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**Canine hip dysplasia in a prospective cohort study - Incidence, risk factors and long-term effects in four large breeds**



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Norwegian School of Veterinary Science

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**Errata list – Canine hip dysplasia in a prospective cohort study – Incidence, risk factors and long-term effects in four large breeds**

Page 41, line 12: All breeders of these four breeds...

Page 43, line 13: .....presented in Figure 2.

Page 64, Figure 4: Y-axis title is “Survival”

Page 65, Figure 5: Y-axis title is “Survival”

Page 67, Figure 6: Y-axis title is “Survival from clinical signs”

**Canine hip dysplasia in a prospective cohort study**

- **Incidence, risk factors and long-term effects in four large breeds**

Randi Ingebjørg Krontveit

Thesis for degree of Philosophiae Doctor (PhD)

Norwegian School of Veterinary Science

Department of Companion Animal Clinical Sciences

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Til Trask og Turbo og alle andre firbente venner

.....

Kom da min kjære kamerat,

jeg dele vil mitt brød.

Min hånd skal tjene deg til fat,

min venn i liv og død!

Så lærer snille Henrik og klapper hunden sin,

jeg vil og gjøre slik som han og stelle godt med min.

Henrik Wergeland

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## **List of abbreviations**

ADG – average daily weight gain

BW – body weight

CHD – canine hip dysplasia

DDH – developmental dysplasia of the hip, humans

DJD – degenerative joint disease

DKK – The Dansk Kennel Club (the Danish Club for dog owners)

FCI – The Fédération Cynologique Internationale

HR – hazard ratio

ICC – intraclass correlation coefficient

IW – Irish Wolfhound

LEO – Leonberger

LR – Labrador Retriever

NF – Newfoundland

NKK – The Norwegian Kennel Club

OR – odds ratio

SKK – The Swedish Kennel Club

TVE – time varying effect

## Summary

The present study is based upon material from a cohort study of four large dog breeds in Norway, aimed at investigating skeletal lesions in growing dogs (the *main study*). The included breeds were Newfoundland (NF), Labrador Retriever (LR), Leonberger (LEO), and Irish Wolfhound (IW).

The main objectives of this thesis were to estimate the incidence risk of radiological canine hip dysplasia (CHD) in these four breeds and to study environmental factors associated with the risk of a radiological CHD diagnosis, as well as to study the effect of the CHD diagnosis and environmental factors on time to death and time to hip related clinical signs.

Among the dogs included in the *main study*, 501 dogs from 103 litters were radiographically screened for CHD at the ages 12 (LR, IW) or 18 (NF, LEO) months and subsequently included in the present study. The breed distribution was 125 NF, 133 LR, 180 LEO, and 63 IW. The dogs were privately owned and had their feeding and environment decided by their breeder and subsequently their owner. The dogs were followed from birth and until 10 years of age. The breeder, owner, and veterinarian recorded information about bodyweight (BW), environmental conditions, and health status for each dog at regular intervals.

The incidence risk of radiological CHD was 36% in NF, 20% in LR, 25% in LEO, and 10% in IW. In LEO and IW, the estimated incidence risks were higher than previously assumed. Growth patterns were described by the Gompertz function. According to the variables from this function, NF was the slowest growing breed whereas LR was the fastest growing breed. Growth in IW was faster than LEO. Only minor differences in growth rate were found between dogs affected and unaffected by CHD within breed. Generalized linear mixed models with random effects for litter were used to evaluate factors associated with

radiological CHD. Higher BW at three months of age, season of birth being spring or summer, breeder house type being farm/small farm, and off-leash exercise in park terrain at three months of age decreased the risk of a radiological CHD diagnosis. Season of birth being fall or winter, breeder house type being single family house, and daily use of stairs at three months of age increased the risk of CHD. The clustering at litter level was high and statistically significant.

Kaplan-Meier curves were used to describe differences in time to death or time to hip related clinical signs. Cox proportional hazards models with a shared frailty for litter were applied to measure the effects of environmental factors on time to death or time to hip related clinical signs until 10 or nine years of age, respectively. IW had the shortest mean time to death and the effect of belonging to this breed increased linearly over time. Severe radiological CHD grade increased the hazard of dying, but the effect on the hazard of dying decreased logarithmically with time and disappeared by eight years. Increasing severity of radiological CHD grade increased the hazard of hip related clinical signs while both off- and on-leash exercise at 12 months of age reduced the hazard. The time to clinical signs varied over time for the breeds. Litter level clustering was high and statistically significant in the latter study, but for time to death the clustering was low and non-significant.

In conclusion, incidence risks and the growth patterns differed between the breeds, but only minor differences were observed in BW or growth between dogs affected or unaffected by CHD. Several environmental factors were found to affect the risk of a CHD diagnosis, time to death, and time to hip related clinical signs. The time window until three months of age appeared important regarding the risk of radiological CHD, while the age of 12 months appeared important regarding the time to when hip related clinical signs were reported. Preventive efforts can be focused on the identified time-periods.

## Sammendrag (Summary in Norwegian)

Denne studien er basert på et materiale fra en kohort studie av fire store hunderaser i Norge (kalt *hovedstudien*). *Hovedstudiens* mål var å undersøke skjelettlidelser hos hunder i vekst. Hunder fra rasene newfoundlandshund (NF), labrador retriever (LR), leonberger (LEO) og irsk ulvehund (IW) ble inkludert i studien.

Hovedmålene for denne avhandlingen var å regne ut insidens risikoen for radiologisk diagnostisert hoftedeledsdysplasi (CHD) hos de fire rasene, og å undersøke hvilke miljøfaktorer som påvirket risikoen for å få en slik diagnose. Et annet mål var å undersøke om CHD diagnosen og miljøfaktorer påvirket livslengden og forekomst av hofterelaterte kliniske symptomer senere i livet.

Blant *hovedstudiens* hunder, ble 501 hunder fra 103 kull røntgenologisk undersøkt for CHD ved 12 (LR, IW) eller 18 (NF, LEO) måneders alder, og disse hundene ble inkludert i denne studien. Rasefordelingen var 125 NF, 133 LR, 180 LEO og 63 IW. Hundene var privateide, og oppdretteren og senere eieren bestemte fôring og andre miljøforhold for hunden sin. Hundene ble fulgt fra fødsel og til 10 års alder. Oppdretterne, eierne og veterinærene registrerte mange faktorer, blant andre kroppsvekt (BW), miljøfaktorer og helsetilstand for hver hund med jevne mellomrom.

Insidens risikoen for CHD var 36 % hos NF, 20 % hos LR, 25 % hos LEO og 10 % hos IW. Hos både LEO og IW var insidensen høyere enn tidligere antatt. Gompertz funksjon ble brukt til å beskrive vekstmønstrene, og bedømt ut fra Gompertz funksjonens variabler, var NF den rasen som vokste saktest mens LR vokste raskest. IW vokste raskere enn LEO. Det var bare små forskjeller i BW og veksthastighet mellom hunder med og uten radiologisk CHD. Logistisk regresjon med kull som systematisk tilfeldig effekt ble anvendt for å studere uavhengige faktorerers virkning på den avhengige variabelen radiologisk CHD. Høyere BW



ved tre måneders alder reduserte risikoen for CHD. Andre faktorer som reduserte risikoen for CHD var hvis oppdretter bodde på gård/småbruk, hvis kullet var født på våren eller sommeren og hvis valpen fikk løpe løs i parkområder ved tre måneders alder. Hvis valpen var født på høsten eller vinteren, hvis oppdretter bodde i enebolig/rekkehus og hvis valpen brukte trapper daglig ved tre måneders alder, var risikoen for CHD økt. Kullvariansen var høy og statistisk signifikant.

Kaplan-Meier kurver ble brukt for å beskrive overlevelsestiden og tiden til tidspunktet når symptomer relatert til hoftene ble registrert første gang. For å måle hvilken effekt miljøfaktorer hadde på overlevelsestid eller når hofterelaterte symptomer ble observert, ble det anvendt Cox regresjon med kull som systematisk tilfeldig effekt. IW hadde den korteste livslengden, og effekten av å være IW økte lineært med tiden. Sterk grad av CHD ga svært høy risiko for død, men denne effekten sank logaritmisk over tid, og ved åtte års alder var effekten nesten borte. Økende alvorlighetsgrad av CHD økte også risikoen for hofterelaterte symptomer, mens lufting både i og uten bånd ved 12 måneders alder reduserte risikoen. Antall måneder til symptomer opptrådte varierte over tid for rasene (en tids-varierende effekt). Kullvariansen var høy og statistisk signifikant i den sistnevnte analysen, mens i beregningen av overlevelsestiden var kullvariansen lav og ikke signifikant.

Insidens risikoen for CHD og vekstmønstrene varierte mellom rasene, men det var bare små forskjeller mellom hunder med og uten CHD. Flere miljøfaktorer hadde innvirkning på risikoen for en CHD diagnose, på overlevelse og på tid til hofterelaterte symptomer. Tidsperioden frem til tre måneders alder var av betydning for risikoen for å få en radiologisk CHD diagnose. Når det gjelder tiden til symptomer ble registrert, var faktorer ved 12 måneders alder av betydning. Forebyggende tiltak kan fokuseres på de tidsperiodene.

## List of papers

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

### Paper I

Krontveit R. I., Nødtvedt A., Sævik B. K., Ropstad E., Skogmo H. K., Trangerud C. (2010). A prospective study on Canine Hip Dysplasia and growth in a cohort of four large breeds in Norway (1998-2001). *Preventive Veterinary Medicine*, 97, 252-263.

### Paper II

Krontveit R. I., Nødtvedt A., Sævik B. K., Ropstad E., Trangerud C. (2011). A prospective study of the effect of housing and exercise conditions on radiographically diagnosed Canine Hip Dysplasia in a cohort of four large breeds in Norway (1998-2001). *American Journal of Veterinary Research*, in press.

### Paper III

Krontveit R. I., Trangerud C., Nødtvedt A., Dohoo I., Moe L., Sævik B. K. (2011). The effect of radiological hip dysplasia and breed on survival in a prospective cohort study of four large dog breeds followed over a 10 year period. *Veterinary Journal*, doi:10.1016/j.tvjl.2011.10.015.

### Paper IV

Krontveit R. I., Trangerud C., Sævik B. K., Skogmo H. K., Nødtvedt A. (2011). Risk factors for hip-related clinical signs in a prospective cohort study of four large dog breeds in Norway. *Preventive Veterinary Medicine*, doi:10.1016/j.prevetmed.2011.09.018.

## Introduction

Canine hip dysplasia (CHD) is considered to be a common developmental disease affecting many breeds of dogs with varying frequency and severity (Todhunter and Lust, 2003). It can be uni- or bilateral and possibly represent a generalized condition (Citi et al., 2005; Keller and Corley, 1989; Lust et al., 1973; Olsewski et al., 1983; Todhunter et al., 1997). The condition was first described in 1935 by Schnelle as congenital hip dysplasia in the dog. This condition was thought to correspond to congenital dislocation of the hip in humans (Henricson et al., 1966; Schnelle, 1959). Henricson et al. (1966) stated that “joint laxity as the causal factor of hip dysplasia seems to be firmly established”, and defined hip dysplasia in the dog as a condition with varying degree of laxity of the hip joint permitting subluxation during early life, giving rise to varying degrees of shallow acetabulum and flattening of the femoral head, and finally leading to degenerative joint disease (DJD). In humans, the term developmental dysplasia of the hip (DDH) has replaced the term congenital dislocation of the hip as it describes the full range of abnormalities affecting the immature human hip joint more accurately (Noordin et al., 2010).

Hip dysplasia has also been described in several other species: cats, horses, cattle, pigs, and rabbits (Haakenstad, 1953; Keller et al., 1999; Owiny et al., 2001; Punto and Puranen, 1978; Weaver, 1978). Occurrence in wild animals is reportedly rare (Riser, 1975), but hip dysplasia has been described in a wolf (Douglass, 1981).

CHD is a quantitative trait with phenotypic expression ranging from normal to severely abnormal, and the expression is a reflection of both the genotype and the many varied influences imposed by the environment (Leighton et al., 1977; Todhunter and Lust, 2003). The exact mechanisms and factors influencing the development of CHD are not fully

understood. The introductory part of this thesis provides an overview of the current understanding of CHD development and the contributing factors in varying detail.

## ***Etiology and pathogenesis***

### *Normal hip joint development in dogs*

In mammalian embryos, the hip joint is laid down as a single unit from mesenchymal tissue. The hip joint of dogs is composed of two parts; the femoral portion is made up by the femoral head, while the pelvic portion, the acetabulum, is composed of os ilium, os pubis, os ischium, and small acetabular bones. The acetabulum and femoral head are not mineralized at birth, but are composed mostly of cartilage. Despite of this, the canine hip joint is functional and stable even at birth, and the joint capsule, chondro-osseous conformation, and the teres ligament along with pelvic musculature are major contributors to the stability of the hip joint (Alexander, 1992; Henry, 1992; Riser, 1975). A thin layer of synovial fluid is believed to produce a suction-like pressure (a hydrostatic-like pressure) in the hip joint and contribute to maintaining joint stability (Smith et al., 1990).

Mineralization starts in the femoral head by approximately two weeks of age and somewhat later in the different parts of the acetabulum. The attachment site for the teres ligament at the femoral head appears as a notch by six to seven weeks and remains as a flattened area (fovea capitis) in the mature dog. By six to seven months of age the acetabular physes have closed and most of the endochondral growth of the pelvis is completed, while the time of closure of the femoral head physis varies between breeds and ranges from six to nine months of age (Alexander, 1992; Dyce et al., 2010; Henry, 1992; Riser, 1975). An outline of the normal anatomy of the mature canine pelvis and hip joints is presented in Figure 1.

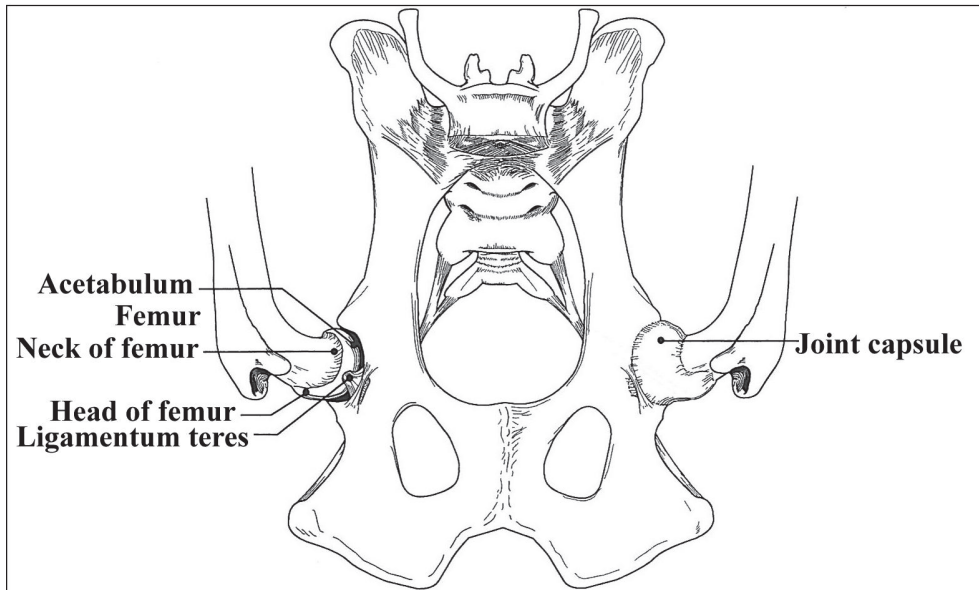


Figure 1. An outline of the anatomy of the normal canine pelvis and hip joints (ventral view). Modified from W. D. Anderson and B. G. Anderson: *Atlas of Canine Anatomy*, Lea & Febiger, Malvern, Pennsylvania, 1994).

