAcrp30 (i.e., adiponectin) caused weight loss in mice despite the fact that food consumption was unaffected. This is a phenotype which appears homologous to the one found in activity-selected animals (Swallow et al., 1999; Swallow et al., 2001). Fruebis et al. (2001) attributed the effect of adiponectin on body mass to increased fat oxidation, specifically in liver and muscle, and this was confirmed in subsequent studies (Berg et al., 2002; Yamauchi et al., 2002; Bruce et al., 2005). In addition, we observed a decrease in the respiratory quotient (RQ) of S mice compared to C mice measured over a period of 24 hours in non-fasted animals (see Chapter 5), which indeed indicates higher levels of fat oxidation in selected mice. We speculate that an increased capacity to downgrade lipids in muscular tissue contributes the increased physical activity displayed by activity-selected mice. The effects of adiponectin on fat oxidation are believed to arise through stimulation of AMP-activated protein kinase (AMPK) (Berg et al., 2001; Yamauchi et al., 2002). Zhang et al. (2006) have shown increased levels of phosphorylated AMPK in the aorta of male activity-selected mice compared with controls, which is consistent with the observed elevated levels of adiponectin in our study (Chapter 4). Another interesting finding was that the distribution of the fat over the body differ between control and selected male mice, even though the total amount of fat did not. Activity-selected mice stored fat more viscerally and one can imagine that this shift in fat distribution is beneficial for mice that run intensively.

The important role of adiponectin in insulin sensitization (Yamauchi et al., 2001; Baratta et al., 2004; Schondorf et al., 2005) makes the selected mice an interesting model to study factors related to the metabolic syndrome. In chapter 5 we investigated whether selected mice were less prone to develop diet-induced obesity. We found the most striking differences between control and selected females. Selected females on the fat diet did not develop obesity. Control mice reduced their food intake, because their food efficiency was higher on the fat diet, but selected females showed opposite results. Selected males did show an increase in fat mass, similar to that of control males on the fat diet. In the plasma levels of several metabolic hor-
mones, we also observed significantly different responses between control and selected males. On the fat diet, both selected males and females had increased adiponectin levels.

Because of their abnormal response to the fat diet, which does not lead to develop obesity, these mice are an interesting model to further study the metabolic syndrome. Future studies should establish whether increased adiponectin levels protect the selected mice against developing insulin resistance on a high-fat diet.

We also measured hormone levels in the cold-exposed mice (see Box 4.1). Cold-exposure is perhaps experienced by the mice as a chronic stressor. Chronic stress is known to have adverse effects on health and longevity (Paré, 1965). We measured basal corticosterone levels in the mice at various ages and found no differences between the groups, indicating that the HPA-axis was not upregulated in response to the cold exposure. Plasma leptin and adiponectin were significantly decreased in cold-exposed mice relative to warm mice throughout life (see Box 4.1). When corrected for fat mass the decrease in leptin, but not adiponectin remained significant.

No studies investigating the direct effect of adiponectin or leptin on ageing have been undertaken. Adiponectin is known to improve factors associated with the metabolic syndrome, which is quickly becoming one of the most important factors compromising human health. Circulating adiponectin levels are reduced in obese humans compared with lean individuals (Arita et al., 1999) and this is associated with increases in cardiovascular risk factors such as insulin resistance and atherogenic lipid profiles. Adiponectin protects against vascular diseases by inhibiting local proinflammatory signals, preventing preatherogenic plaque formation, and by impeding arterial wall thickening (Schondorf et al., 2005). Also in nonobese, healthy adults hypoadiponectinemia results in increased cardiovascular risk factors (Im et al., 2006). Opposite to adiponectin, leptin levels in obese humans are increased in compared with lean individuals (Park et al., 2004). Leptin may play an important role in the pathogenesis of hypertension related to obesity and metabolic syndrome. Furthermore, the lipotoxic effect of leptin resistance may cause insulin resistance and _ cell dysfunction, increasing the risk of type 2 diabetes and leptin has also been shown to possess proliferative, pro-inflammatory, pro-thrombotic, and pro-oxidative actions (Correia and Rahmouni, 2006). Low levels of leptin and high levels of adiponectin thus seem to protect against developing several pathologies.

Differences we observed in adiponectin and/or leptin levels between the groups (see Table 10.2), may thus have had a role in protecting or making animals more vulnerable to develop associated pathologies. If so, these effects can not explain the differences we observed in life span between the groups, since selected mice (with high adiponectin levels, and low leptin) actually lived shorter than control mice. In the cold-exposed mice the picture is also complex since they had low adiponectin and low leptin levels.
The role of fat in ageing

One property common to exercising, cold-exposed and calorically-restricted mice is that they all have a significantly reduced fat content compared to their respective controls. Ad libitum fed animals in captivity usually develop obesity because of their sedentary life style, which may have adverse affects on their health (e.g., developing metabolic syndrome and associated diseases, see Chapter 4 and 10.3) and reduce their life span. A negative relationship between body mass and life span (Miller et al., 2002) is an indicator of these effects.

