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Fasting in rodents

Clarification of terms

This document is primarily concerned with the effects of sudden fasting *(acute food deprivation)* on laboratory rodents that are accustomed to the continual presence of food. The document does not address the consequences of the removal of drinking water *(water deprivation),* nor the effects of rationing the feed *(food restriction)*.

An animal satisfies its needs for food and water, not just because it experiences an acute feeling of thirst or hunger, but also because it has learned to realise its needs and knows how to meet them within the constrictions of the environment in which it lives. If the absence of food or water is not dramatic or longlasting, the temporary effects this has on the animal's metabolism or hydration status will have little effect on the animal's welfare. The critical factor is whether the animal learns to change its behaviour to adapt to the availability of food and water over time (Toth & Gardiner, 2000). This situation is common in the wild and also exists in laboratory experiments when food is rationed. These adjustments are often well tolerated as long as they are within limits to which the animal can adjust. A totally different situation arises if the animal is subjected to sudden, unexpected and chronic food deprivation.

Interpretation of borderline cases

There are many examples of unclear boundaries between procedures that are defined as animal experiments and those that fall outside the definition. Researchers and departmental heads alike wish for more detailed guidelines, or examples of procedures that fall into one or the other category. The situation becomes complicated when decisions are taken at several levels, especially if the parties concerned have different opinions. Their subjective opinions, which are coloured by personal experience (not necessarily in the same species or procedure), will also influence the decision-making. In addition, well-known psychological interactions between the parties will play a significant role: the people involved in the decisions can have different agendas, or may hesitate to challenge others, particularly those who are assumed to have a high degree of competence, rank or longer experience.

Legal issues

Norwegian Animal Protection Act

Paragraph 21 of the Norwegian Animal Protection Act states that animal experiments must be carried out in such a manner that the animal is not exposed to the risk of suffering more than is strictly necessary for the purpose.

Norwegian Animal Welfare Act

Norway's new Animal Welfare Act, which will replace the Animal Protection Act on January 1st 2010, gives clear guidelines on how animals are to be treated when they are used for research or teaching purposes (§13):

Permission cannot be given if the aim can be achieved without the use of animals, or if the animals are in danger of being subjected to unnecessary stress. As few animals as necessary shall be used, and the animals shall be subjected to as little stress as possible. (Norecopa's translation)

Norwegian Regulation on Experiments with animals

§2 in this Regulation offers dispensation from the definition of an animal experiment for, among other things, experiments within breeding, nutrition and environmental needs if there

is no cause to believe that the experiment will produce an unphysiological state in the animal. In cases of doubt, the Norwegian Animal Research Authority is to make the decision.
In other words, a decision on whether to fast an animal must be based upon:

- current knowledge on what affects the animal's physiology and behaviour (i.e. to be able to have 'cause to believe')
- an assessment of whether the animal is still within normal physiological limits
- whether or not the local competent person ('*ansvarshavende*') is in doubt or not

Physiology is the study of the function of living organisms. An 'unphysiological state' is therefore a condition in which this function is abnormal. However, the body continually makes innumerable large and small adjustments to internal and external changes in its environment, to offset changes that threaten to create an abnormal state. Many of these adjustments are easily visible and relatively dramatic, but still well within what may be defined as 'normal'. The regulation of cellular nutrition during and between meals is an example of these adaptations. As long as the animal is given sufficient food as often as it is accustomed to, these changes are physiological. If, however, the supply of food deteriorates significantly (in quality or quantity), the body will no longer be able to regulate its functions within normal limits, and pathological (non-physiological) conditions will arise.

The interpretation of legal phrases in the Regulation such as 'slight pain or discomfort of a highly temporary nature' and 'the animal's normal way of life' can also be difficult. An animal will notice changes in its supply of food rapidly, and this may lead to psychological stress. The degree of stress, in relation to fasting, will depend upon the species (how often they eat, when they eat during the 24 hour period and whether periods of fasting are a normal part of the animal's life history) and also, for animals in captivity, the individual's past experience (when and how often it is fed).