### *Development of hip dysplasia in dogs*

As long as full congruity is maintained between the femoral head and the acetabulum, the hip joints are assumed to develop normally. However, if the joint components are pulled apart, the development follows an abnormal course that correlates with the degree of incongruity and CHD can develop (Alexander, 1992; Riser, 1975; Riser and Miller, 1966). DDH in humans can be diagnosed clinically at birth as hip joint instability. The condition is considered congenital although some children have normal femoro-acetabular relationship at birth and only later develop a dysplastic hip joint (Dezateux and Rosendahl, 2007; Noordin et al., 2010). Signs of incongruence or instability of the hip joints have not been found on post mortem examination of newborn and two weeks old puppies which were offspring of CHD

affected parents (Gustafsson et al., 1975; Norberg, 1961; Riser, 1975; Riser and Miller, 1966; Riser and Shirer, 1966). Riser (1975) also followed four German Shepherd Dogs from birth to maturity and found no recognizable evidence of CHD before seven weeks of age. All four dogs developed severe CHD later (Riser, 1975). The hip joints of newborn puppies are thus suggested to be normal and stable. A later study can be considered to support this by finding stable hip joints in two month old puppies, some of which subsequently developed unstable hip joints at four months of age and later signs of CHD (Smith et al., 1998). During the first two to three months of life the muscles and nerves are immature, the tissues are soft and plastic, and the joints are vulnerable to injury and abnormal development (Alexander, 1992; Riser, 1975). Stress on the joints starts when the puppy begins to move towards the mother and when it starts to walk (Alexander, 1992; Riser, 1975). Changes in biomechanical balance, stress, compression, traction, muscle pull, lubrication, or congruity between the joint components can affect the normal development. It has been suggested that by six months of age, function, tissue strength, and ossification have progressed sufficiently to prevent development of CHD under normal circumstances (Alexander, 1992; Riser, 1975). This is supported by the findings in other studies where the period between three and eight months of age appeared important in the development of CHD (Lust et al., 1973; Olsewski et al., 1983).

Stretching of the joint capsule as well as oedema and torn fibers in the teres ligaments have been reported as the first observed pathological changes of the hip joints in puppies and were detected as early as four to eight weeks of age (Norberg, 1961; Riser, 1975; Riser and Shirer, 1966). The earliest radiographic sign of CHD is delayed ossification and subluxation of the femoral head, as well as delayed development in the craniodorsal rim of the acetabulum, and this can be detected at four to eight weeks of age (Henry, 1992; Norberg, 1961; Riser, 1975). An increased volume of joint fluid will abolish the suction-like pressure of the synovial fluid, which can contribute to lateral displacement of the femoral head due to

increased joint laxity and subsequent subluxation (Lust et al., 1980; Lust and Summers, 1981; Smith et al., 1990). Lateral displacement of the femoral head leads to abnormal wear of certain areas of the joint surfaces during weight-bearing, and this can result in microfractures of acetabular bone, joint deformity with flattening of the femoral head, thickening of the femoral neck, and shallowness of the acetabulum (Brass, 1989; Henry, 1992; Riser, 1975; Todhunter and Lust, 2003). During the period from three to eight months further development of CHD leads to thickening of the joint capsule, loss of lubricant function of the synovial fluid, stretching and swelling of the teres ligament, and signs of new bone formation (Riser, 1975). Inflammation and DJD characterized by loss of articular cartilage, fibrosis, bone remodeling, and loss of function can occur eventually (Alexander, 1992; Brass, 1989; Fries and Remedios, 1995; Todhunter and Lust, 2003). At this stage it can be difficult to differentiate the changes representing true hip dysplasia from those representing secondary DJD (Riser, 1975). The described changes are consistent with the severe grades of CHD. In milder forms, the degrees of change are milder and appear later, varying between five and 14 months of age (Riser, 1975). DJD of the hips secondary to CHD have been found to progress throughout life, but some breeds can possibly be more prone to develop DJD in the hips than others (Kealy et al., 2000; Kealy et al., 1997; Runge et al., 2010; Smith et al., 2006).

The exact mechanism behind the development of CHD is not clear, but increased hip joint laxity and abnormal progression of the endochondral ossification have been proposed as important pathogenetic mechanisms (Bardens and Hardwick, 1968; Lust et al., 1980; Norberg, 1961; Riser, 1975; Riser and Shirer, 1966; Smith et al., 1990; Smith et al., 1993; Todhunter and Lust, 2003). These two mechanisms are not mutually exclusive and can both create an abnormal mechanical environment in the hip joints that eventually results in CHD and secondary DJD (Todhunter and Lust, 2003).

Passive hip joint laxity is the form of laxity which can be detected by palpation or measured on hip radiographs, while functional hip joint laxity is the pathologic form of laxity that occurs when the limbs are weight-bearing (Smith et al., 1993). It has been suggested that passive hip joint laxity precedes functional hip joint laxity (Smith et al., 1993). Increased volume of synovial fluid can increase the laxity of the hip joint and can result in subluxation of the femoral head (Lust et al., 1980; Lust et al., 1993; Smith et al., 1990). Lust et al. (1981) described that synovial inflammation with increased synovial fluid and ligament volumes in the hip joints of CHD prone dogs coincided or preceded the microscopic cartilage changes in the hip joints. It is not clear, however, whether the increased synovial fluid volume is an etiological factor, a response to mechanical stress, a response to synovitis, or a combination of these (Lust et al., 1980; Smith et al., 1998). There are some indications that, in breeds with high CHD prevalence, collagen alterations localized to the hip joint capsule and the teres ligaments can decrease the support given from the capsule and ligaments and thus contribute to the hip joint laxity (Madsen, 1997; Todhunter and Lust, 2003).

Abnormal progression of the endochondral ossification can affect different joints, including the hips, and is also reported in dogs which subsequently develop CHD (Madsen, 1997; Todhunter et al., 1997). Ossification of the femoral head is detected later in dysplastic hip joints than in non-dysplastic hip joints of dogs and delayed closure of the physis of the femoral head has also been reported (Madsen et al., 1991; Norberg, 1961; Todhunter et al., 1997).

It is not clear when or why passive hip joint laxity converts to functional laxity, whether laxity precedes the observed subluxation and disconformity, or whether these and the endochondral abnormalities are caused by tissue immaturity, injury, endocrine imbalances, or genetic abnormality (Lust et al., 1993; Todhunter and Lust, 2003). There is some indication



that hip joint laxity might have a genetic basis because offspring from dogs unaffected by CHD had less laxity in the hip joints compared to those from affected parents (Lust et al., 1993). Environmental factors can probably be involved in lateral displacement of the femoral head when passive hip joint laxity converts to functional hip joint laxity during weight-bearing (Kapatkin et al., 2002) and this might subsequently affect the endochondral ossification.

Investigation of factors in the environment of newborn and young puppies with regards to CHD can be useful for further understanding of the mechanisms behind abnormal hip joint development and perhaps generate hypotheses for future research.

#### *Multiple joint involvement?*

Studies have found joint changes and abnormalities of endochondral ossification similar to those described for CHD in other joints of both young and mature dogs (Kealy et al., 2000; Olsewski et al., 1983; Todhunter et al., 1997). Increased risk of radiological elbow dysplasia in dogs with a radiological CHD diagnosis has also been found (Cachon et al., 2010; Gnudi et al., 1998). It has been suggested that CHD might be a systemic disease which is manifested predominantly in the hip joints, although gait and stance alterations and subsequent stress on other joints in dogs with CHD cannot be excluded as a possible explanation for multiple joint involvements (Kealy et al., 2000; Olsewski et al., 1983; Riser, 1975).

### ***Clinical signs and diagnosis of CHD***

Clinical signs related to CHD can range from mild or intermittent lameness with difficulty rising after rest, to nonambulation in severely affected dogs (Dassler, 2003; Fry and Clark, 1992). These signs are likely linked to hip joint pain and secondary DJD. Pelvic muscle atrophy is common and often results in an overdimension of the thoracic limb musculature. A bimodal age distribution of clinical signs related to CHD is often observed. Commonly, younger dogs develop signs between three and 12 months of age due to stretching of the joint capsule and acetabular microfractures, while mature dogs develop gradually increasing severity of signs due to progression of DJD. The onset of signs in mature dogs can vary from two to 12 years of age (Dassler, 2003).

Before relating clinical signs to the diagnosis of CHD, other causes of lameness or gait abnormalities must be ruled out (Fry and Clark, 1992). Other causes of hind limb lameness in young dogs are Legg-Calvé-Perthes disease, stifle joint abnormalities, osteochondrosis of different joints, hypertrophic osteodystrophy, panosteitis, and fractures unique to immature animals (McLaughlin, 2001; Powers et al., 2005). In mature and aging dogs, DJD, trauma, and neoplasia are important differential diagnoses to DJD related specifically to CHD (Powers et al., 2005; Roush, 2001; Vaughan, 1990). Additionally, muscle and neuromuscular diseases must be ruled out.

A complete clinical and orthopedic examination should include observation of the dog at rest, during walk and trot, and re-examination after exercise (Dassler, 2003; Fry and Clark, 1992). In young dogs, hip joint laxity can be the primary cause of pain and the Ortolani, Bardens, and Barlow tests can provide information about passive hip joint laxity (Dassler, 2003). However, these tests rely on operator experience and individual patient factors, and sedation is often necessary (Dassler, 2003; Fry and Clark, 1992). In adult dogs, secondary

DJD is often the cause of pain, and palpation of the hip joints and evaluation of the range of motion of the joint can give further information about DJD in such dogs (Dassler, 2003; Fry and Clark, 1992; Ginja et al., 2010). Standard ventrodorsal hip-extended radiography can be used to evaluate joint congruence and signs of DJD (Dassler, 2003; Fry and Clark, 1992; Ginja et al., 2010). Radiographic stress-techniques can be used to evaluate passive hip joint laxity, while functional hip joint laxity is not readily measurable (Farese et al., 1998; Flückiger et al., 1999; Smith et al., 1993). The radiographic examinations also require sedation and classification relies on experience. The severity of clinical signs does not necessarily correspond with the radiographic findings, and some dogs with a radiographic CHD diagnosis will never develop clinical signs (Brass, 1989; Dassler, 2003; Fry and Clark, 1992). Several authors have found that the changes characteristic of DJD secondary to CHD progress throughout life (Kealy et al., 2000; Kealy et al., 1997; Smith et al., 2006).

Early diagnosis based on accurate examination of the hip joints could be clinically useful for possible primary prevention of CHD, but also for selection of breeding dogs (Vezzoni et al., 2005). It might then be possible to intervene and prevent CHD by adjusting environmental conditions or by preventive surgical procedures (Vezzoni et al., 2005). In humans, the hip joints are palpated for signs of instability after birth, and infants with known predisposing factors are additionally examined by ultrasonography, and further detrimental hip development is sought corrected (Dezateux and Rosendahl, 2007; Noordin et al., 2010). Several studies have been undertaken to evaluate different clinical tests and imaging methods for early detection (ages ranging from 16 days to eight months) of hip joint laxity and CHD in dogs (Adams et al., 2000; Fischer et al., 2010; Ginja et al., 2008a; Lust et al., 1973; Lust et al., 1993; Ohlerth et al., 2003; Smith et al., 1993; Smith et al., 1998; Vezzoni et al., 2005). It appears that milder forms of CHD can be difficult to diagnose precisely before the age of four months. In the age-period from four to eight months, radiographic stress-techniques seem

more sensitive than standard hip radiography for diagnosis, but a combination of different techniques and performing re-evaluations will increase the accuracy of the diagnosis (Adams et al., 2000; Fischer et al., 2010; Ginja et al., 2008a; Ginja et al., 2008b; Henry, 1992; Kishimoto et al., 2009; Lopez et al., 2009; Lust et al., 1973; Lust et al., 2001; Lust et al., 1993; Ohlerth et al., 2003; Smith et al., 1993; Smith et al., 1998; Todhunter et al., 2003; Vezzoni et al., 2005). For primary non-surgical prevention it is, however, important to increase the knowledge regarding the detailed etiology and which environmental factors that might influence the development of CHD.

### ***Screening programs for CHD***

Efforts to reduce the occurrence of CHD have been made by selection of desired phenotypes based on radiographic screening of dogs at one to two years of age and subsequently imposing breeding recommendations for dogs with radiographic diagnosis of CHD. This can be seen as a pass/fail system for hip joint status given once during the dogs' life. The screening is voluntary, and the results are recorded in national registries. Selection based on radiographic screening has been applied for several decades in a number of different countries.

The most widely used radiographic technique applied in the screening programs is the standard ventrodorsal hip-extended view, which evaluates hip joint congruence and radiographic features of DJD. The screening procedure has been standardized worldwide, and there are three somewhat different radiographic scoring modes in use; the Fédération Cynologique Internationale (FCI) scoring mode, the Orthopedic Foundation for Animals scoring mode, and the British Veterinary Association/The Kennel Club scoring mode (Flückiger, 2007; Gibbs, 1997; OFA, 2010). Radiographic stress-techniques are methods

which measure the amount of passive hip joint laxity and this laxity-measurement has been suggested to approximate the functional laxity that would be present during weight-bearing (Farese et al., 1998; Flückiger et al., 1999; Smith et al., 1990; Vezzoni et al., 2005).

In Norway, the standard ventrodorsal hip-extended view is used for CHD screening in combination with the FCI scoring mode. Hip radiographs are taken by practicing veterinarians and subsequently sent to The Norwegian Kennel Club (NKK) for evaluation by the NKK-panelist. The hip joints are graded in 5 grades A, B, C, D, or E. The grades A and B denotes free of CHD, while the grades C, D, and E denote mild, moderate, and severe CHD respectively (Figure 2). The percentage of dogs radiographically screened for CHD in Norway varies by breed, but for the large breeds approximately 60% of the registered puppies are screened (Heim, 1999; Indrebø, 2008).

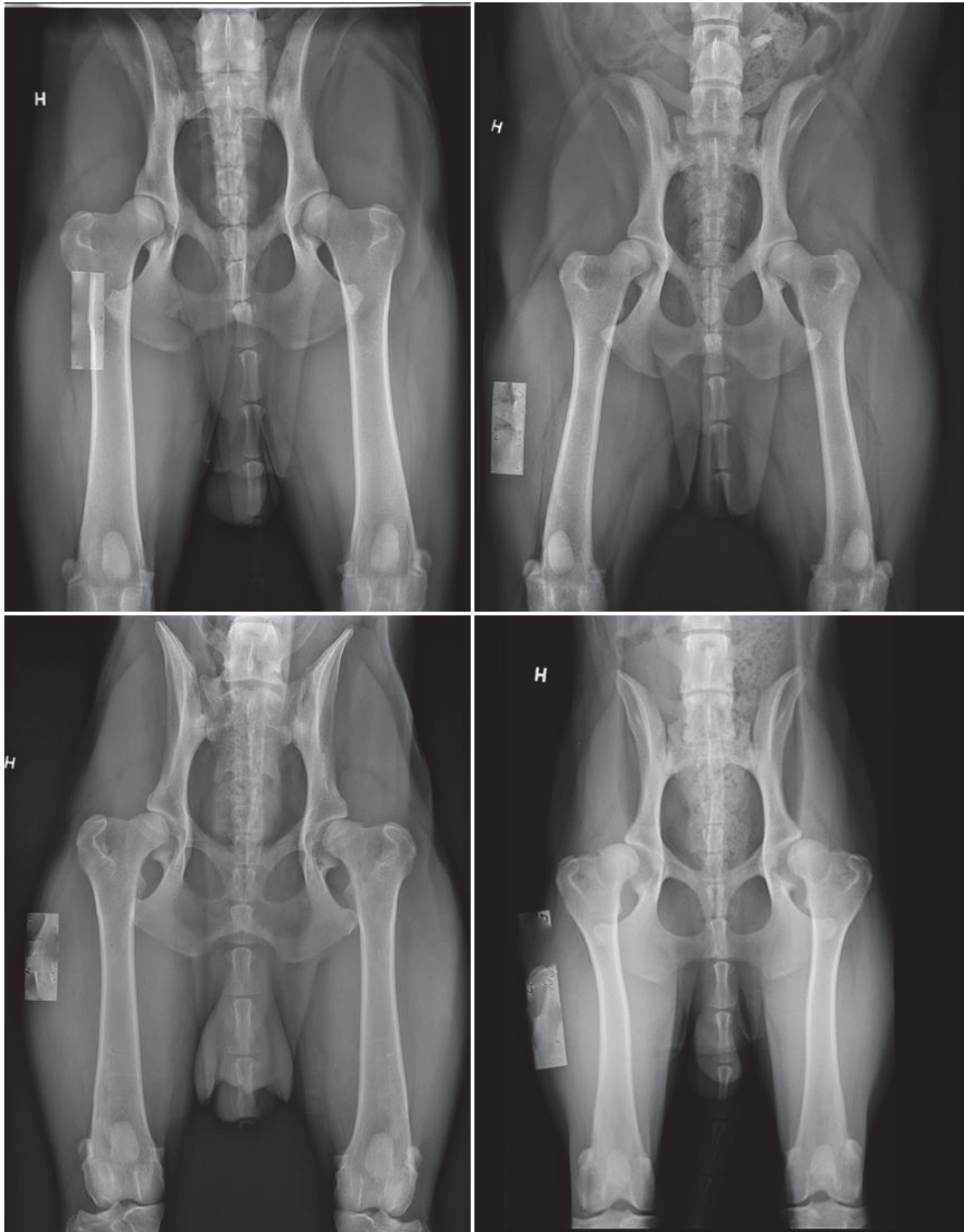


Figure 2. Radiographs of hip joints graded as free (A or B) (top left), mild (C) (top right), moderate (D) (bottom left), and severe (E) (bottom right) hip dysplasia. The letter H represents the right leg. The identification number of the dogs (here shaded) is placed lateral to the right femur (Pictures provided by Hege K. Skogmo, the Norwegian School of Veterinary Science).