Fat is a major source of nitric oxide (NO) stimulated by leptin, and as fat stores increase, leptin and NO release increase in parallel. NO is highly toxic and can cause damage to macromolecules. NO may be responsible for increased coronary heart disease as obesity progresses (McCann et al., 2005). It has further been shown that fat cells increase carcinogenesis in mice (Lu et al., 2006). In this study, voluntary wheel running activity stimulated UVB-induced apoptosis in the epidermis and in tumours of mice. This effect was related to the reduced fat content in exercising

Table 10.2. Overview of our results.

<table>
<thead>
<tr>
<th>S+ vs. S-</th>
<th>Table/Fig</th>
<th>Cold vs. Warm</th>
<th>Table/Fig</th>
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<tr>
<td>Energy expenditure</td>
<td>+</td>
<td>T6.4, F6.4</td>
<td>+</td>
</tr>
<tr>
<td>Life span</td>
<td>=</td>
<td>T6.1, F6.3</td>
<td>=</td>
</tr>
<tr>
<td>LEPBM</td>
<td>+</td>
<td>T6.4</td>
<td>+</td>
</tr>
<tr>
<td>LEPOM</td>
<td>=</td>
<td>T6.4</td>
<td>+</td>
</tr>
</tbody>
</table>

**Body composition:**
- Body mass - T6.2 - T8.2
- Dry lean mass + T6.2 + T8.2
- Fat content - T6.2 - T8.2
- Organ mass = T6.2 + T8.2

**Defense systems:**
- Antioxidant enzyme activity = F7.1-F7.3 =- F9.1, F9.2
- Protein synthesis = T7.1 = T9.1
- Uncoupling proteins = Not shown = F8.5

**Hormones:**
- Corticosterone = F4.2 = Box 4.1
- Leptin = F4.2 - Box 4.1
- Adiponectin = F4.2 - Box 4.1

Overview of the results from the ageing study in exercising and cold-exposed mice. We compare mice that have been selected for high wheel-running activity that were housed with a running wheel (S+) with their sedentary controls (S-). We do not include the comparison between C+ and S+ mice, because in this comparison, differences between the animals that have occurred during the selection process are a confounding factor. In addition, we compared cold-exposed mice to warm mice. + indicates an increase in S+/Cold mice compared to S-/warm mice, - indicates a decrease in S+/Cold mice compared to S-/warm mice, and = indicates no differences between the groups. The Table/Fig columns show in which tables and figures of the thesis the results are shown.
animals; removal of the parametrial fat pads (partial lipectomy) 2 weeks before UVB irradiation also enhanced UVB-induced apoptosis. The enhancement of apoptosis eventually resulted in a 30% lower incidence of tumours in the lean exercising mice. Fat cells may thus secrete substances that inhibit apoptosis in cells with DNA damage, and possibly also in tumours, thereby increasing the incidence of cancer (Lu et al., 2006). It is known in humans that the incidence of certain cancers increases in obese subjects (Bray and Bellanger, 2006).

The reduced fat content in cold-exposed, exercising and calorically restricted animals compared to their controls, may thus counteract the effects of their energy expenditure, and thereby result in a longer life span than expected based on predictions from the rate of living theory. Indeed all three factors have been shown to reduce tumour growth and the incidence of cancer: cold-exposure (Holloszy and Smith, 1986), exercise (Kritchevsky, 1990; Lu et al., 2006) and caloric restriction (Kritchevsky, 2001). Whether there is a direct link between fat content and tumour incidence under these conditions needs to be examined.

In summary

Between species there is a general pattern showing an inverse relationship between energy expenditure and longevity. We show that within a species (mice), manipulating energy expenditure by increased activity or decreasing ambient temperature, did not affect life span in the direction predicted, and the quantitative expectation of constancy of the Lifetime Energy Potential was not upheld. These results may refute the strict rate of living theory. Yet they are consistent with a causal influence of energetics on ageing, and they highlight the importance of understanding how different systems work together in ageing animals. ROS that are produced during normal aerobic metabolism are known to have an important role in ageing, because they can cause damage to DNA, lipids and proteins. There are different ways to prevent ROS damage to cellular macromolecules, i.e., by reducing ROS production (by reducing metabolic rate or increasing uncoupling), by reducing the amount that can cause damage (antioxidant enzymes), and by repairing damage that does occur (DNA repair, protein turnover). We showed large differences in daily energy expenditure between exercising (+14%) and cold-exposed mice (+50%), but we did not find strong evidence that differences in uncoupling, antioxidant enzyme activity or protein synthesis occurred. The observed differences in life span between the groups could thus not be explained by differences in these processes. Also, differences in hormone levels could not explain differences in longevity between the groups. Other factors involved in ageing, may have enabled the mice to expend large amounts of energy without a change in life span. One of these factors may be the reduced fat content in exercising and cold-exposed mice, since it seems to protect against developing tumours.
Ageing from an evolutionary perspective