Rats consume about 80% of their daily food intake at night (Rowland, 2007; Strubbe *et al.*,1986), with peaks around 8 p.m. and 6 a.m. (Vermeulen *et al.*, 1997). Most laboratory rodents are have a continual supply of food. Rodents adjust their behaviour and thus their metabolic activity to predictable rhythms in their surroundings, among other things to changes in light intensity and food supply. The sudden removal of this predictability, particularly when it affects factors related to animals' basal needs, can be expected to be particularly distressing, and it has been demonstrated that unforeseen environmental changes can lead to an increase in cortisone release in a number of vertebrates (Wingfield & Kitaysky, 2002).

Many of the signs of developing stress and 'non-physiological states' will only be observed by people with a close relationship to the animal. Procedures such as fasting should therefore be discussed in a forum where both researchers, animal caretakers and the head of the animal facility are present.

Tradition or science?

Animal experiments are often built on the results of earlier studies, and procedures are often imported from these. These procedures are not necessarily optimal (such as the widespread use of albino animals, which are not good models of pigmented species). Acceptance of these suboptimal techniques can hinder the establishment of alternative models, even if they clearly are better, because results must be able to be compared with previous studies. In this way, suboptimal conditions may be perpetuated, "the unacceptable has become the accepted".

In some cases it may be claimed that there are good scientific reasons to fast the animal. Some (but not all) pathologists claim, for example, that histological studies of the liver are easier after fasting overnight (the hepatocytes contain less glycogen). Most laboratories conducting trials involving pathological assessment of the animals fast animals overnight before autopsy, to make it easier to observe toxicological effects (personal communications). Reactions in isolated cells or tissue will also be influenced by whether the animal was fasted or not before it was killed, and some studies (e.g. on the effects of insulin) necessitate fasting. Food deprivation overnight is widely used in pharmacological research, to empty the stomach before oral gavage. This also increases the standardisation of studies on bioavailability and absorption. In cases where large volumes of fluid are to be administered orally, prior emptying of the stomach can be essential.

In other cases, however, it can appear as if the period of fasting has been influenced more by routines (e.g. working hours), as food is often removed at the end of the day. In a Norwegian facility, where animal technicians often finish work at 3.30 p.m. and do not resume work until 8 a.m. the following day, the period of fodd deprivation can easily be 17 hours, unless special arrangements (involving overtime or automated feeding) are made.

One must be prepared for very different opinions when discussing the necessity of food deprivation.

The effects of fasting

In contrast to the intake of fluid, which is a response to cellular dehydration and hypovolemia (Toth & Gardiner, 2000), our knowledge of why animals feel hunger is still poor. The size of the last meal is probably more important than the length of time since that meal, which suggests that calorie intake is the most important factor (Friedman & Stricker, 1976). A feeling of satiation is probably also important for animals, as it is for humans – this feeling is in turn influenced by a range of factors, not just the degree of distention of the stomach. The loss of the feeling of satiation is probably more important for the re-initiation of feeding than a feeling of hunger.

The lack of knowledge about the factors controlling feeding behaviour makes it difficult to evaluate the effects of fasting, which is again complicated by the fact that even adult animals often exhibit marked variations in body weight and feed intake. For example, even genetically identical mice (B6C8F1) can vary in weight from 30 to 48 g (Allaben *et al.*, 1996). Feed intake and calorie demands can in addition vary between genetic lines, agegroups, environments (effects of, among other things, room temperature), physiological status and the opportunity to undertake physical exercise. Animals will also often overeat when large amounts of food are continually available, especially if there is little else to do. The body has better mechanisms for avoiding overhydration than it does for limiting overeating.

The effects of fasting on emptying of the digestive tract and body weight

If researchers consider it necessary to fast their animals in order to empty portions of the gastro-intestinal tract, it is important to collect information on how long it is necessary to fast the animal to achieve their goal. In this way, the period of fasting can be reduced to that which is totally necessary for the study, and thereby prevent unnecessary stress and suffering. As mentioned earlier, a certain degreee of fasting may be necessary in some situations to avoid overfylling the stomach in cases of oral gavage.