### ***Prevalence and incidence of CHD***

Prevalence is defined as the proportion of a population that has a disease at a specific point in time, while the incidence risk is the probability that an individual animal will develop a disease within a specific time period. Incidence rate on the other hand is a measure of the number of new cases in a population per animal-time unit (Dohoo et al., 2009).

Considering CHD, prevalence and incidence risk have been reported interchangeably as measures of disease frequency. The prevalence of the standard radiographic phenotype from a national registry is what is most commonly reported.

In general, large and giant breeds usually have the highest prevalences although breeds like Irish Wolfhound (IW), Borzoi, and Greyhound are large and giant breeds with low prevalence. Medium and small sized breeds are reported to have lower prevalence of CHD (Bouw, 1982; Coopman et al., 2008; Corley and Hogan, 1985; Distl et al., 1985; Flückiger et al., 1995; Genevois et al., 2008; Ginja et al., 2009; Henricson et al., 1972; Janutta et al., 2008; Kaneene et al., 2009; Leppanen and Saloniemi, 1999; Lingaas and Heim, 1987; Martin et al., 1980; Ohlerth et al., 1998; Paster et al., 2005; Rettenmaier et al., 2002; Swenson et al., 1997; Worth et al., 2011). Flückiger et al. (1995) reported a radiological CHD prevalence of 42% of all purebred dogs, and Swenson et al. (1997) reported prevalences of 18% to 57% depending on the breed. In Norway, the large breeds IW, Newfoundland (NF), Leonberger (LEO), and Labrador Retriever (LR) have a radiological CHD prevalence of 5.4%, 38.5%, 14.8%, and 17.3%, respectively (average figures from NKK in the period 1980-2009).

In humans, screening for DDH is performed in all newborn babies in many countries, and reported incidence rate is 1 to 34 per 1000 live births depending on time and the diagnostic modality and criteria used (Dezateux and Rosendahl, 2007; Noordin et al., 2010).

The CHD prevalence estimates based on registries in which participation is voluntary are not necessarily representative of the general population of dogs. The prevalence is probably underestimated in many breeds. The measures of CHD frequency depend upon the diagnostic method, and it is important to relate the frequency measures to the actual diagnostic method because different methods have different sensitivity and specificity. In a study comparing standard hip-extended radiography with a radiographic stress-technique, the radiological CHD prevalence was 14.8%, while 82.8% were considered susceptible for DJD of the hips by the radiographic stress-technique applied in that study (Powers et al., 2010).

### ***Efficacy of the screening programs for CHD***

Although phenotypic improvements have been reported in some populations of dogs, the reported prevalences of CHD are still high for many breeds although radiographic screening programs with subsequent breeding recommendations have been used for decades (Coopman et al., 2008; Corley and Hogan, 1985; Flückiger et al., 1995; Ginja et al., 2009; Indrebø, 2008; Janutta et al., 2008; Kaneene et al., 2009; Leppanen and Saloniemi, 1999; Lingaas and Heim, 1987; Willis, 1997; Worth et al., 2011). There might be several reasons for the low observed efficacy of the screening programs. The standard hip-extended radiographic view is considered relatively insensitive at detecting hip joint laxity and hip joint laxity is considered an important factor in the development of CHD (Bardens and Hardwick, 1968; Farese et al., 1998; Flückiger et al., 1998; Flückiger et al., 1999; Henricson et al., 1966; Kapatkin et al., 2004; Lust et al., 1993; Norberg, 1961; Powers et al., 2010; Smith et al., 1990; Smith et al., 1993). Additionally, there are indications that dog breeds might have different susceptibility to development of CHD and secondary DJD for a given degree of hip joint laxity (Flückiger et al., 1998; Popovitch et al., 1995; Runge et al., 2010; Smith et al., 2001). It



has also been reported that DJD of the hips develops continuously throughout life (Kealy et al., 2000; Kealy et al., 1997; Smith et al., 2006). Selection based on standard radiographic view can thus yield false negative dogs because it is performed at a relatively young age (Farese et al., 1998; Flückiger et al., 1999; Genevois et al., 2008; Kaneene et al., 2009; Paster et al., 2005). Furthermore, the prevalence of CHD in many registries might not be truly representative of the general or breed specific populations because a relatively small proportion of dogs are examined. Reasons for this can be that radiographs with obvious signs of CHD often are not sent in for assessment, and that screening of dogs intended for breeding only is common in many countries (Genevois et al., 2008; Kaneene et al., 2009; Paster et al., 2005). Additionally, dog breeding is to a large extent individual-based, and the screening for CHD is voluntary, and breeders can prioritize other traits than CHD when selecting breeding animals.

The use of so-called estimated breeding values has been recommended to improve the current screening programs (Ginja et al., 2008b; Hou et al., 2010; Leppanen et al., 2000; Lewis et al., 2010; Lingaas and Klemetsdal, 1990; Malm et al., 2008; Zhang et al., 2009; Zhu et al., 2009). Estimated breeding values are calculated based on information from all known relatives (simultaneously adjusting for systematic environmental factors) and will utilize all available information about a dog and not only the phenotype of the dogs at radiographic screening (Malm, 2010). By including more than just one phenotypic measurement of CHD in the breeding value, more information about an individual dog is provided (Hou et al., 2010; Zhang et al., 2009). In the future, so-called genomic breeding values (based on single nucleotide polymorphisms or sequence variants) might also be developed and applied to aid the selection of breeding dogs, in addition to estimated breeding values (Guo et al., 2011).

### ***Factors influencing the development and manifestation of CHD***

Understanding the factors that contribute to CHD development is necessary in order to be able to prevent new cases in the future. Several such risk factors have been identified and those commonly considered most important are presented in the following sections:

#### *Genetic background*

Schales (1957) reported that CHD was inherited and that the manifestation varied between individuals. Henricson et al. (1966) reported extensive hereditary data based on hip radiographs from the breeding stock of the Swedish Army Dog Training Center and reported a heritability of 0.4 to 0.6. CHD is considered a quantitative genetic trait with a complex and largely unknown inheritance pattern.

Heritability estimates for radiological CHD phenotype vary considerably both within and between breeds and often lie between 0.10 and 0.60 (Janutta and Distl, 2006a; Zhu et al., 2009). The highest estimates are often found in closed kennel populations with low environmental variance, but the nature of the CHD phenotype used for estimation, the estimation method, and the amount of available data also contributes to variation in heritability estimates (Janutta and Distl, 2006a; Leighton, 1997; Mackenzie, 1985; Zhang et al., 2009).

Underlying molecular genetic basis for CHD has been investigated in several studies. Major genes are identified in some populations of breeds (Janutta et al., 2006b; Leighton, 1997; Mäki et al., 2004), and quantitative trait loci (QTL) for CHD traits and DJD of the hips have been identified on several chromosomes in different breeds (Chase et al., 2005; Hays et al., 2007; Mateescu et al., 2008; Phavaphutanon et al., 2009; Todhunter et al., 2005). QTL

mapping can be used for fine resolution mapping of candidate genes, genetic testing and marker-assisted selection of potential breeding dogs (Phavaphutanon et al., 2009).

### *Body type and breed*

The large diversity in size and conformation among dog breeds is a reflection of genetic variation between breeds. A study by Riser and Larsen (1974) described that breeds with high prevalence of CHD were heavy, with a rounded stocky conformation, less developed muscles, and early physical maturity. Diminished pelvic muscle mass, alterations in size of specific pelvic muscles, and altered muscle fiber size and composition has been found in dogs that developed CHD (Bardens and Hardwick, 1968; Cardinet 3. et al., 1997; Lust et al., 1972a; Lust et al., 1972b; Riser and Shirer, 1967). Hypotrophy of the pectineus muscle is a developmental neuromuscular disease occurring at around two months of age in some breeds predisposed to CHD (Cardinet 3. et al., 1997). This condition is considered to possibly promote hip joint laxity and subluxation of the femoral head (Cardinet 3. et al., 1997). Differences in pelvic musculature could be genetic in origin and directly related to the development of CHD (Cardinet 3. et al., 1997), but muscle disuse or altered gait secondary to CHD can also give alterations in pelvic musculature (Lust et al., 1972a; Lust et al., 1972b) as well as in other body regions.

Breeds which are well-muscled and with a straight limb stance are reported to be less susceptible to DJD associated with CHD compared to breeds with less muscle mass and a more angled limb stance (Flückiger et al., 1998; Popovitch et al., 1995; Smith et al., 2001). Large muscle mass and a straight limb stance are considered to inhibit transformation of passive hip joint laxity to functional laxity when the dog moves, and thus the forces that promote conversion to functional hip joint laxity can be decreased.

Riser (1975) reported that unstable hip joints were found frequently in the toy breeds. However, the bone changes characteristic of hip dysplasia in large breeds did not develop in most of these small dogs presumably because of the low weight of the toy dog breeds (Riser, 1975).

Gustafsson et al. (1975) used the age of appearance of ossification centers and closure of physes to assess skeletal maturity in dogs and observed that the Greyhounds were more skeletally mature at birth and the first seven weeks of life compared to the German Shepherd Dogs. By four months of age, however, the German Shepherd Dogs had developed a more mature skeleton than the Greyhounds. Higher occurrence of CHD was found in the German Shepherd Dogs compared to the Greyhound, and it was hypothesized that CHD could be a manifestation of accelerated skeletal development (Gustafsson et al., 1975).

Breed specific CHD prevalences from national registries have been used to find associations between body size and shape (given in the breed standard) and prevalence of radiological CHD (Comhaire and Snaps, 2008; Priester and Mulvihill, 1972; Roberts and McGreevy, 2010). Greater body weight and body mass index, as well as body shapes that are longer than they are tall, apparently increased CHD prevalence (Comhaire and Snaps, 2008; Priester and Mulvihill, 1972; Roberts and McGreevy, 2010).

### *Sex*

In some studies, male dogs have been found to have higher occurrence of CHD than females, while other studies have found the opposite. The results seem to differ between different breeds and populations (Hedhammar et al., 1979; Martin et al., 1980; Swenson et al., 1997; Wood et al., 2000a; Wood et al., 2000b; Wood et al., 2002). Several studies have failed

to detect an obvious sex predisposition for CHD (Keller and Corley, 1989; Ohlerth et al., 1998; Priester and Mulvihill, 1972; Rettenmaier et al., 2002; Riser, 1975).

### *Hormonal influences*

The hormones relaxin and estradiol have been investigated as possible contributors in the development of CHD through increased hip joint laxity. High doses of estradiol and combinations of estradiol and relaxin have been shown to induce CHD (Gustafsson, 1975; Månsson and Norberg, 1961; Pierce et al., 1965), but it was concluded that primary hyperestrogenism is unlikely in dogs with hip dysplasia (Kasström et al., 1975). CHD affected bitches seem to have different metabolism of relaxin during pregnancy and the fetuses can thus be exposed to abnormal amounts of relaxin before birth (Goldsmith et al., 1994). In addition, relaxin and estrogens in colostrum and milk are absorbed into the circulation of puppies and might play a role in inducing hip joint laxity in CHD predisposed puppies (Goldsmith et al., 1994; Steinetz et al., 2008). The mechanism behind this hip joint laxity might be caused by premature or inappropriate expression of hormone receptors in the connective tissues of the hip joints in CHD predisposed puppies (Steinetz et al., 2008).

### *Rate of growth*

The role of the growth rate on the development of CHD has been investigated in several studies. Among German Shepherd Dogs, it was concluded that heavier puppies of both sexes at 60 days of age had a higher frequency of radiographic CHD than lighter puppies (Riser et al., 1964). However, approximately 40% of the dysplastic dogs were under average weight for their group and 42% of the non-dysplastic dogs were over average weight for their

group and the dogs were only weighed once (at 60 days) (Riser et al., 1964). In a study by Kasström (1975), radiographic CHD was more frequent, severe, and occurred earlier among dogs with rapid weight gain caused by increased caloric intake, than among dogs with restricted feeding and lower weight gain. Another study, however, found that only among caesarean-delivered dogs which were deprived of colostrum and hand reared at greatly reduced rates of growth, did fewer dogs develop radiographic signs of CHD (Lust et al., 1973). Kealy et al. (1992) studied the effect of limited food consumption on radiographic incidence risk of CHD in LRs followed until two years of age. The dogs on restricted diet had lower mean weight gain and lower incidence risk of radiographic CHD and the difference in CHD incidence risk between restricted-fed and *ad libitum*-fed dogs increased until two years of age (Kealy et al., 1992). Lopez et al. (2006), however, did not find that *ad libitum* feeding had an adverse effect on hip joint laxity in puppies from six weeks up to four months of age. The apparent differences between the studies concerning feeding regimen could be explained by differences in study design (Lopez et al., 2006a). In the study by Lopez et al (2006), the CHD phenotype was assessed by passive hip joint laxity, and the weight gain was assessed weekly in the immediate post-weaning period. In the other studies, standard hip radiographs were used to assess CHD phenotype, and the studies were of longer duration with greater intervals between weight measurements. The dogs included in all these studies were offspring from parents with high frequency of CHD, and at least one of the parents had CHD (Kasström, 1975; Kealy et al., 1992; Lopez et al., 2006a; Lust et al., 1973). The observed positive correlation between rapid weight gain and development of CHD does not necessarily mean that increased weight as such is the causal factor inducing CHD development (Kealy et al., 1992). Overfeeding might maximize the expression of CHD in genetically susceptible dogs (Todhunter and Lust, 2003).

The studies by Hedhammar et al. (1979) and Ohlerth et al. (1998) investigated factors associated with radiological CHD among military working dogs and guide dogs, respectively. Body weight and growth measurements were not associated with increased radiological CHD frequency in these studies of dogs where growth was not manipulated by feeding intensity (Hedhammar et al., 1979; Ohlerth et al., 1998). Further studies of growth patterns among “normally” fed dogs of several breeds might further elucidate the relationship between CHD and growth.

### *Nutrients*

Few specific nutrients have been identified as having a direct influence on the development of CHD, and the role of nutrition is probably as multifactorial as the disease itself (Richardson, 1992). Over-supplementation of energy, vitamins, minerals (especially calcium), and possibly acid/base imbalance can create imbalances that especially the large and giant breeds cannot cope with and therefore subsequently develop abnormal bone remodeling and CHD (Hazewinkel, 2004; Hedhammar, 1974; Richardson, 1992). Life-long diet restriction was found to increase life span and delay onset of late life diseases in a kennel population of LR. Furthermore, reduced prevalence of DJD of the hips and other joints was found among the restricted fed dogs (Lawler et al., 2008; Smith et al., 2006).

### *Exercise*

The effects of different exercise regimens have not been extensively studied with regard to development of CHD. Riser and Miller (1974) found that confinement of one month old puppies in small cages where they stayed seated for long periods prevented development

of CHD. These puppies were predisposed to CHD. It was thought that the confinement allowed the femoral heads to remain well seated in the acetabulum and thus hip joint congruity was maintained (Riser and Miller, 1966). However, this regimen cannot be recommended as a preventive measure for animal welfare, as well as for practical, reasons. Lust et al. (1973) did not find that restricted exercise or exercise on-leash or running on a treadmill three times weekly up to three years of age had an effect on CHD development in an experimental study. Running after a ball or stick thrown by the owner was identified as a risk factor for radiological CHD in a retrospective cross-sectional study of one to two year old LR's (Sallander et al., 2006). The effect of different exercise conditions should be studied further to increase the understanding of exercise and physical activity on the development of CHD.

