S | SUMMARY & SAMENVATTING

English summary

The research described in this thesis has investigated undernutrition during development and its acute and long-term effects on energy balance. This was studied by raising rats in litters that were manipulated to contain an increased number of pups: an animal model for early postnatal food restriction.

In the introductory **Chapter 1**, background information on the regulation of energy balance and developmental programming was presented. The concept of developmental programming entails that characteristics of the environment encountered during early development can permanently alter physiology in later life. This developmental plasticity does not only provide an explanation for the distribution of chronic diseases in human populations, but is primarily a developmental process that allows organisms to adjust their phenotype to the environment. It only turns into a harmful phenomenon if the environment changes substantially within the lifetime of the organism.

Body adiposity and obesity are among the features that are believed to be programmed by the early environment (and in an adverse way in humans with aberrant perinatal nutrition). Obesity results from prolonged periods of positive energy balance (intake of energy exceeds its expenditure), when excess body fat is stored. In the brain, an important part of the regulation of the intake and expenditure of energy occurs in the hypothalamus. The adipocyte hormone leptin plays important roles both in energy balance regulation and in the development of the hypothalamic circuitry. Therefore, research on developmental programming of energy balance has focussed on effects on three categories of parameters: body composition measurements, assessment of energy intake and -expenditure, and indicators of hypothalamic functioning. In both humans and rodent models, evidence has been reported for effects that persist long after the window of plasticity has closed. However, the direction and duration of these changes has been quite inconsistent between studies, emphasising the importance of consistent methodology and of combining measurements of different parameters within a group of subjects.

Chapter 2 concentrated on the aims and methods of the research conducted in this thesis. To investigate whether energy balance can be programmed by the early-life nutritional environment, we chose to induce early postnatal food restriction by raising rats in litters of 20 pups. Rats raised in litters of 10 pups (which is a common litter size in rats) served as controls. After weaning on day 25, all animals were allowed unlimited access to food and water. To investigate programming effects, representatives from each of the three categories (body dimensions, energy balance, and hypothalamic regulation) were measured in food-restricted (FR) and control rats at different ages. The methods used to perform these

measurements were explained in this chapter, as well as our motives to choose the FR rat as a model for developmental programming. The next four chapters focussed on the experiments and their outcomes. Here, these are arranged by the type of measurements in the studies.

Body dimensions and growth patterns were described in **Chapter 3**. These provide information on the effects of early postnatal food restriction on body composition; and hence indirect information about the effects on energy balance status. In addition, they describe the general phenotype of rats subjected to this model.

Immediately after the pups were rearranged in control litters and large FR litters, the growth rate of all body dimensions dropped dramatically in the large-litter pups. This resulted in significantly reduced body weight, body-, tail-, and total length, and body mass index (BMI). Body weight was most heavily affected: on day 21 of life, FR body weight was only 65% of that in control rats. After weaning, FR rats showed a period of compensatory growth, which lasted until around day 70. At that point, FR body weight had reached almost 90% of control values, and then remained stable until the end of the experiment at the age of one year. The other body dimensions stabilised at 95% to 97% of control values, but all remained significantly lower than in control rats.

Serum concentrations of leptin were measured at four different ages throughout life. At all time-points, its levels were significantly reduced in FR rats. On day 10, about a week after the pups were allocated to a control or FR litter, mean leptin levels in the FR pups were only 20% of those in control pups. By the time of weaning (day 25), this had increased to over 60% of control levels. Until adulthood, leptin levels further recovered to about 80%, and then stabilised. Leptin gives an indirect indication of the animals' fat mass. BMI, which represents body weight irrespective of length, is also commonly used as a proxy for body composition. In the present study, the persistent effects on both parameters suggest that early postnatal food restriction 'programmes' rats for a lean and thin phenotype.

The effects of early postnatal food restriction on energy balance were also investigated more directly by measurements of energy intake and energy expenditure. Energy intake was determined from daily (24-hour) food intake, whereas indirect calorimetry was used to measure daytime resting energy expenditure.

In **Chapter 3**, chow intake per cage was measured regularly throughout life: from weaning until the end of the experiment at one year. In both male and female FR rats, total energy intake over this period was significantly lower than in control rats. Because energy requirements vary with size, energy intake was also analysed after adjustment for body size. When corrected for body weight, intake in newly weaned male FR rats was actually higher than in their controls. The difference was not significant for females and in older males. These results suggest that although the absolute amount of food consumed may be smaller in FR rats, it is mostly appropriate for their body size. The elevated intake in FR males just

after weaning may have facilitated catch-up growth. Because catch-up growth also occurs in females and lasts much longer than this elevated relative food intake, FR rats must also have another means to increase growth. This is illustrated by the fact that this compensatory growth (between the time of weaning and around the age of 10 weeks) was accompanied by an increase in the efficiency with which the FR rats converted food into growth. When a larger proportion of the available energy is invested in growth, it seems likely that proportionally less energy will be available to invest in other processes (e.g. reproduction, activity), which may be affected as a result.