In their natural environment, animals do not survive long enough to reach a very long life span due to extrinsic mortality (i.e., predation, starvation, disease). For this reason it is suboptimal to invest indefinitely in maintenance of the body and protection against ageing. There is a trade-off in the investment of resources between reproduction and survival. This is described by the disposable soma hypothesis (Kirkwood, 2002). The theory states that ageing results from the twin principles that (1) the force of natural selection declines with age, and (2) longevity requires investments in somatic maintenance and repair that must compete with investments in growth, reproduction and activities that enhance fitness. Animals with low extrinsic rates of mortality are thus expected to invest more in maintenance than animals with high extrinsic mortality rates (e.g., predation). During evolution, animals have developed various systems that can protect them against damage that occurs due to aerobic metabolism. The disposable soma hypothesis should predict that species will adjust their investment in these systems to the forces of natural selection, and this may lead to differences in life span.

Birds on average expend energy at a rate on average 1.5 times faster than a mammal of similar mass (Daan et al., 1991), but have yet greater longevity. Has the specific way of living of birds, possibly associated with reduced risks resulted in increased investment in maintenance? There is evidence that birds have evolved mitochondria that produce less ROS per ml oxygen consumed, are better protected against ROS and have lower oxidative damage (Barja et al., 1994; Herrero and Barja, 1998; Herrero and Barja, 1999). Comparing long- and short-lived mammalian species, the long-lived species also generally have lower levels of ROS production and higher protection against ROS (Barja, 1998; Perez-Campo et al., 1998; Barja, 2002b). During evolution differences in ROS production at a certain metabolic rate and in the capacity to defend against oxidative stress have thus emerged. Also within species, manipulations that increase life span like caloric restriction enhance maintenance processes (e.g., protein turnover, antioxidant enzymes, DNA repair).

Aging could thus be defined as the failure of maintenance and repair. Different maintenance mechanisms exist and most of them have been shown to decrease with age. They depend on many genes and a considerable investment of metabolic resources is necessary to keep up their activity. Individual theories of ageing revolve around the failure of given maintenance systems, and highlight different aspects of a complex process rather than being mutually exclusive explanations. Ageing does not result in a given cause of death; the system that fails first is largely a matter of chance. Ageing should be viewed as a multi-factorial process. No manipulation will affect only one factor of this process at the same time and this causes complications when testing a single theory such as the rate of living theory.

Most manipulations of energy expenditure usually lead to changes in body mass (which by itself affects life span) and in body composition. The expression of energy expenditure then requires a correction for body size. The best way to do this is
still under debate (Ramsey et al., 2000; Speakman, 2005a). Changes occur in numerous physiological parameters (e.g., hormone levels, fat content, antioxidants, protein turnover) that may affect life span. This makes the attribution of differences in life span to a single overall process such as energy expenditure nearly futile. Our studies reject the rate of living theory in its simplest quantitative form that states that the lifetime energy turnover per gram (whether body, lean or organ mass) remains constant when the instantaneous rate of turnover changes. They do not prove the absence of a negative effect of energy turnover on life span. In fact too many studies have demonstrated such an effect in one form or another.

Taking different consequences of energy turnover into consideration implies that the relationship between energy turnover and lifespan can not be a simple unidirectional process. In Figure 10.1 we conclude with a more realistic, if schematic proposition. Energy turnover increases deleterious effects (e.g. ROS) as proposed in the rate of living theory that will cause a decline in expected lifespan. Simultaneously, a decrease in energy turnover rate may also have deleterious effects on survival, for instance because it causes excess body fat, which is a health risk and will negatively affect life span (positive relationship). These two opposing processes then result in an optimal survival at a certain energy expenditure (striped line). See text for further explanation.

Figure 10.1. The relationship between energy expenditure and survival. The graph shows the presence of two separate processes that occur with changing energy expenditure and can affect life span. An increase in energy expenditure can have deleterious effects on survival, e.g. via the increased production of ROS, shown as a negative relationship. On the other hand, a decrease in energy expenditure may also have deleterious effects on survival, for instance because it causes excess body fat, which is a health risk and will negatively affect life span (positive relationship). Together these two opposing processes will generate an intermediate optimum as far as the maximization of life span is concerned (This needs not be the same energy expenditure that maximizes individual fitness, since fitness, i.e. the expected rate of gene propagation to the next generation, includes the additional component of reproductive output). On this basis of opposing processes we should not even expect to find evidence for the rate of living theory across the whole range of energy metabolic rates.