#### *Season of birth*

Several studies have addressed the effect of season of birth on the frequency of CHD. One study used the NKK hip dysplasia-registry and found that puppies of certain breeds had lower occurrence of CHD if they were born between March and August compared to those born between September and February (Hanssen, 1991). The suggested mechanism was that adequate physical exercise would improve muscle strength around the hips to decrease the risk of development of CHD, and that the amount of such exercise was limited for puppies born during winter months due to climatic conditions (Hanssen, 1991). Others have found similar results regarding season of birth (Leppanen et al., 2000; Ohlerth et al., 2001; Wood and Lakhani, 2003a). A new study of season of birth in combination with exercise and housing conditions for puppies could increase the understanding of the effect of both season of birth and outdoor exercise.



### *Gene-environment interactions*

If a specific environmental exposure has different effect on different genotypes, gene-environment interaction is present (Falconer and Mackay, 1996; Ottman, 1996). Epigenetic changes are environmentally influenced modifications of the DNA or associated proteins which regulate gene expression without changing the DNA sequence (Feinberg, 2008). Different environmental factors are considered important in the development and manifestation of CHD (Todhunter and Lust, 2003) possibly through modification (down or up regulation) of the expression of genes. Both gene-environment interactions and epigenetic changes can possibly be involved in the phenotypic development and expression of CHD.

### *Clinical relevance and prognostic value of radiological CHD diagnosis*

The exact mechanism behind development of CHD is still not clear despite decades of research. CHD has been reported as a reason for euthanasia for several breeds (Adams et al., 2010; Bonnett et al., 1997; Malm et al., 2010; Proschowsky et al., 2003), but the association between radiological CHD phenotype and development of clinical signs has not been well researched.

DJD is a common complaint in aging dogs (Roush, 2001; Vaughan, 1990). Several authors have found that DJD of the hips secondary to CHD continue to develop throughout life, although there might be differences between breeds regarding the susceptibility to development of DJD (Flückiger et al., 1998; Kealy et al., 2000; Kealy et al., 1997; Popovitch et al., 1995; Runge et al., 2010; Smith et al., 2001; Smith et al., 2006). Furthermore, different breeds and individual dogs can have different pain thresholds which might affect the

likelihood of being diagnosed with hip related problems. Malm et al. (2010) studied insured dogs to investigate the association between radiological CHD status and insurance claims for veterinary care or life claims due to CHD. A strong association between moderate and severe CHD status and subsequent insurance claims were found and demonstrated the impact of these CHD grades on prognosis (Malm et al., 2010). Studies based on insurance claims have inherent sampling biases, and the quality of the diagnosis might be questionable (Adams et al., 2010; van Hagen et al., 2005b). Van Hagen et al. (2005a) studied the incidence of and risk factors for “clinical CHD” in a birth cohort of Boxers, but a standardized CHD phenotype was not defined which makes comparisons difficult (van Hagen et al., 2005a).

Prospective longitudinal studies of the relationship between CHD phenotypes and end-of-life outcomes would help elucidate the relationship between CHD phenotypes and long- as well as short-term prognosis. If the radiological CHD phenotype could be used to predict prognosis for an individual dog, the available CHD screening programs could be useful at the individual dog level in addition to at the population level.

### ***The need for further research on CHD development***

CHD is a disease that affects many breeds and causes varying degrees of pain and disability in dogs. The etiology of CHD is considered complex, and the association between the genetic predisposition and environmental factors influencing the development is poorly understood. Prevalence and heritability estimates vary depending on the phenotype evaluated. For the conventional radiological CHD phenotype the heritability estimates are often relatively low, and the prevalence estimates are often considered to be underestimated. Which environmental factors that contribute to the development and manifestation of CHD, and to what extent, is largely unknown. Most studies regarding CHD have been retrospective or

experimental in relatively small kennel populations. If the aim is to prevent disease at the individual as well as the population level, prospective studies of dogs in real-life situations are needed for identification of environmental factors that can influence the development of CHD.

## Objectives

The aim of this thesis was to increase the understanding of factors related to development of CHD and evaluate factors that influence the prognosis of CHD. Prospective studies in a large cohort of privately owned dogs from four breeds which were followed from birth and throughout life were used. The breeds were of large size, assumed to be fast growing, and represented both high- and low-risk breeds regarding CHD. Various environmental factors registered during the natural growth period were investigated for influence on CHD phenotype and long-term prognosis. Specific research questions were

- What is the incidence risk of radiological CHD among the four included breeds?
- Do body weight and growth related measurements influence the incidence risk of radiological CHD?
- Which housing and exercise conditions influence the incidence risk of radiological CHD?
- Does radiological CHD status obtained at approximately one year of age affect overall survival over a 10 year period?
- Do radiological CHD status, body weight, housing and exercise conditions during growth have an influence on time to clinical signs related to the hip joint over a nine year period?
- Does litter have an influence on the incidence risk of radiological CHD status and prognosis?
- Can the results from this study be used to develop preventive efforts and practical recommendations for breeders and owners of large breed dogs?
- Can the findings generate new hypotheses regarding the etiology of CHD?

## Materials and methods

The dogs included in the present thesis come from the study described in detail below (Paper I-IV).

### *Study design and inclusion of dogs*

The present thesis is based upon material from a larger cohort study of four dog breeds in Norway (called the *main study*). This representative cohort was followed from birth and through a 10 year period. The purposes of the *main study* were to investigate diseases in growing dogs related to nutrition, growth rate, environmental, and genetic factors. Four skeletal diseases were of primary interest: CHD, elbow dysplasia (ED), osteosarcoma, and panosteitis. Dogs from four breeds which were considered to represent both high incidence and low incidence breeds regarding these skeletal diseases were included: NF, LR, LEO, and IW. All breeders of these 4 breeds in Norway were invited to participate in the study. The plan was to include dogs as they were born if the breeder was willing to comply with the project protocol. Litters of puppies were therefore included at the time of mating of the bitch. Seven hundred puppies from 107 different litters were enrolled in the *main study*. The owners who purchased puppies from these litters as well as their local veterinarians were encouraged to participate in the study. All participants (breeders, dog owners, and 140 veterinarians) signed a written agreement of consent and cooperation.

The eligibility criterion for entering the study was that the puppies were born in Norway between November 1998 and June 2001. The breeding stock consisted of dogs born in Norway as well as dogs that had been imported, and all were registered in NKK. A litter was included at birth, and all puppies were also registered in NKK. The puppies/dogs were followed prospectively from birth and throughout their lives. Not all dogs enrolled in the *main*

*study* continued to completion (Trangerud et al., 2007), and as a result, 647 dogs were potentially eligible for inclusion in the present study sample.

All dogs were privately owned and had housing, exercise, and feeding regimens decided for them by the breeder and subsequently the owner without any influence from the researchers.

### ***Study sample-inclusion criteria***

The inclusion criteria for the studies reported in this thesis were that the dogs were radiographically screened for CHD at the official NKK ages (Papers I-IV), that they had weight measurements, housing and exercise conditions recorded at least once during their first year of life (Papers I-IV). In Paper IV an additional inclusion criterion was that the dogs were still present in the cohort at three months of age. Dogs that were radiographically examined for CHD before the ages for official screening (due to clinical signs of hip joint disease) were included if their hip radiographs were graded by the panelist in the NKK.

### ***Radiographic screening for CHD***

The written consent encouraged dog owners to have their dogs radiographically screened for CHD, but this was not required by the project protocol. LR and IW were radiographed at 12 months of age, and LEO and NF at 18 months of age, which are the recommended screening ages for these breeds in the NKK.

Hip radiographs were taken by a practicing veterinarian and subsequently sent to NKK for grading irrespective of whether the dog showed any clinical signs or not. More than 90% of the radiographs were scrutinized by one experienced panelist at NKK. Some dogs in a few litters were sold by the breeder to owners living in Sweden or Denmark. The hip radiographs

of these dogs were therefore graded by the panelists in the Swedish Kennel Club (SKK) and the Dansk Kennel Club (DKK) (the Danish club for dog owners), respectively. The radiographic panel of the Nordic Kennel Union consists of panelists from the Nordic countries and ensures that the hips are evaluated and graded according to the same protocol, to minimize differences in grading between the Nordic countries.

All dogs were sedated before the radiographic examination to achieve complete and similar muscle relaxation. The identity of the dogs (NKK registration number, ear tattoo or microchip number) was photographed onto the film before developing the radiographs. The radiographs were made at 100-cm film to focus distance.

The dogs were placed in dorsal recumbency with the hind limbs extended and abducted so that the patellae were superimposed over the femurs. The entire pelvis and femurs including the patellae were included on the radiographs. The correct position is presented in Figures 2. The FCI five class grading scale was used to classify the hip status of the dogs: A (excellent), B (normal), C (mild dysplasia), D (moderate dysplasia) and E (severe dysplasia). The CHD grades are defined descriptively based on the measurement of the Norberg angle, degree of subluxation, shape and depth of the acetabulum, and signs of DJD (Flückiger, 2007). Each hip joint was graded separately, and the final grading of the dogs hips was based on the most severely affected hip joint. Dogs graded C (mild dysplasia), D (moderate dysplasia), and E (severe dysplasia) were considered affected by CHD in the present studies and dogs with A (excellent) and B (normal) were classified as unaffected (free).

### ***Questionnaires and clinical registrations***

The variables evaluated in this study are presented in Table 1 Parts a-d (Appendix 1).

Information for each included dog was obtained from three sources: 1) the breeder of the litter, 2) the owner of the dog, and 3) the veterinarian examining the dog. All three sources completed questionnaires and recorded information in a booklet prepared for each included dog. All questionnaire sheets appeared in duplicates in the booklet, so that one sheet could be mailed to the researchers and a copy retained in the booklet. The breeder and owner booklets (in Norwegian) are available at:

[http://www.nvh.no/Documents/PDF/SportFaMed/breeder\\_booklet.pdf](http://www.nvh.no/Documents/PDF/SportFaMed/breeder_booklet.pdf) and

[http://www.nvh.no/Documents/PDF/SportFaMed/owner\\_booklet2.pdf](http://www.nvh.no/Documents/PDF/SportFaMed/owner_booklet2.pdf), respectively.

The breeder was asked to record the body weight (BW) in grams for each puppy at birth and on day three and seven, and then weekly until 56 days of age. The breeder decided the feeding and housing regimen of the litter, and this information along with information about any medications given (including vaccinations and antiparasite treatments), were recorded in the booklet. The puppies stayed with the bitch until approximately eight weeks of age when they were sold to their new owner.

The owners reported information regarding feeding, housing, exercise, and any signs indicative of disease in their dogs. The owner completed the questionnaires and reported information regarding the dog at specific ages, called “the observational ages”: 3, 4, 6, 12, 18, and 24 months. A few owners refused to follow the project protocol and their dogs were therefore lost to follow-up at different ages.



The owners agreed to have their dogs examined by a veterinarian at visits scheduled especially for this project at each observational age. Clinical examination, blood sampling, measurement of BW in kg, and measurement of the circumference of the distal radius and ulna in cm were done at each of the veterinary visits. Vaccinations and antiparasite treatments were administered at the veterinary visits. During these veterinary visits any additional clinical signs and treatments were recorded. The dog owners were encouraged to contact their veterinarian for examination if their dog had any signs of disease between the scheduled visits.

After 24 months of age, annual questionnaires were mailed to the dog owners until death of the dog or the end of the observation period. There were no pre-scheduled veterinary visits after 24 months of age, but owners would contact a veterinarian as they thought needed. The owner reported information about feeding, exercise conditions, the dog's BW, and health status on the annual questionnaires including information about any diagnoses and treatments given by a veterinarian and if relevant the natural death or euthanasia of the dog. The owners were encouraged to include copies of any veterinary records from the preceding year. Owners who did not respond for various reasons were contacted by telephone to regain contact.

The breeders and owners submitted the questionnaires from the booklets and the annual questionnaires at regular intervals in prepaid envelopes.

### ***Laboratory analyses***

Hematologic analyses were carried out at ages 3, 4, 6, 12, 18, and 24 months, and the blood serum was analyzed for 20 biochemical variables at the same ages (Table 1 part c). The analyses were conducted at the Central Laboratory, Norwegian School of Veterinary Science.

### ***Outcome variable Paper I and II***

Radiological CHD status was the outcome in Paper I and II and it was reclassified into unaffected (free) (grades A, and B) and affected (grades C, D, and E). Thus, the dependent variable CHD was a dichotomous variable.

### ***Outcome variable Paper III***

The owners continued to report information using the annual questionnaires at the approximate ages 3, 4, 5, 6, 7, 8, 9, and 10 years of age. Participants who did not respond for various reasons were contacted by telephone, and the age of euthanasia or death was recorded. Factors related to the dog and the owner as well as the veterinarian examining the dog can influence decisions regarding euthanasia (Yeates and Main, 2011). Therefore, the endpoint in the analysis of Paper III was defined as euthanasia or natural death irrespective of the reported main cause and the outcome is referred to as death. Cases which were lost to follow-up or were still alive at the end of the study, were included in the survival analysis up until the last time point at which they were known to be alive and were thereafter censored. For technical reasons, 6 months were subtracted from the time at risk, and the outcome variable in Paper III was termed “time from six months to death until 10 years of age”.

### ***Outcome variable Paper IV***

During the veterinary visits scheduled especially as a part of this project, the dogs were examined by a veterinarian and any clinical signs and treatments were recorded. The dog owners could also contact their veterinarian for an examination if their dogs had any signs of disease between the scheduled visits. Health status and information about any diagnoses and treatments given by a veterinarian was recorded by the owner on the annual questionnaires

after the age of 24 months. The owners were encouraged to include copies of any veterinary records from the preceding year. Definition of the endpoint in Paper IV was hip related clinical signs (called “the event”) and was based on fulfillment of the following blindly applied criteria (without knowledge regarding exposure status like breed and radiological CHD status): 1) owner-reported clinical signs of hip disease (e.g. difficulties in standing up after rest, stiffness, exercise intolerance, lameness) and 2) clinical signs of hip disease and findings at veterinary examination (e.g. hip-joint laxity, pain, and reduced movements of the hip joints) reported either from a scheduled veterinary visits at one of “the observational ages” or as stated by the owner in the annual questionnaires. The impact of any concurrent causes of hind-limb lameness (as diagnosed by the veterinarian), were also taken into consideration: if other musculoskeletal conditions were reported (by the veterinarian or stated by the owner) as the dog’s main problem, this dog was not included as having the event at that time. A few dogs were reported by the owner to have been euthanized due to hip dysplasia/hip-related problems without veterinary reports of clinical signs prior to euthanasia. These dogs were also considered as having the event, and the time of occurrence of clinical signs was considered the midpoint between the last observation point and the time of euthanasia (Dohoo et al., 2009). Dogs that died or were euthanized due to other causes, or were lost to follow-up, or were still alive at the end of the study, were included until the last time at which they were known to be alive without the event and were thereafter censored in the analysis. Based on these data and assumptions the outcome variable in Paper IV “time from birth to owner reported veterinary-diagnosed hip-related clinical signs” (the event) was obtained.

### *Statistical analyses*

The software package Stata 11 (Stata Corporation, 4905 Lakeway Drive, College Station, TX 77845, USA) was used for all analyses.

Breed and sex distribution as well as breed distribution by CHD grade were calculated. The incidence risks of radiological CHD were calculated by relating the number of CHD-affected dogs to the total number of dogs in each breed.

### *Description of growth*

The Gompertz function is a sigmoid function with a point of inflection at 36.8% of mature BW (Helmink et al., 2000), and this function was used for description of the dogs' growth pattern (Paper I). BW data were separated by breed, sex, and CHD status, and were then fitted to the Gompertz function by the following equation (Helmink et al., 2000) using the NLIN procedure (SAS statistical software):

$$W_t = W_{\max} \exp(-e^{[-(t-c)/b]}), \text{ where}$$

$W_t$  is the BW at time  $t$ ,  $W_{\max}$  is the mature BW,  $b$  is the proportional for duration of growth (a constant),  $c$  is the age at the point of inflection (36.8 % of mature BW is reached), and  $t$  is the age in days. Analyses were carried out separately for the subgroups created by: breed, sex, and CHD status. Duration of growth was estimated by  $(4b + c)$ , which describes 98 % of the growth duration (Helmink et al., 2000). The derivative of the Gompertz function describes the growth rate.

### *Multivariable analyses*

In Papers I and II the dependent variable CHD is a dichotomous variable (CHD affected or unaffected), and a logistic regression model of the relationship between predictors and CHD was considered. The dogs were clustered into litters which violate the assumption of independence between observations, and a random effect for litter was therefore included in a generalized linear mixed model (Dohoo et al., 2009). A random effect for breeder was considered, but since the breeder: litter ratio was close to one, the breeder level was omitted.