Vermeulen *et al.* (1997) demonstrated that the stomach of male rats (200-250g) is empty as soon as 6 hours after the initiation of fasting. There was no significant difference in intestinal emptying between animals fasted for 6 or 18 hours.

Large changes in body weight have been reported following fasting: Vermeulen *et al.* (1997) recorded a weight reduction of approx. 10% after 18 hours food deprivation. Others have reported weight loss of up to 48g in male rats that weighed 264g, which is over 18% of their bodyweight (Dohm *et al.*, 1983). Claassen (1994) cites studies where weight loss in rats varied between 3,3% and 18% after 24 hours, but it was not possible in all cases to determine whether the weight loss was also due to water deprivation.

The reduction in liver weight during a fast can be relatively larger than tthe reduction in body weight, and the liver's ontent of free fatty acids increases markedly (Claassen, 1994).

It is also important to be aware that fasting can lead to reduced basal metabolic rate, which can persist after food has been re-introduced, while body weight is returning to a normal range (Penicaud & Le Magnen, 1980; Bjørntorp & Yang, 1982).

Whether or not an animal loses weight during food deprivation will also depend upon the presence or absence of bedding or nesting material, since a hungry animal may well eat these. If body weight is to be used in an experiment as a health indicator or humane endpoint, it is important to consider this factor. This also means that, in order to achieve complete emptying of the gastro-intestinal tract, the animals must be housed in cages without such material. In addition, they must be equipped with neck collars or similar devices to prevent coprophagy (uptake of caecotrophs, the partially digested products of fermentation in the appendix). Both of these procedures are likely to be stressing to the animals.

The effects of fasting on pharmacological parameters and behaviour

Claassen (1994) cites many studies that demonstrate changes in pharmacokinetics as a consequence of fasting. He has written a comprehensive textbook describing the factors that influence animals used in pharmacological and neurophysiological research. He concludes (summary, chapter 13):

Fasting causes severe changes in the physiological and biochemical processes of the animal which become more serious with longer duration of food withdrawal. However, also the often used fasting periods of 16-24 hours bring about important changes which may significantly affect the responsiveness towards experimental stimuli. Fasting effects may vary to a great extent between strains and individuals. The use of fasting animals in experiments seems therefore only to be permitted when the feeding condition functions as a factor in experimental design.

Fasting can be expected to cause, among other things, stress, aggressive behaviour (which may in turn lead to the need for individual housing, which is stressfull for many rodents), as well as reductions in body weight, body temperature and plasma glucose levels.

A reduction in basal metabolic rate, which persists after the period of fasting, has been reported (Penicaud & Le Magnen, 1980). The effect of food deprivation is greatest when it occurs in the dark phase, when the animals are most active – significant reductions in liver weight and glycogen content, as well as increases in levels of glycerol, free fatty acids and acetoacetate have been measured after 3 hours (Palou *et al.*, 1981). Claassen (1994) cites a number of studies that show increases in plasma levels of glucose, urea, lactate and amino acids, a range of hormones including insulin, glucagon and corticosterone, as well as increased activity in the sympathetic nervous system.

Vermeulen *et al.* (1997) investigated activity patterns in rats during a period of fasting by filming the animals and by placing them in cages mounted on platforms that registered movement (vibrations). Animals that were fasted for 18 hours showed increased locomotory behaviour and an increase in the time spent grooming, which also resulted in the accumulation of significant amounts of hair in the stomach. Shorter periods of deprivation resulted in smaller changes in the animals' physiology and behaviour.

Toth & Gardiner (2000) cite several publications that demonstrate that activation of the sympathetic nervous system and moderate increases in plasma glucocorticoids can be measured in rats fasted for 24 hours, but these changes are less than those observed when animals are stressed with other stimuli. These changes can therefore be interpreted as normal adaptive responses to reduced food supply. After 48 hours of food deprivation, glucocorticoid levels increase dramatically and the animal's natural diurnal rhythm is obliterated. This suggests that periods of fasting that last longer than 24 hours are associated with increasing metabolic stress and perhaps worsening of psychological stress (Toth & Gardiner, 2000). These studies are, however, of less interest in the current discussion, since food deprivation is commonly practised for a maximum of 24 hours. The question remains as to whether animals simply experience increased focus on food when confronted with an abrupt and unforeseen period of food deprivation, or whether they enter a 'non-physiological state' and are seriously affected by it.