Individual food intake of male rats was determined at two time-points in adult life in **Chapter 4**. Similar to the per-cage measurements, energy intake was significantly lower in FR animals. This difference persisted after adjustment for body composition (BMI or estimated fat-free mass). Because lean body mass is generally considered to be a more suitable parameter for adjustment of energy requirements (rather than crude body weight as was used in Chapter 3), these results suggest that energy intake in adult FR rats is actually programmed to be lower than could be expected from their body size.

Chapter 4 also describes individual resting energy expenditure in the same adult male rats. At both ages, this was significantly lower in FR rats than in control rats. However, the differences between the groups disappeared after adjustment for body composition (BMI or estimated fat-free mass). This suggests that adult size was a more important factor than neonatal nutrition; and therefore, unlike energy intake, resting energy expenditure does not seem to have undergone developmental programming in these rats. The difference between energy intake and resting energy expenditure, or the energy available for activity, was not affected by adult body composition and was significantly reduced in FR rats.

The data so far have shown that both body dimensions and energy balance were persistently affected by raising rats in large litters. Therefore, the hypothalamic regulation of energy balance was hypothesised to be altered in these FR rats.

Two main types of neuropeptides are involved in this regulation: orexigenic and anorexigenic peptides. The former type of neuropeptides stimulates feeding and promotes energy storage, whereas the latter type inhibits feeding and leads to a more negative energy balance. Within the hypothalamus, the arcuate nucleus (ARC) produces the orexigenic neuropeptide Y (NPY) and agouti-related protein (AgRP), as well as the anorexigenic peptides α-melanocyte-stimulating hormone (α-MSH) and cocaine- and amphetamine-regulated transcript (CART). Two other nuclei, the paraventricular nucleus (PVN) and the lateral hypothalamic area (LHA), produce anorexigenic and orexigenic peptides, respectively, with corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH) expressed in the former and melanin-concentrating hormone (MCH) and orexin (ORX) in the latter. In **Chapter 5**, quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to measure mRNA levels of NPY, AgRP, pro-opiomelanocortin (POMC, the precursor for α-MSH), and CART in the ARC; CRH and TRH in the PVN; and MCH and ORX in the LHA of

neonatal, weanling, and middle-aged male and female control and FR rats. **Chapter 6** investigated NPY and POMC expression levels using *in situ* hybridisation in the ARC of young adult male control and FR rats.

In the ARC, CART expression was significantly decreased in FR rats of all three ages tested by qRT-PCR (neonatal rats on day 10, weanling rats on day 25, and middle-aged rats around day 380). In middle-aged FR rats, the other anorexigenic ARC peptide, POMC, also showed reduced expression. The orexigenic ARC peptides, NPY and AgRP, did not differ between control and FR rats at any of the three ages. In young adult male rats (sacrificed around day 77), NPY expression was significantly higher in the FR group, with no difference in POMC expression. Of the four peptides that were tested in the PVN and LHA on days 10, 25 and, 380, MCH expression was significantly increased in neonatal FR rats.

The ratio of orexigenic to anorexigenic expression in the ARC (i.e. NPY {and AgRP[*]} *vs.* POMC {and CART[*]}) was transiently elevated in FR rats: it was significantly higher on days 10 and 25 and around day 77, but in middle-aged FR rats, the balance between orexigenic and anorexigenic expression had normalised.

In the last chapter, **Chapter 7**, the results of the preceding chapters were combined and discussed. Because all measurements were performed in animals that originated from the same experiment (and hence had experienced very similar circumstances), the results for the three categories of measurement (body dimensions, energy balance, hypothalamic neuropeptides) can easily be related to each other. The overall phenotype that emerged from these studies was remarkably similar between males and females. FR rats:

1) were persistently lighter, shorter, and leaner than control rats;
2a) had lower energy intake throughout life,
2b) with adult resting energy expenditure that was appropriate for their smaller body size,
2c) leaving less energy for activity;
3a) had an increased ratio of orexigenic to anorexigenic gene expression in juvenile life,
3b) which normalised in adult life (between day 77 and day 380).

In brief, all three categories were persistently affected, until long after the original stimulus (i.e. food restriction until weaning) was removed. Since developmental programming requires the occurrence of long-lasting changes in response to a transient stimulus during a period of plasticity, it can be concluded from these results that raising rats in large litters has actually induced programming of energy balance.

In the remainder of the chapter, I speculated about the implications of these results; about possible mechanisms for programming, the relevance of our results for the human situation, and about experiments that might further clarify the phenotype of FR rats. One aim for future investigations would be to search for more permanent changes in the

[*] AgRP and CART: included on days 10, 25, and 380 (qRT-PCR in Chapter 5); not available on day 77 (*in situ* hybridisation in Chapter 6).

hypothalamus and other parts of the system that regulates energy balance (besides those in gene expression of POMC and CART in the ARC), that could explain the lean phenotype of adult FR rats in view of the normalised ratio of orexigenic to anorexigenic gene expression in the ARC. An important candidate to be investigated is the leptin sensitivity of FR rats.

With this study, we have shown that early nutritional manipulations in rats can have permanent effects on energy balance and homeostatic energy balance regulation. Extrapolating these results to the human situation, it seems likely that a (temporarily) increased orexigenic drive may offer at least a partial explanation for the increased risk of obesity in perinatally malnourished humans. For the future, this animal model offers ample possibilities to further investigate the underlying mechanisms of developmental programming of energy balance.