In Paper III and IV the outcome was time from birth until death or clinical signs related to the hip joint, respectively. The aim was to measure the effects of predictors on time to these events, and Cox proportional hazards models with shared frailty terms for litter were used to account for the clustering of dogs in litters (Dohoo et al., 2009).

Associations between the dependent variable and the predictor variables were first screened based on unconditional associations with univariable random litter-effect logistic regression (Papers I and II) or univariable Cox proportional hazards regression with shared litter-frailty (Papers III and IV). The variables were tested for collinearity by Goodman and Kruskal's gamma for ordinal and dichotomous variables, the Phi coefficient for nominal variables, and pair-wise correlations for continuous variables. Associations above 0.7 or below -0.7 were considered evidence of collinearity. Variables with a univariable P-value  $\leq 0.20$ , provided that there was no collinearity between them, were then considered for further multivariable analysis. For screening of the blood parameters (Paper I) a more restrictive unconditional P-value of 0.10 was applied due to the large number of variables (Table 1 Part c). When collinearity was detected between two predictors, the predictor with least missing data was selected. The linear relationship between the continuous variables and the logit of the outcome was assessed by lowess curves (Paper I and II) and by plotting of martingale

residuals against the continuous predictor of interest (Paper IV). Variables with many missing values (> 20% missing observations) were not retained in the multivariable analysis.

Multivariable random effects logistic models (Papers I and II) and multivariable Cox proportional hazards models with shared frailty for litter (Paper III) were constructed using both manual forward selection and backward elimination. In Paper IV, only forward selection was applied due to convergence problems. Predictors were retained in the model when the P-value of the likelihood ratio test (LRT) was < 0.05. Potential confounding and intervening variables were evaluated based on tentative causal diagrams. Changes of more than 20% in the coefficients in the model with the potential confounder present were also judged as indications of confounding. A variable was considered to be intervening if adding it substantially altered the effect of another variable, and if the intervening variable lay on the causal path between the other variable and the outcome. All possible two-way interactions between significant predictors in the models were tested by adding an interaction term. Interaction terms were retained if  $P < 0.01$ , judged by the LRTs. The LRTs were used to evaluate significance of categorical predictors in the final models. The significance of the random litter effects and shared frailty terms was also evaluated through a LRT.

From the final multivariable random effects logistic regression models (Papers I and II) the between litter variance ( $\sigma^2_{\text{litter}}$ ) was estimated. The intraclass correlation coefficient (ICC) was calculated by the latent variable approach, assuming that the dog level variance ( $\sigma^2$ ) is constant at  $\pi^2/3$  (Dohoo et al., 2009)

$$\text{ICC} = \sigma^2_{\text{litter}} / \sigma^2 + \sigma^2_{\text{litter}}$$

To quantify the amount of clustering in the proportional hazards models (Papers III and IV), Kendall's  $\tau$  for the shared gamma frailty was given by

$$\tau = \theta/\theta+2$$

### *Model evaluation*

To evaluate and assess the fit of the final random effects logistic regression models (Papers I and II) the residuals at the dog level were estimated, and the residuals at the litter level were estimated and evaluated by plotting of residuals against both predicted values and against fitted values to evaluate homoscedasticity and normality.

To evaluate the Cox proportional hazards models (Paper III and IV) the assumption of proportional hazards was evaluated based on the Schoenfeld residuals for each variable in the model. If there was violation of the proportional hazards assumption and graphical assessment indicated a time-varying effect (TVE) of a variable, an interaction term between the variable and time (on the appropriate time scale) was included in the model. The assumption of independent censoring was evaluated by sensitivity analyses based on both complete positive and complete negative correlation between censoring and outcome. Plots of the deviance residuals, score residuals, and scaled score residuals against time at risk were used to identify outlying and influential observations, and the models were fit with and without any outlying observations.

## Results and discussion

The reported results are derived from an observational study of four large dog breeds (NF, LR, LEO, and IW). The incidence risk of radiological CHD is described, and dog signalment data and environmental factors that influence the risk of radiological CHD are identified. Signalment data, radiological CHD status and environmental factors are additionally found to influence time to death or time to clinical signs related to the hip joints. The results are presented and discussed in more detail in the following sections.

### *Study sample description (I-IV)*

The study sample consisted of dogs that were radiographically screened for CHD and had weight measurement, housing and exercise conditions recorded at least once during their first year of life. In total, 647 dogs from 106 litters from the *main study* were eligible for inclusion in the present study. This sample consisted of 7.7% of the total number of LR-litters born during the sampling period. For NF, LEO, and IW the corresponding percentages of included litters were 36.4, 41.3, and 66.7. For LR, the proportion was quite low even though this was numerically the largest breed among the four in Norway. For the other breeds almost half of the population was included and the sample can be considered to be a very good representative for the overall population of these breeds in Norway. Of these eligible dogs, 501 dogs from 103 litters were radiographically screened for CHD and thus included in the study sample. It follows that 146 were not screened and thus not included. Reasons for not screening were commonly due to death of the dog at early age, loss to follow-up, or that the owner did not want to cover the cost of radiographing the dog. The 103 litters were the offspring of 94 dams from 86 different breeders.



The breed distribution of dogs was 180 LEO, 125 NF, 133 LR and 63 IW. The vast majority of the hip radiographs were scrutinized by the panelist in NKK (N=491), while 10 dogs had their radiographs scrutinized by the panelists in SKK and DKK. Distribution of dogs by breed and radiological CHD grade is presented in Table 2. In Paper IV the additional inclusion criterion gave the study sample of 494 dogs.

**Table 2.** Descriptive statistics of radiological canine hip dysplasia (CHD) grade in a study of four large breeds.

Breed	Radiological CHD grade, n (%)					Total n (%)
	A	B	C	D	E	
Newfoundland	62 (49.6)	18 (14.4)	16 (12.8)	18 (14.4)	11 (8.8)	125 (25)
Labrador Retriever	88 (66.2)	18 (13.5)	11 (8.3)	12 (9.0)	4 (3.0)	133 (26.5)
Leonberger	116 (64.5)	19 (10.6)	15 (8.3)	24 (13.3)	6 (3.3)	180 (35.9)
Irish Wolfhound	50 (79.4)	7 (11.1)	3 (4.8)	3 (4.8)	-	63 (12.6)
Total	316 (63.1)	62 (12.4)	45 (8.9)	57 (11.4)	21 (4.2)	501 (100)

### ***Incidence risk of radiological CHD (I)***

Among the 501 dogs (260 females, 241 males) that were included in Paper I, 123 (24.6%) were affected by CHD as diagnosed at radiological screening. There were breed differences in incidence risk of radiological CHD, and NF had the highest with an 18 month incidence risk of 36%. In LEO, the 18 month incidence risk was 25%, and in LR and IW the 12 month incidence risks were 20%, and 10%, respectively. Based upon the final logistic regression model (Paper I) it appears that the risk of radiological CHD among both LR and IW are about one fifth (Odds ratio (OR) 0.22) of the risk for NF, while the risk for LEO (OR 0.60) is not statistically significant different from NF. Several studies have found differences in CHD prevalence between breeds (Coopman et al., 2008; Corley and Hogan, 1985;

Flückiger et al., 1995; LaFond et al., 2002; Leppanen and Saloniemi, 1999; Lingaas and Heim, 1987; Martin et al., 1980; Priester and Mulvihill, 1972; Swenson et al., 1997). Large and giant appear to be more susceptible to CHD, but exceptions are breeds like the Collie, Borzoi, and IW, which are large breeds considered to be of low risk (Corley and Hogan, 1985; LaFond et al., 2002; Martin et al., 1980; Priester and Mulvihill, 1972). IW had the lowest incidence risk in the present study when compared to the other breeds, but in the multivariable analysis the risk of radiological CHD in IW was identical to LR (using NF as the baseline for comparison). Small and miniature breeds are to a much lesser extent studied with regard to CHD prevalence, but Martin et al. (1980) found that 25% of radiographed miniature poodles had CHD. Mixed breed dogs are also affected by CHD (Lust et al., 1973; Rettenmaier et al., 2002). When selecting breeds for the present study, IW and LEO were assumed to be low incidence breeds regarding CHD, based on the prevalence in NKK and other national registries. However, in the current analysis the incidence risk proved to be higher than previously assumed. On average, approximately 50% the IW, NF, and LEO litters born during the sampling period were included in this study and considering this large proportion of included litters, the estimated incidence risks can be considered representative estimates for the overall population of dogs from these breeds. The incidence risk estimate for radiological CHD in LR is more uncertain due to the fact that only approximately 8% of the born litters were included.

No difference in the incidence risk of radiological CHD between males and females was detected among the breeds included in the present study. This finding is in accordance with a review of a large number of studies, where no differences between the sexes were detected in 21 breeds (Dietschi et al., 2003). There is no clear evidence of any breed-specific sex-disposition, as different studies of the same breed have generated divergent results (Dietschi et al., 2003; Swenson et al., 1997; Wood et al., 2000a; Wood et al., 2002).

## Factors associated with radiological CHD (I, II)

### Body weight and growth (I)

In Paper I, a large number of growth-related variables were investigated for influence on radiological CHD diagnosed at screening (Table 1, Part a-c, see Appendix 1), and the growth patterns of affected and unaffected dogs were described by use of the Gompertz function (Table 3, Figure 3).

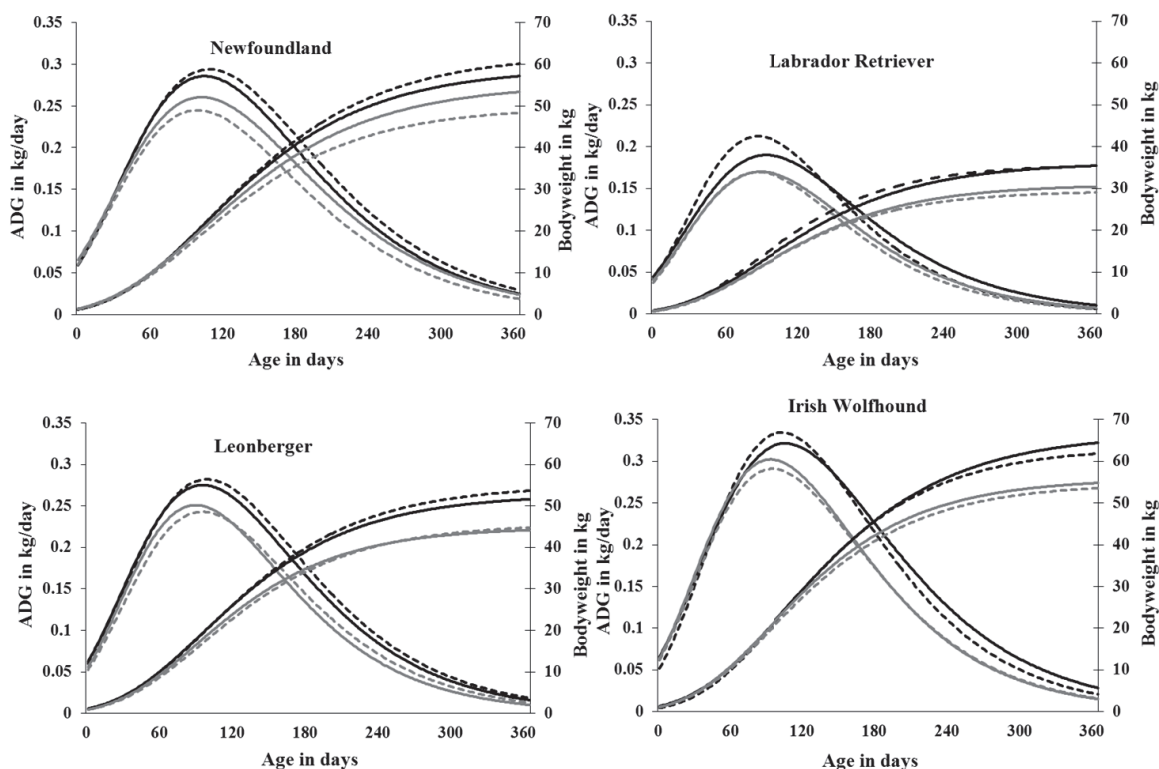


Figure 3. Gompertz derivative (left axis), average daily weight gain (ADG) in kg/day, and average growth curves of body weight in kg (right axis) estimated with Gompertz function for female and male Newfoundland, Labrador Retriever, Leonberger, and Irish Wolfhound separated by canine hip dysplasia (CHD) status.

Key: — male unaffected by CHD, - - - male affected by CHD, — female unaffected by CHD, - - - female affected by CHD.

During the growth period from birth to 12 months of age the differences in BW and average daily weight gain (ADG) were only modest between CHD affected and unaffected dogs. The difference was most obvious in affected female NFs, which were lighter and had smaller ADG than unaffected female NFs (Figure 3). Vanden Berg-Foels et al. (2006) found that increased birth weight and increased early postnatal body weight increased the probability of degenerative changes in the hip joint later in life among LRs. In the present study, CHD affected male LRs seemed to have a peak in ADG approximately between 60 and 90 days of age (Figure 3) and thus later than in the previously reported study (Vanden Berg-Foels et al., 2006).

NF reached maximum growth rate (the variable  $c$ ) between 99 and 109 days of age and had duration of growth (the estimate  $4b+c$ ) estimated to 400 to 420 days according to the variables of the Gompertz function (Table 3). Compared to the corresponding estimates for the other breeds, NF reached maximum growth rate as well as maturity at the highest age and was thus the slowest growing breed. However, NF had the highest incidence risk of radiological CHD. IW had a lower incidence risk of radiological CHD and reached maximum growth rate and maturity at younger ages than NF (Table 3). LR reached maximum growth rate at ages 87 to 94 days and maturity at 335 to 374 days and was thus the fastest growing breed in this study. Considering LEO, this breed reached maximum growth rate at ages 90 to 99 days and maturity at ages 353 to 386 days thus a slower growth than LR but faster than NF. The incidence risk of radiological CHD in LEO was higher than LR and lower than NF.

**Table 3.** Least squares means of the estimated variables<sup>a</sup> from the Gompertz function and mean birth weight by radiological canine hip dysplasia (CHD) grade and sex in four large breeds. Standard error of the mean is given in parentheses behind the estimates.

Breed	CHD <sup>b</sup>	Wmax in kg	c in days	b in days	4b+c in days	Birth weight in g
Newfoundland						
Male	0	59.04 (0.35)	105.20 (1.07)	76.05 (1.32)	409.40 (6.24)	611 (14.3)
	1	62.44 (0.89)	109.10 (2.29)	78.17 (2.78)	421.78 (18.27)	637 (28.9)
Female	0	55.22 (0.42)	102.80 (1.47)	77.99 (1.94)	414.76 (9.90)	584 (17.3)
	1	49.77 (0.53)	99.23 (1.74)	74.79 (2.15)	398.39 (12.20)	579 (20.3)
Labrador Retriever						
Male	0	36.21 (0.20)	94.11 (0.90)	70.07 (1.18)	374.40 (5.27)	406 (12.6)
	1	35.80 (0.55)	87.26 (2.30)	61.97 (2.93)	335.14 (16.67)	417 (38.2)
Female	0	30.95 (0.21)	89.61 (1.06)	66.90 (1.41)	357.19 (6.45)	390 (11.6)
	1	29.44 (0.26)	87.12 (1.29)	63.87 (1.63)	342.60 (8.01)	374 (15.4)
Leonberger						
Male	0	55.76 (0.28)	95.68 (0.91)	70.55 (1.16)	377.95 (5.22)	510 (12.5)
	1	55.00 (0.53)	98.96 (1.71)	71.77 (2.27)	386.05 (12.01)	513 (23.9)
Female	0	44.84 (0.23)	89.80 (0.84)	65.75 (1.09)	352.83 (4.84)	464 (10.9)
	1	45.70 (0.46)	95.09 (1.71)	69.20 (2.27)	371.90 (11.79)	469 (31.1)
Irish Wolfhound						
Male <sup>c</sup>	0	66.64 (0.43)	106 (1.18)	76.26 (1.50)	411.10 (7.23)	603 (26.3)
	1	63.31 (1.15)	103.00 (2.91)	69.62 (3.62)	381.49 (27.14)	580 (-)
Female	0	55.82 (0.52)	93.80 (1.50)	67.93 (1.89)	365.51 (9.81)	614 (21.3)
	1	54.66 (1.16)	95.30 (3.14)	69.07 (4.18)	371.56 (30.89)	680 (10.0)

<sup>a</sup> Wmax= mature body weight in kg, c=age in days at point of inflection (36.8% of mature body weight), b= proportional for duration of growth in days, 4b+c=duration of growth in days (98% of the growth duration), <sup>b</sup> 0 = unaffected by CHD, 1 = affected by CHD, <sup>c</sup> only one male affected by CHD

The only weight variable investigated in Paper I which was significantly associated with radiological CHD diagnosis (P = 0.044) was BW at three months of age. The OR was

0.89 for a one-unit (kg) increase in BW, indicating a protective effect of higher BW at three months of age on the risk of radiological CHD. The upper limit of the 95% CI approached one (CI 0.80-0.99) and the effect seemed modest. The OR for BW at three months decreases by 0.10 for an increase across the interquartile range (13.6-18.7 kg). It can be speculated that the heavier dogs at three months of age have increased general muscle mass compared to lighter dogs. Increased muscle mass can possibly be beneficial for maintaining hip joint stability and prevent development of CHD (Popovitch et al., 1995; Riser and Shirer, 1967; Smith et al., 2001).