The effect of a short period of fasting will also be influenced by whether it occurs in the dark phase (when rodents eat most) or not. Claassen (1994) quotes studies showing that many plasma values fall, including glucose, urea, lactate and amino acids, while glycerol and free fatty acids increase, and that rats killed at the beginning of the light phase have significantly lower liver weights and liver glycogen levels after only 3 hours of fasting. These changes will affect internal cellular biochemistry and one should therefore expect differences in the effects of preparations on isolated cells, tissue or organs removed from animals that have, or have not, been fasted.

Welfare indicators and fasting

Since it is relatively difficult to detect signs of restlessness and stress in animals during the early stages of a fasting period, most published experimental studies have employed relatively long deprivation periods to provoke negative welfare indicators such as gastric ulcers and other pathological changes. Albee *et al.* (1987) demonstrated, however, that rats fed 15% or 50% less food than the previous day, had significant changes in a range of neurophysiological parameters, and that they were more excitable when handled.

It should be stressed that there are probably large differences between how animals experience unexpected food deprivation and how they react when exposed to shorter or regular periods of fasting to which they have gradually becom accustomed through training.

Claassen (1994) makes the point that fasting can be expected to influence metabolic status greatly in animals that eat relatively large amounts of food during the day, and that the processes that are initiated to accustom the animal to the new situation will in all probability include both hormonal and nervous activity.

Guidelines on fasting

There are a number of existing guidelines for food deprivation, although not all specify whether or not the animal is given access to bedding material.

1. The British authorities (Home Office, 2003) demand a clear rationale for studies involving a weight loss of more than 15%, and such studies must be sought approval for. As long as no other factors are altered, approval does not have to be sought for studies in mice, hamsters and rats under 100g involving deprivation of 16 hours or less, and likewise for periods of 24 hours or less for rabbits and rats weighing over 100g. These guidelines state that fasting should be avoided at all in guinea-pigs, ferrets and shrews.
2. The American Animal and Plant Health Inspection Service (APHIS, 1997) has published policy documents on, among things, procedures that can be painful. APHIS state that the deprivation of food or water beyond that which is necessary for normal surgical preparations can be examples of procedures that cause more than transient or mild stress.
3. In their internal guidelines, many American laboratory animal facilities cite a bibliography produced by the Animal Welfare Information Center (AWIC, 2005). These guidelines expect researchers to apply for permission for studies where a weight loss of more than 15% is expected.
4. The IACUC Handbook (Silverman *et al.*, 2006) has similar guidelines and suggests that rats are given half of their calculated daily ration in the afternoon, so they have eaten by around 3 a.m. The handbook points out that the intestinal tract will not be empty, due to coprophagy.

5. OECD Guidelines for toxicity testing (Acute Oral Toxicity, www.oecd.org/dataoecd/17/51/1948378.pdf) contain the following detailed suggestions:
   Animals should be fasted prior to dosing (e.g., with the rat, food but not water should be withheld overnight; with the mouse, food but not water should be withheld for 3-4 hours). Following the period of fasting, the animals should be weighed and the test substance administered. The fasted body weight of each animal is determined and the dose is calculated according to the body weight. After the substance has been administered, food may be withheld for a further 3-4 hours in rats or 1-2 hours in mice. Where a dose is administered in fractions over a period of time, it may be necessary to provide the animals with food and water depending on the length of the period.
   It should be emphasised that this text uses the words 'should' and 'may', instead of issuing absolute demands.

Matsuzawa & Sakazume (1994) point out that many guidelines for regulatory toxicity testing demand food deprivation overnight for both rodents and non-rodents before bloodsampling. They document the effects of 16 hours' fasting on a range of blood parameters. The authors conclude that the need for food deprivation should be assessed in each case, and that it should no longer be a standard requirement in toxicity testing.