The between-breed differences in the described growth patterns are in accordance with another study modeling growth of dogs in the *main study* in which dogs were separated by breed and sex (Trangerud et al., 2007a). When the breeds in the present study are compared with each other, the findings are, however, not in accordance with the belief that breeds with rapid growth and early physical maturity (among other breed characteristics), are high prevalence breeds for CHD (Riser, 1975; Riser and Larsen, 1974). Although the breed with most rapid growth in the present study, the LR breed, had incidence risk of radiological CHD of 20%, two other breeds (LEO and NF) with considerably higher incidence risks had slower growth than LR. It is important though, to separate skeletal maturity from maturity measured by body weights as these two measures are not the same. The results from Paper I cannot be used to assess skeletal maturity.

Age of appearance of ossification centers and age of closure of physes have been used to compare skeletal maturity of Greyhounds (with low CHD risk) with German Shepherd Dogs (with high CHD risk) (Gustafsson et al., 1975). It was reported that the low-risk breed Greyhound is more skeletally mature at birth than the high-risk breed German Shepherd Dog, but that as they grow the maturation of the skeletons of German Shepherd Dogs accelerate (Gustafsson et al., 1975). By about four months of age, the skeletons of the German Shepherd

Dogs were more mature than those of the Greyhounds. Crossbreeds of these two had more similarities with Greyhounds than with the German Shepherd Dogs, and it seemed that the Greyhound trait for normal hip development dominated over the German Shepherd Dog trait for dysplastic hip development (Gustafsson et al., 1975).

The prevalence of radiological CHD is reported to be higher in dog breeds selected for body shapes being longer than they are tall and in dogs with high body mass index (Comhaire and Snaps, 2008; Roberts and McGreevy, 2010). Selection of body shapes that are longer than they are tall, in combination with high BMI can act together giving too much load on the hip joint thus exacerbating characteristics of CHD such as subluxation and osteoarthritis (Roberts and McGreevy, 2010).

Several studies have compared dogs fed *ad libidum* with dogs fed a restricted diet with respect to frequency and severity of CHD and found that dogs fed *ad libidum* have a higher incidence of CHD than dogs on a restricted diet (Kasström, 1975; Kealy et al., 1992; Riser et al., 1964). However, a study in a cohort of “mixed breed hounds” fed *ad libidum* did not reveal any significant effect of growth on hip distraction index (Lopez et al., 2006a). Hedhammar et al. (1979) and Ohlerth et al. (1998) investigated ADG and BW measurements among dogs without manipulated growth rates. Neither of these studies reported significant associations between increased risk of CHD and high BW or rapid growth.

In the present study, growth and BW and the association with radiological CHD are assessed at the breed level by the Gompertz function and at individual dog level (while controlling for breed and litter) in the multivariable logistic regression model. It is obvious from the present study that the incidence risk of radiological CHD was not increased by rapid growth or high BW neither on breed level nor dog level in these dogs where the growth rate was not manipulated by the investigators. Most of the dog owners in the present study probably fed their dogs according to the dog-food manufacturers’ guidelines and did probably

not overfeed these growing dogs. Manipulating growth rate by restricted or *ad libitum* feeding might not cause CHD, but can perhaps maximize the expression of the radiological CHD phenotype (Todhunter and Lust, 2003). Classifying breeds as high or low risk breeds for CHD based on the assumption that being large implies (too) rapid growth might be wrong. Growth and BW is influenced by the genotype and other breed specific characteristics probably in interaction with and influenced by the environment. Associations between growth or BW and CHD development must be interpreted with these other factors in mind.

### *Exercise and physical activity (II)*

In the study of the effect of housing and exercise conditions on radiological CHD (Paper II), a large number of variables from different ages (the breeder period and the observational ages 3, 4, 6, and 12 months) were investigated (Table 1 Part a and d) and analyzed in separate age-specific sub-models. Finally, only variables from the breeder period and the age of three months were found to have a significant effect on the risk of CHD. Off-leash exercise in park terrain more than halved the risk (OR 0.31,  $P=0.002$ ), and the daily use of stairs almost doubled the risk (OR 1.96,  $P=0.015$ ) of radiological CHD. Riser (1974) found that confinement of puppies in small areas where they stayed seated for long periods prevented development of CHD reportedly due to forced hip joint congruence mediated by this confinement. Another study did not find any effect of restricted exercise on development of CHD (Lust et al., 1973). Anatomic studies have found that dogs/breeds with high pelvic muscle mass are more likely to have a normal development of the hip joints (Cardinet 3. et al., 1997; Riser and Shirer, 1967). Controlled stair descent and uphill walking has proven useful as therapeutic exercises for dogs with musculoskeletal diseases (Holler et al., 2010; Millard et al., 2010). The beneficial effect of off-leash exercise until three months of age in the present



study might be due to increased muscle development and strength associated with such exercise. Gait inconsistencies due to un-developed neuromuscular function and coordination have been observed in puppies (Lopez et al., 2006b). Stairs are rarely designed for dogs, and the steps can be too high and slippery for a small puppy. Thus, stair climbing and decent until the age of three months, in combination with the immature neuromuscular function and coordination, might create too much load on the immature joints and thus promote abnormal development.

### *Housing conditions (II)*

The risk of radiological CHD was more than halved (OR 0.34,  $P = 0.010$ ) if housing conditions at the breeders were characterized as farm or small farm/holding (rural area). An explanation for the effect of breeder house-type could be related to outdoor environment and exercise conditions. Puppies reared in rural areas on a farm or small farm/holding might get outdoor off-leash exercise on a more regular basis compared to puppies reared in a single family house in more urban areas. Housing conditions for dogs have not been extensively studied with respect to radiological CHD, but box rest and irregular exercise have been found to increase severity of osteochondrosis lesions in foals (Barneveld and van Weeren, 1999).

### *Season of birth (II)*

A seasonal effect was obvious in that dogs born in the fall had twice the risk of radiological CHD (OR 2.13) compared to dogs born in the winter. Furthermore, a protective effect of being born during spring and summer was observed with the risk of radiological CHD being about one half (OR 0.54 and 0.62 respectively) compared to being born during

winter. Previous studies have found similar effects (Hanssen, 1991; Leppanen et al., 2000; Ohlerth et al., 2001; Wood and Lakhani, 2003a). A possible explanation for the observed seasonal effects might be that housing and exercise conditions could change depending on season, such that puppies born during spring and summer probably get more free exercise on soft ground than puppies born in the fall and winter. However, other unmeasured factors might be present and could bias the result. As a sensitivity analysis in the present study, March was merged into spring which led to the protective effect of spring becoming less obvious (OR close to one) while the effect of fall was increased (OR 3.2). March is a winter month in most parts of Norway (by meteorological definition) (SMHI, 2011; YR, 2011). Merging March into spring will therefore probably diminish the differences between winter and spring and is probably in support of the hypothesis that the seasonal effects observed are a result of less outdoor exercise during the winter months.

#### *Effect of age (I, II)*

In the present study, ADG was estimated to reach its maximum at approximately three months of age (Paper I, Figure 3, Table 3), and that is in line with previous studies of dogs in this cohort (Trangerud et al., 2007a; Trangerud et al., 2007b). Among all the other potential environmental risk factors (exercise, housing, season of birth), only factors in the period from birth to three months of age were found to be significantly associated with the risk of a radiological CHD diagnosis (Paper II).

Human DDH can be diagnosed at birth as hip joint instability, and the condition is thus congenital (Dezateux and Rosendahl, 2007; Noordin et al., 2010). No signs of abnormalities of the hip joints have been detected in studies of newborn and two weeks old puppies considered likely to develop CHD, and it was hence concluded that dogs are probably born

with normal hips (Gustafsson et al., 1975; Norberg, 1961; Riser, 1975; Riser and Shirer, 1966).

Considering the normal development of the hip joints in dogs it appears that the hips joints are most susceptible to negative as well as beneficial environmental effects during the period from birth until four to six months of age (Norberg, 1961; Riser, 1975; Riser and Shirer, 1966). It has also been reported in foals that the first months of life are when the musculoskeletal system is most vulnerable to adverse influences that may result in developmental orthopedic disease (Barneveld and van Weeren, 1999; Lepeule et al., 2009). Rapid growth and development might make the musculoskeletal apparatus of the dog even more susceptible to environmental influences, and beneficial or negative effects of different kinds of exercise might be most pronounced during time of rapid growth. The detection of significant associations in the period from birth until three months of age in the present thesis is important. It probably results from this period being particularly vulnerable in the development of the hip joints and due to the rapid growth during this period. Further research on the etiology of CHD should thus include this time period.

### ***Long-term effects of radiological CHD status (III, IV)***

#### ***Effect of radiological CHD status on time to death (III)***

The 501 dogs included in the study sample were followed until 10 years of age, and during this time period 279 dogs died or were euthanized. Breed differences in time to death were observed (Figure 4). For LR more than 50% of the dogs were alive at 10 years of age, while for IW only about 5% of the dogs were alive at this age. The effect of being IW on the hazard of dying increased linearly over time.

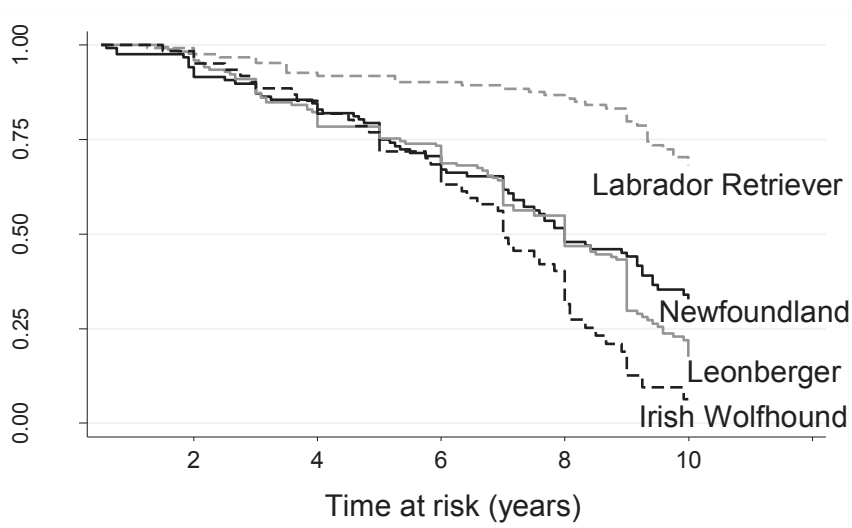


Figure 4. Kaplan-Meier survival curves by breed describing survival in a prospective cohort of 4 large dog breeds. Time at risk is from 6 months to 10 years of age.

Severe radiological CHD status had an effect on survival, but this effect on the hazard of dying decreased over time on a logarithmic time scale. At six months of age the effect of severe CHD on the hazard of dying was very high (Hazard ratio (HR) 270) compared to dogs free of radiological CHD. Already by the age of two years the effect had dropped substantially (HR 7) and it was equal to the other radiological CHD grades by eight years of age (HR 0.94). This indicates that dogs with severe radiological CHD died (were euthanized) at younger ages than dogs graded free, mild, or moderate. The Kaplan-Meier curves for each breed by radiological CHD grade demonstrated that the breeds NF and LEO contributed most to the observed effect of severe radiological CHD on the hazard of dying before two years of age (Figure 5).

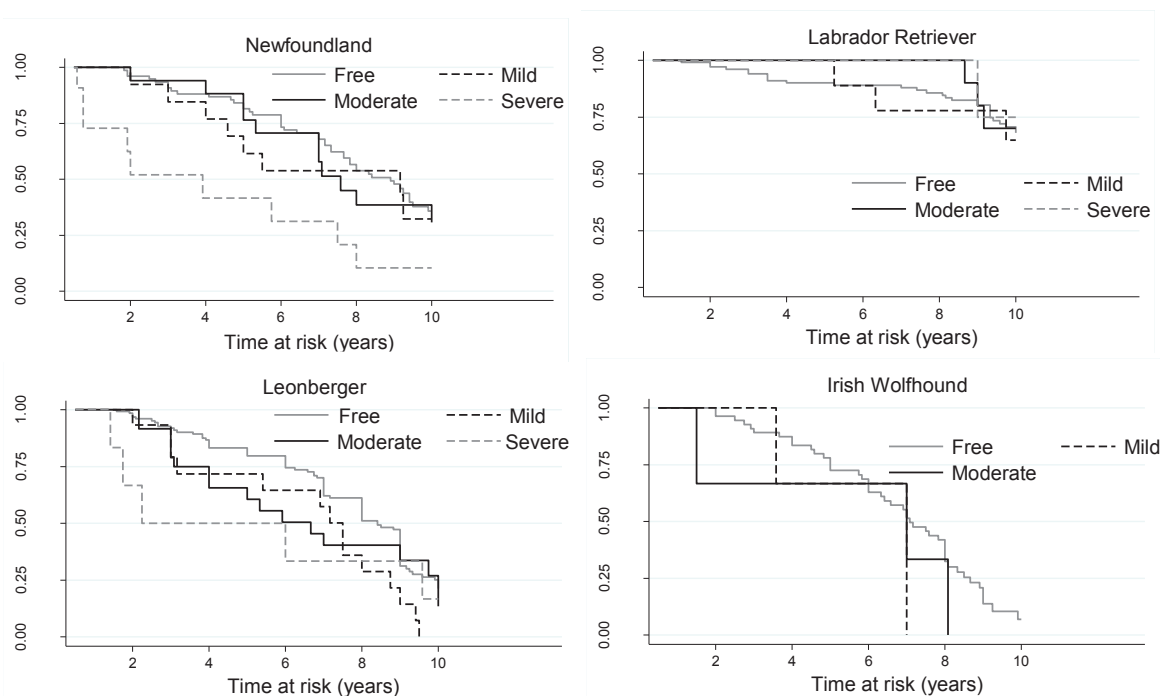


Figure 5. Kaplan-Meier curves for Newfoundlander, Labrador Retriever, Leonberger, and Irish Wolfhound by radiological canine hip dysplasia (CHD) status. The figures illustrate the effect of radiological CHD status on overall survival in each breed. Time at risk is from 6 months to 10 years of age.

The impact of radiological CHD on overall survival in dogs has not been reported previously, but insured German Shepherd Dogs with severe CHD were found to have a high incidence risk of CHD life-insurance-claims shortly after screening (Malm et al., 2010). Radiographic CHD was not found to have a substantial effect on the service longevity among military working dogs predominantly of the breeds German and Belgian Shepherd Dog in another study. However, dogs with the more severe CHD grades were not procured for military service (Banfield et al., 1996; Moore et al., 2001). In the study by Malm et al. (2010) the authors suggested that owners of German Shepherd Dogs might be more prone to euthanize their dogs based on the radiographic screening result due to the widespread use of

this breed as working dogs in Sweden. In Paper III time to death irrespective of cause was chosen as the outcome to provide a measure of relative mortality because reasons for choosing euthanasia are diverse and often complex and the vast majority (> 95%) of the dogs in this cohort died following euthanasia.

*Effect of radiological CHD status and exercise conditions during growth on time to hip related clinical signs (IV)*

Because radiological CHD status had an effect on time to death as described in Paper III, a study of factors (Table 1 Part a, b, and d) that could influence time to clinical signs related to the hips was performed (Paper IV). The event was defined as owner-reported hip-related clinical signs based on predefined criteria and assumptions. Dogs that did not have recordings after the breeder period i.e. up to three months of age were excluded from this analysis. The reason for this was that it is difficult to determine whether or not such young puppies could be considered to have hip related clinical signs (or not). Among the 494 dogs included, 46 dogs (9.3%) were registered as having the event during the observation period from birth to nine years of age. The distribution of events by breed and radiological CHD grade is presented in Table 4 and Kaplan-Meier curves for each breed by radiological CHD grade in Figure 6. For both NF and LEO 10.7% of the dogs had reports of hip related clinical signs while for LR and IW there were fewer dogs with such reports, 7.6% and 6.3%, respectively.

**Table 4.** Number of dogs with owner reported veterinary-diagnosed hip-related clinical signs / total number within radiological canine hip dysplasia (CHD) status by breed in a prospective cohort study of four large breeds.

Breed	Radiological CHD status			
	Free	Mild	Moderate	Severe
Newfoundland	0 / 79	2 / 14	4 / 18	7 / 11
Labrador Retriever	4 / 106	2 / 10	2 / 11	2 / 4
Leonberger	5 / 133	4 / 15	7 / 24	3 / 6
Irish Wolfhound	1 / 57	1 / 3	2 / 3	-
Total	10 / 375	9 / 42	15 / 56	12 / 21

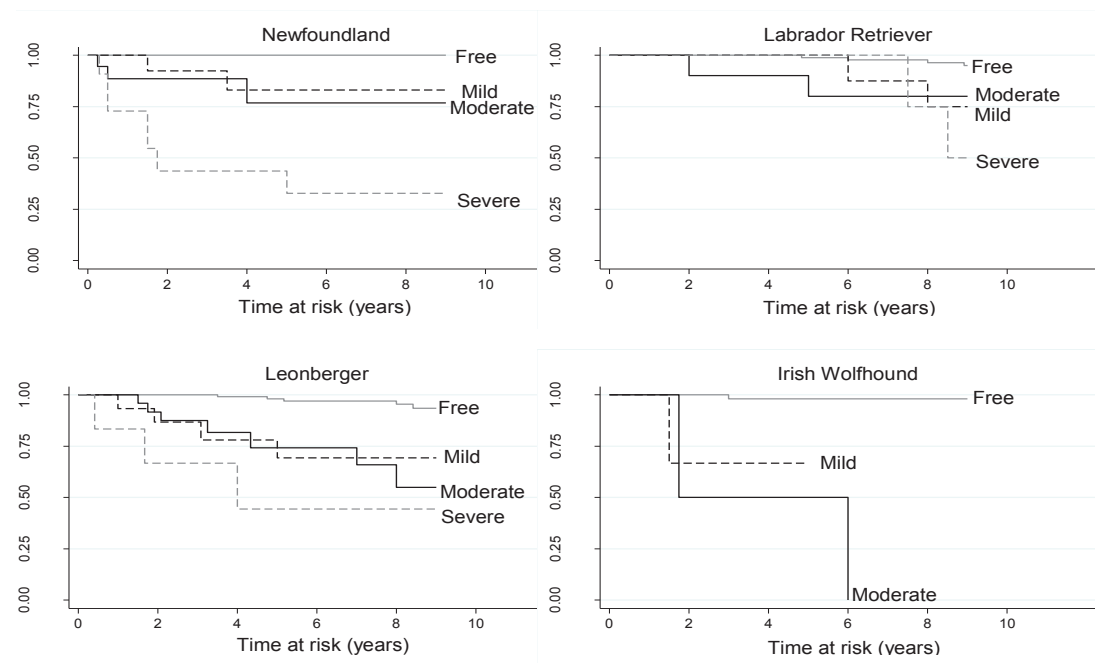


Figure 6. Kaplan-Meier curves for Newfoundland, Labrador Retriever, Leonberger, and Irish Wolfhound by radiological Canine Hip Dysplasia grade describing time to occurrence of owner reported veterinary-diagnosed hip-related clinical signs. Time at risk is from birth to 9 years of age.

Time to event differed between the breeds with NF having the shortest mean time to event (1.9 years) and LR the longest (6.5 years), IW and LEO were in between (3.1 and 3.9 years, respectively) meaning that there was a protective effect of being LR, LEO, and IW compared to NF. Additionally, the effect of breed on time to event changed over time as described by the TVE for breed. Compared to NF, the protective effect of being LR disappeared by four years and of being LEO by two and a half years of age. Increasing severity of radiological CHD status increased the numbers of dogs with the event and the hazard of the event increased markedly with HRs being 12, 18, and 92 for the grades mild, moderate, and severe, respectively. Malm et al. (2010) found breed differences in time to CHD-related insurance claims as well as a strong and significant effect of the more severe radiological CHD grades. CHD is a developmental condition that can produce secondary DJD of the hips, but secondary DJD can also be a result of other primary joint conditions that interfere with the normal joint mechanics (Vaughan, 1990). DJD in the hip joints progresses throughout life (Kealy et al., 2000; Kealy et al., 1997; Smith et al., 2006). Additionally, DJD in the hips can be primary as a consequence of wear-and-tear and differentiating between primary and secondary DJD can be difficult when examining joints of old dogs (Vaughan, 1990). In Paper IV, the event was therefore termed “hip-related clinical signs”. The dogs were followed until the age of nine years and signs at least in the later stage of the study period, might be attributed to primary DJD and not necessarily DJD secondary to CHD. A few dogs (n = 10) with the event were graded “free” of CHD at radiological screening, which was similar to what was found among insured dogs (Malm et al., 2010). These dogs could have had increased joint laxity that was not detected on conventional radiography and developed DJD secondary to CHD later in life, but the presence of DJD from other causes or injuries to the hip joints after radiographic screening cannot be ruled out. Several authors have indicated that different breeds can have different susceptibility to development of DJD over time



(Flückiger et al., 1998; Popovitch et al., 1995; Runge et al., 2010; Smith et al., 2001) which can explain the breed differences in time to event in the present study. Individual dog and breed factors such as pain threshold as well as owner attitude towards their dog might also influence the observed breed differences in time to event.

Exercise conditions found to delay time to event were off-leash exercise in garden or yard (HR 0.47,  $P=0.03$ ) and in rough terrain (HR 0.35,  $P = 0.005$ ) both at age 12 months, and on-leash exercise on asphalt at age 12 months (HR 0.33,  $P = 0.003$ ). Exercise in moderately rough terrain at three months of age was protective against a radiological CHD diagnosis in the analyses of Paper II. It seems reasonable that exercise conditions at 12 months of age can reflect how the dogs were exercised later in life. These kinds of exercise might be beneficial by strengthening muscle mass and improving range of motion in the hip joints thus possibly delay time to event. Both off-leash and on-leash exercise is recommended for dogs with DJD, but excessive stress should be avoided to prevent flare-ups of clinical signs (Vaughan, 1990). It has been hypothesized that given the same level of functional hip joint laxity, heavy exercise will impose higher stresses on the joint cartilage and thus induce severe DJD of the hips earlier in life (Smith et al., 2001). The types of exercise found to delay time to event in the present study may be thought of as normal activities for dogs held as pets and thus appear to be at a level which is beneficial rather than to impose excessive stress on the joints.

Exercise conditions at three months of age were not found to influence time to hip related clinical signs possibly because these do not reflect exercise conditions later in life and most dogs had events later than 12 months of age. Another explanation could be that factors associated with the development of CHD are different from those associated with development of DJD and that the latter occurs later in life than the former. The important

time-period for development of or protection against DJD appears to be from 12 months of age and onwards.

### ***Methodological considerations (I-IV)***

#### ***Choice of analytical tools and models (I-IV)***

The design of this study as a prospective cohort, the large amount of information registered through the questionnaires, and the clustered nature of the data posed several analytical challenges. Multivariable regression models were chosen to be able to investigate several factors simultaneously and account for the clustering, evaluate and control for confounders, and detect intervening factors as well as interactions.

In the regression analyses of Paper I and II the outcome variable was radiological CHD status in 5 categories (A-E). Treating the outcome as an ordinal outcome and applying a proportional odds (or another ordinal regression) model or treating the outcome as a linear outcome and applying linear regression was considered. However, the logistic regression model was chosen because the diagnosis was based on radiographic screening and it is the phenotypic status unaffected (A+B) or affected (C+D+E) that is currently considered in selection of animals for breeding and becoming guide dogs for the blind or rescue/working dogs.

In the regression analyses of Paper III and IV the outcome was time to event (death or clinical signs). Non-parametric analyses (Kaplan-Meier function) were used for descriptive purposes, but this function cannot be used for continuous variables or for multivariable analyses. Parametric survival models were considered because such models make better use of the data than semi-parametric models provided that the baseline distribution of survival time (baseline hazard) is correctly specified. However, the commonly used semi-parametric

Cox proportional hazards models were chosen because the baseline distribution was unknown and the aims were to measure effects of predictors and not make predictions about the future.

The Gompertz function is a parametric model and can be used for mathematical modeling of growth. A baseline sigmoid shape with the point of inflection at 36.8% of mature BW is assumed, thus the growth patterns of the dogs are forced to follow this curve. Plotting of crude data indicated that a sigmoid shape could be appropriate. This function has previously been used to model growth in dogs (Helmink et al., 2000; Trangerud et al., 2007a; Trangerud et al., 2007b). Modeling growth using mathematical functions summarizes growth data for an individual or a group of individuals (Helmink et al., 2000). Many data points taken over time are reduced to a few parameters and the resulting curve visualizes growth. Interpretation of the estimated parameters provides an explanation for what is occurring biologically.

#### *Hierarchy and clustering (I-IV)*

The study design with inclusion of litters of puppies from different breeders resulted in a hierarchical structure of the data with the following levels from low to high: dog-litter-dam-breeder/owner. The litter:dog ratio was 0.2 and accounting for litter in the analyses by including a random litter effect (I and II) or a shared frailty term (III and IV) was necessary as there was violation of the assumption of independence between observations. However, the ratios dam:litter, breeder:litter, and owner:dog were close to one, and these levels were therefore omitted from the models. The ICCs estimated in Paper I and II represent the proportion of variance in the data at the litter level and they were high and significant at 22.6% and 18.3%, respectively. In the survival analyses, the random litter effects were represented as frailties, and the shared frailty variance for litter was high and significant in

Paper IV ( $\tau = 0.27$ ) indicating a strong association between dogs in the same litter regarding time to clinical signs, while it was small in Paper III ( $\tau = 0.035$ ) indicating a very weak association between dogs in the same litter regarding time to death.

Intra-herd correlations for infectious diseases have been observed to range from 4% to 42%, but mostly below 20% (Otte and Gumm, 1997). In a study relating preweaning mortality in puppies to additive genetic, common-litter, and within-litter factors, the litter factors and especially the within-litter factors explained most of the variation (van der Beek et al., 1999). The high level of clustering (litter variance) found in Paper I and II is most probably a result of the common genetic background for dogs in a litter as well as common environment within a litter both before and after birth. Breeders commonly have recommendations for the owners who buy puppies regarding dog management and this might make litter effects important even after weaning. Also, in Paper IV the common genetic and environmental background for dogs in a litter is the most probable reason for the pronounced effect of litter on time to clinical signs. The environmental variance, i.e. all variation of non-genetic origin, is most probably greater in our study population than in dogs living in kennels under standardized conditions. Genotype-environment interactions are considered important when individuals of a population are reared under different conditions (Falconer and Mackay, 1996) such as the dogs in our cohort, and might contribute to the observed high litter clustering. In Paper III, however, the outcome (event) was time to death and the litter variance was low. Several factors influence the decision to euthanize a dog and thus owner, other environmental, and veterinary related effects are probably more important than the litter effects in Paper III. For methodological reasons the additive genetic effect could not be separated from the litter effect and thus the magnitude of the contribution from genetic versus other litter effects on the total litter level variance could not be estimated.

### *Validity (I-IV)*

All Norwegian breeders of the four breeds in this study were invited to participate in the *main study* during the inclusion period, and there were no restrictions regarding household or environment. The NKK registered population is believed to include more than 90% of the purebred dogs. Of the total registered population of dogs in NKK, the *main study* included 36.4% of NF litters, 7.7% of LR litters, 41.3% of LEO litters, and 66.7% of IW litters born in Norway during the sampling period. The study is a large scale prospective cohort study of dogs living in private homes. Cohort studies are generally considered to have high relevance to real-world situations and a high external validity (Dohoo et al., 2009). A potential limitation of the study is the restricted inclusion of only purebred dogs of large size, and the results derived from the study might not be relevant for smaller breeds.

The study sample consists of approximately 80% of the dogs included in the *main study*. The inclusion criterion of radiological CHD screening might be a source of selection bias. If dog owners who screen their dog have different attitudes towards their dogs than dog owners who do not, and if the probability of being screened also varied across the levels of other factors of interest such as breed or living region, then a bias would occur. However, the most common cause for not screening was death of the dog at young age. High socio-economic status can increase willingness to participate in observational studies (Dohoo et al., 2009) and if the socio-economic status of the dog owners is associated with both the dogs' exposures (e.g. breed) and outcome (e.g. hazard of death) then a selection bias is present. This source of selection bias is less probable in Paper I and II where the outcome is radiological CHD status, but it might be present in Paper III and IV where the outcome to a larger extent relies upon owner reports. Other sources of selection bias are non-response and loss to follow-up especially in Paper III and IV which have long duration (Pfeiffer, 2010). Strategies aimed

to reduce these sources of bias were signed owner contracts, regular follow-up during the first two years of the study, and thereafter annual questionnaires, following which non-responders were contacted by telephone. The most common cause of non-response was death of the dog.

Misclassification of the outcome might be a source of bias in the reported analyses. In Paper I and II the standard hip-extended radiographic method was the basis for the outcome variable. The degree of hip joint laxity cannot be evaluated reliably using this method, and false negative diagnoses can occur although breed differences are likely to be present (Farese et al., 1998; Flückiger et al., 1999; Kapatkin et al., 2004; Powers et al., 2010; Smith et al., 1990; Smith et al., 1993). The low sensitivity can be a source of non-differential misclassification, and if the number of false negative diagnoses also varies across predictors of interest, e.g. breed, then differential misclassification can occur. The use of a stress-radiographic method for the CHD diagnosis would probably increase the proportion of affected dogs through detection of increased hip joint laxity and subluxation of the femoral head. On the other hand, if not all dogs with hip joint laxity develop CHD and secondary DJD of the hips, the stress-radiographic method may be too sensitive and create false positive diagnoses. In Paper IV, 10 dogs diagnosed free of CHD by the standard hip-extended radiographic method developed hip-related clinical signs over time. This can indicate that false negative CHD diagnoses were present, and these findings are in line with several other studies (Farese et al., 1998; Flückiger et al., 1999; Kapatkin et al., 2004; Powers et al., 2010; Smith et al., 1990; Smith et al., 1993). In one study, 82.8% of the dogs were considered susceptible to develop DJD of the hips measured by a stress-radiographic method, while only 14.8% were considered affected by CHD by the standard hip-extended radiographic method (Powers et al., 2010). Radiographic findings regarding CHD and DJD of the hips do not always correlate well with the degree of clinical signs. In the analyses of Paper IV, the hip related clinical signs had to be severe enough to make the owner contact a veterinarian for a

clinical examination of the dog. This can explain the relatively low percentage (2.7%) of probable false negative diagnoses in Paper IV.

The vast majority of the hip radiographs in the present study were, however, graded by a single panelist and this reduces the inter-observer variation and thus some of the potential misclassification bias (Verhoeven et al., 2009).

The age of the dog at screening influence the screening result. Older dogs tend to get more severe CHD grades than younger because secondary changes in the hip joints are more common with increasing age (Hou et al., 2010; Kealy et al., 1997; Leppanen et al., 2000; Mäki et al., 2000; Ohlerth et al., 1998; Swenson et al., 1997; Wood and Lakhani, 2003b). Thus, the fact that NF and LEO were six months older when they were screened can be an important source of differential misclassification.

In Paper III the outcome was time to death irrespective of cause and the amount of misclassification was probably low, with the exception that some of the censored dogs could in fact be dead, but instead were recorded as lost to follow-up. The test of independent censoring gave only minor changes in the estimated effects and this source of bias is therefore assumed to be low.

Both non-differential and differential misclassification of the outcome variable could be present in Paper IV. Non-differential misclassification could be present if dogs were classified as having (or not having) hip related clinical signs (the event) when they in fact did not (or did) have these signs. The amount of non-differential misclassification in Paper IV was sought reduced by including dogs from birth (free of clinical disease), by having criteria for being considered as an event, and by evaluating all information about the individual dogs “blindly” with only a non-informative dog identifier available when evaluating the records.