Alternatives to fasting

This document has mentioned several refinements that can be implemented to reduce or avoid a period of food deprivation. Pilot studies should also be carried out, to determine whether or not fasting is essential.

Prior *et al.* (2009) describe a refinement of the Charcoal Meal Test, where rodents are routinely fasted overnight (18-24 timer), before being dosed with ny candidate drugs against diarrhoea or constipation, and then given activated charcoal orally. The animals are killed 15 minutes later for measurements of the degree of emptying of the stomach and intestinal movement. The authors fasted the animals for either 0, 3, 6 or 18 hours before dosage with a control substance (vann) or a known antagonist of intestinal movement (atropine), followed by a dose of activated charcoal one hour later. The results from the animals fasted for 6 hours were comparable with those from the animals fasted overnight, except that the animals exhibited less weight loss and aggression, enabling them to be housed in groups.

Levine & Saltzman (1998) report favourable results of using sugar solutions in the period of food deprivation, to reduce the feeling of hunger.

The IACUC Handbook (Silverman *et al.*, 2006) cites studies on rats where half of the daily ration was given in the afternoon, so that it had been consumed by around 3 a.m, resulting in a shorter period of fasting before the animals were used the next day. The handbook points out that the intestinal tract will not be totally empty due to coprophagy.

Norecopa's recommendations

1. It is unrealistic to assume that one standard can be made for all cases where fasting is required, but the physiological and behavioural consequences of fasting in the species and age griup in question should always be assessed.
2. In all cases where fasting is proposed, for example prior to oral gavage, the real need for fasting, the degree and length of the deprivation period and the potential consequences for the animals should be investigated thoroughly, if necessary by means of pilot studies if the literature is scarce. This assessment should be made by persons who know the animal species (and preferably the individuals themselves) well.
3. Sudden unexpected food deprivation should be avoided. Gradual adaptation to periods with limited or no access to feed are probably tolerated much better.
4. The deprivation period should if possible be during the daytime, when rodents normally eat less food. Adequate emptying of the gastrointestinal tract may well be achieved after 6-8 hours' fasting, enabling the experiment to start during the afternoon or evening the same day, avoiding the need for overnight fasting.
5. Food deprivation of 15 hours or more should be considered as an animal experiment and permission should be applied for in the normal manner. *This should also apply to periods of food deprivation where there is a real danger that the animals may be fasted for 15 hours or longer, for example in situations where unexpected delays may occur.* Notwithstanding this guideline, the local competent person (animal welfare officer) should still have the authority to decide whether or not a period of food deprivation falls within the definition of an animal experiment or within the bounds of an 'unnecessary burden' on the animals.
6. All food deprivation (regardless of length) should be considered to be an exception rather than the rule, and weighty scientific arguments should be present before it is allowed. Arguments based on tradition or convenience for the personnel, to avoid working out of normal hours, are inadequate.
7. The final decision should be made in cooperation with the animal technicians, researchers, attending veterinarian and the local ethical committee.
8. Conflicting interests between study design and animal welfare should be discussed. It should always be clear who has the final word if consensus cannot be reached. Under the Norwegian system, this should be the local competent person (*ansvarshavende*) or, if necessary, the Norwegian Animal Research Authority (*Forsøksdyrutvalget*). The animals should be given the benefit of any doubt arising in these discussions.
9. Some source of energy (for example, a sugar solution) should be given to the animals wherever possible in the food deprivation period, to satisfy the animals' energy needs.
10. Adequate care and observation of the animals should be undertaken. Studies involving food deprivation should be carried out in periods with adequate staffing (technical and scientific), to enable fastest possible reactions in cases where the animals' behaviour is unexpected.
11. Welfare indicators (e.g. weight loss, glycogen reserves in hepatic cells), preferably using continuous data, should be identified and used actively to follow the effects of food deprivation on the animals.

12. One or more humane endpoints must be established and used actively during the experiment, especially if the animal approaches the limits for these parameters that were set before the start of the experiment.

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