Differential misclassification could occur if dog owners or veterinarians knowledge of radiological CHD status influenced the reporting of hip related clinical signs. Some dogs were in fact screened free of CHD, but had reports on hip related clinical signs which might indicate that the amount of differential misclassification is low.

The effect of misclassification of the outcome can be difficult to predict. Non-differential misclassification of the outcome will bias the measure of association towards null and thus have a conservative effect and possibly lead to underestimation of associations between exposure and outcome (Dohoo et al., 2009). Differential misclassification may, however, bias the measure of association in any direction (Dohoo et al., 2009).

The use of multivariable analyses with random effects for litter allows controlling for several effects simultaneously in addition to accounting for the clustering of the data at litter-level. In Paper III and IV the use of survival analyses reduced the selective survival bias. Because the dogs were followed from birth, a non-differential misclassification of health-status at inclusion (i.e. failure to exclude prevalent cases at the start of the study) was negligible. This kind of bias can lead to either under- or overestimation of associations and is considered a more serious bias (Dohoo et al., 2009).

When comparing the results from this study with other studies it is important to acknowledge inherent design-made differences. In Norway, it is recommended that breeding dogs should be diagnosed unaffected by radiological CHD at screening (at 12 or 18 months of age). Furthermore, the study sample is heterogeneous consisting of dogs from four different breeds living in private homes. Hence, they are under influence of maternal, genetic, and environmental factors which most probably influence both growth and CHD development simultaneously. Most studies regarding growth and other risk factors for CHD have been done in dogs with high parental prevalence of CHD, thus rendering the offspring more likely to be



affected by CHD. Commonly, the studies include dogs preselected for certain purposes (e.g. guide dogs for the blind) and with housing and management controlled and standardized in a manner that will reduce the environmental variance (Kasström, 1975; Kealy et al., 1992; Lopez et al., 2006a; Lust et al., 1973; Ohlerth et al., 1998). Additionally, many studies were done several decades ago and have partly provided the basis for recommendations on feeding of growing dogs, especially the large breeds. There has been extensive research on dog feeds. The feeding of the dogs in our study is therefore likely to be different from what were common 20 to 30 years ago. Puppies and young dogs of large breeds are commonly fed diets designed for heavy, fast-growing dogs and these diets intend to avoid overfeeding and to slow down growth thus reduce the risk of skeletal disorders. Size and body shape of the breeds might also have changed over this time-period due to selective breeding of dogs with desired body size and shape. It is thus interesting that a result from the present study was that the slowest growing breed had the highest incidence risk of CHD and that higher BW at three months was protective against this skeletal disease. The study did not include a grading of body condition score. Such grading would have been beneficial for assessment of the association between overweight and obesity as puppy and increased risk of CHD, hip related clinical signs, and death.

#### ***Future recommendations (I-IV)***

The thesis had two main focuses. The first was to study factors influencing the radiological CHD diagnosis during the first year of life. The second main focus was to evaluate prognostic factors for dogs with a radiological CHD diagnosis. One of the research questions was if the results of these studies could be used to suggest preventive efforts regarding development of CHD and regarding prognosis.

It is important to distinguish between primary and secondary prevention. Primary prevention aims at hindering the disease from developing in an individual and secondary prevention aims at reducing clinical signs in affected patients (Arshad, 2005).

Two important time-periods have been identified through the analyses in this thesis. Factors with significant influence on the diagnosis radiological CHD were identified in the period from birth until three months of age and this may be an important time period for primary prevention by environmental adjustments or perhaps preventive surgical or physical procedures. Factors with significant influence on prognosis were identified at the age of 12 months and this age and onwards may be important ages for secondary prevention related to the clinical effects of CHD.

#### *Primary prevention (I, II)*

Based on the results from Paper I and II, it seems that it is during the first months of life that the anatomic structures of the canine hip joints are most susceptible to damage and thus can develop abnormally. Signs of the more severe CHD grades can occur before the age of three months (Lust et al., 1973; Norberg, 1961; Riser, 1975). The findings in Paper I and II indicate that primary preventive efforts should be instituted early in life; up to three months of age when growth is most rapid and during the critical time of development. The studied factors had significant influence on radiological CHD diagnosis only up to three months of age. Provision of regular exercise on soft ground in moderately rough terrain appeared protective against radiological CHD, while daily use of stairs should be avoided in this period due to its negative effect on radiological CHD. The beneficial effect of higher BW at three months could be used in support of the suggested exercise related preventive strategies. The higher BW might be due to increased muscle mass which subsequently can be beneficial for

hip joint development. Riser (1975) reported that confinement of puppies prevented development of CHD, possibly because this treatment maintained hip joint congruence. It should be possible to develop non-surgical treatment methods to maintain hip joint congruence and prevent CHD, which also allow normal social behavior of puppies.

It was recently reported that CHD is predictable from genomic data (Guo et al., 2011). If such genomic predictions become generally available and puppies can be genotyped at young age, this information could be used to select candidates for primary preventive management and thus possibly provide a good quality-of-life for dogs with high CHD-risk genotypes.

#### *Secondary prevention (III, IV)*

Radiological CHD status was studied for potential influence on longevity and clinical signs in Paper III and IV, respectively. The findings indicate that the severe radiological CHD grade influences survival, especially in NF and LEO, and that increasing severity of radiological CHD status shortens time to development of clinical signs related to the hips.

When considering long-term prognosis for development of clinical signs related to the hip joints, provision of regular exercise both off and on-leash in different terrains at 12 months of age (and probably onwards) can delay time to occurrence of clinical signs and are potential secondary preventive efforts. Regular exercise strengthens muscle function and can improve range of motion of joints and thus be beneficial for dogs with CHD and DJD.

## Conclusions

- The incidence risk of radiological CHD varied by breed.
  - NF had the highest incidence risk of 36% and IW the lowest incidence risk of 10%.
  - LEO and LR had incidence risks of 25% and 20% respectively.
  - Both LEO and IW had higher incidence risk of radiological CHD than previously assumed.
- The findings failed to support the hypothesis that when comparing these large sized breeds, large and fast growing dogs were at increased risk of a radiological CHD diagnosis when compared to lighter and slower growing dogs.
  - NF was the slowest growing breed, but had the highest incidence risk of CHD.
  - IW had faster growth than NF, but had the lowest incidence risk of CHD.
  - LEO had slower growth than LR, but had higher incidence risk of CHD.
  - Higher BW at three months of age decreased the risk of radiological CHD when controlling for breed, and none of the other weight measurements had significant influence.
- Season of birth, housing and exercise conditions were significantly associated with the risk of a radiological CHD diagnosis.
  - Being born during spring or summer halved the risk of radiological CHD.
  - Breeder house type being farm/small farm more than halved the risk of radiological CHD.

- The use of stairs on a daily basis doubled the risk of radiological CHD while off-leash exercise in park terrain more than halved the risk and these two effects were limited to the first three months of life.
- Only the severe grade of radiological CHD status influenced survival.
  - The effect of severe CHD on the hazard of dying varied over time; initially the HR was over 200, but decreased dramatically to seven by two years of age.
  - By eight years of age the effect of severe CHD on the hazard of dying was almost equal to the effect of the less severe CHD grades.
- Radiological CHD status and exercise conditions during growth influenced time to clinical signs related to the hips.
  - There were breed differences and LR and LEO developed these signs later than NF.
  - Increasing severity of radiological CHD status yielded shorter time to hip related clinical signs and HR for severe CHD was five to seven times that for mild and moderate CHD and 92 times that of CHD grade free.
  - Off-leash exercise in garden and rough terrain and on-leash exercise on asphalt at 12 months of age increased time to hip related clinical signs and thus had a protective effect with HR approximately on third compared to dogs not exercised this way.
  - No effect of BW measurements was found.

- The results regarding clustering at the litter level were depending on the outcome of the analyses.
  - A large and statistically significant litter variance of approximately 20% was found on the risk of radiological CHD when considering BW/growth measurements and housing /exercise conditions.
  - A statistically significant litter variance of 27% was also found on time to hip related clinical signs.
  - The litter variance regarding overall survival was as small as 3.5% and non-significant.
  
- Preventive efforts and practical recommendations for breeders and owners of large breed dogs can be suggested based on the findings in this thesis.
  - Growth is most rapid during the first three months of life and preventive efforts aimed at reducing the risk of radiological CHD at screening will probably be most effective during this time-period.
  - Provision of varied physical activity on soft ground and in moderately rough terrain during the first three months of life can be beneficial with regard to radiological CHD, but the use of stairs on a daily basis should be avoided in this period.
  - Provision of varied physical activity at 12 months of age and later seems beneficial with regard to when clinical signs related to the hip joints occur.

- Dogs with severe CHD can be expected to develop clinical signs earlier than dogs with the less severe grades and additionally to have decreased survival, especially in the breeds NF and LEO.

## Future perspectives

The studies in this thesis have provided new insight regarding the environmental factors associated with radiological CHD diagnosis as well as the long-term effects of this diagnosis. The detection of a time-period which seems important regarding the development of CHD can be used in future studies regarding etiology and prevention. However, the studies have also identified areas regarding etiology, development, and prevention of CHD in which further research is needed:

- Evaluate interactions between BW and exercise conditions by combining the significant findings regarding growth and weight with significant findings regarding housing and exercise conditions (Paper I and II).
- Further investigation of the growth patterns by use of other mathematical models studying the rapid growth period from birth to three months of age.
- Separation of the additive genetic effect from maternal, environmental, and litter specific effects to further explore the litter variance.
- Investigate genetic effects, gene-environment interactions, and epigenetic changes.
- One hypothesis is that regular physical activity at soft and non-slippery terrain in the period from birth until three to six months of age improves muscle strength enough to prevent conversion of passive hip joint laxity to functional (weight-bearing) hip joint laxity in genetically predisposed puppies and thus prevent the development of the more severe CHD grades. To test this hypothesis, hip joint laxity measurements and



muscle size measurements could be done at regular intervals during the first year of life in groups of puppies exposed to different housing and exercise conditions.

- Another hypothesis is that maintained hip joint congruity the first three months of life could prevent severe CHD. To test this hypothesis imaging methods applied on the hip joints could be used to compare groups of puppies preferably from different breeds exposed to different non-surgical treatment regimens aimed at maintaining hip joint congruity.

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## A prospective study on Canine Hip Dysplasia and growth in a cohort of four large breeds in Norway (1998–2001)

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### ABSTRACT

The study-objective was to measure the effect of weight and growth related parameters on the risk of development of Canine Hip Dysplasia (CHD). The hypothesis was that heavy and fast growing dogs of large sized breeds were at increased risk of development of CHD compared to lighter and slower growing dogs. A prospective cohort study was conducted among dogs of four large breeds: Newfoundland (NF), Leonberger (LEO), Labrador retriever (LR), and Irish wolfhound (IW). The dogs were privately owned with individualized nutrition and environment, and they were followed from birth and throughout the growth period until the official screening for CHD was performed. The study sample consisted of 501 dogs from 103 litters, with the breed distribution 125 NF, 180 LEO, 133 LR, and 63 IW. Because the dogs were clustered in litters a multivariable random effects logistic regression model was used to assess statistically significant growth-related risk factors for CHD. The estimated incidence risk of CHD was 36% in NF, 25% in LEO, 20% in LR, and 10% in IW. Based upon the final multilevel model it appears that the odds of CHD among both LR and IW (odds ratio (OR) 0.22) are about one-fifth of the odds for NF. The odds for LEO (OR 0.60) are not significantly different from NF. There appeared to be an inverse relationship between body weight at 3 months of age and odds of CHD, with an OR of 0.89 ( $P=0.044$ ). The degree of clustering at the litter-level was high (22.6%) and highly significant ( $P<0.001$ ). Findings failed to support the hypothesis that heavy and fast growing dogs from four large sized breeds were at increased risk for development of CHD. There might be other unmeasured environmental risk factors for CHD in this cohort of dogs, although the contribution of the genetic variance to the litter-level clustering also needs further investigation.

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### 1. Introduction

Canine Hip Dysplasia (CHD) is a common developmental orthopedic disease (Todhunter and Lust, 2003). Although not completely understood, two broad etiological categories have been proposed; hip joint laxity causing hip instability and abnormal progression of the endochondral

ossification in multiple joints (Todhunter and Lust, 2003). In puppies, lesions in the coxofemoral joint can be observed as early as 14–30 days of age (Riser, 1975; Alexander, 1992).

The true occurrence of CHD is not known, although there are several reports on the prevalence of CHD from official registries in different countries. However, it has been suggested that the prevalence of CHD in many registries may not be truly representative of the general or breed specific populations because a relatively small proportion of the dogs are examined for CHD and also because dogs affected by severe forms of CHD often are not officially screened

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(Smith, 1997; Leppanen and Saloniemi, 1999; Paster et al., 2005; Genevois et al., 2008; Kaneene et al., 2009). Reported breed prevalences vary from 2% to 67%, and in general large and giant breeds have the highest prevalences although exceptions exist. Medium and small sized breeds appear to have lower prevalences (Priester and Mulvihill, 1972; Corley and Hogan, 1985; Lingaas and Heim, 1987; Fluckiger et al., 1995; LaFond et al., 2002).

Conventional radiography of the hip joints or distraction index radiography (PennHip) is used to diagnose CHD (Adams, 2000; Adams et al., 2000; Dassler, 2003). Efforts to reduce the occurrence of CHD have been made by radiographic screening of dogs at a certain age and subsequently imposing breeding restrictions for dogs with radiographic diagnosis of CHD (Corley and Hogan, 1985; Lingaas and Klemetsdal, 1990; Willis, 1997; Swenson et al., 1997; Leppanen and Saloniemi, 1999; Heim, 1999; Janutta et al., 2008). The screening procedure has been standardized worldwide, and there are three somewhat different radiographic scoring modes in use; the Fédération Cynologique Internationale (FCI), the Orthopedic Foundation for Animals OFA (OFA), and the British Veterinary Association/The Kennel Club (BVA/KC) (Fluckiger, 2007).

Several studies on heritability of CHD have been based on the radiographic diagnosis from official registries without considering clinical signs and support a genetic basis for CHD with moderate to high heritability estimates (Swenson et al., 1997; Maki et al., 2000; Janutta and Distl, 2006; Malm et al., 2008; Engler et al., 2008; Madsen, 2008). Thus CHD is considered to be a genetic disease, but there also appears to be environmental factors which affect the development of dysplasia in the hip joints of genetically predisposed puppies (Fries and Remedios, 1995; Janutta et al., 2006).

The most extensively studied factors regarding development of CHD are rapid growth rate and high food consumption, and most investigators conclude that overfed dogs grow faster than dogs fed a restricted diet and hence are more prone to the development of CHD (Lust et al., 1973; Kasstrom, 1975; Kealy et al., 1992). Dietary components, such as the amount of protein and calcium in the diet, have also been studied (Richardson, 1992). Furthermore, body type and body condition have been studied as risk factors for development of CHD (Comhaire and Snaps, 2008; Roberts and McGreevy, 2010). Other environmental risk factors for the development of CHD are not extensively studied, but hormonal influences and amount of exercise have been investigated as possible predictors (Lust et al., 1973; Kasstrom et al., 1975; Goldsmith et al., 1994; Steinetz et al., 2008).

Most studies regarding development of CHD are done in populations of dogs preselected for certain purposes (e.g. guide dogs) or as controlled studies. These dogs are often offspring of hip dysplastic parents, and they are living in kennels with feeding, exercise, and housing regimen controlled and standardized and therefore not necessarily representative of privately owned dogs with a more diverse husbandry. We hypothesized that heavy and fast growing dogs in a prospectively followed cohort of four large sized breeds were at increased risk for development of CHD when compared to lighter and slower growing

dogs. The aim of the current study was to measure the effect of weight and growth related parameters on the odds of development of CHD in privately owned dogs followed from birth and throughout the growth period until the diagnosis of CHD was made.

## 2. Materials and methods

The study was carried out in agreement with the provisions enforced by the National Animal Research Authority (NARA)

### 2.1. Study design

The present study is part of a larger study (the so-called *main study*) aimed at investigating the effects of risk factors on the occurrence of skeletal diseases: CHD, elbow dysplasia, panosteitis, and osteosarcoma. The *main study* included dogs from four large breeds: Newfoundland (NF), Labrador retriever (LR), Leonberger (LEO), and Irish wolfhound (IW). A prospective single cohort study was conducted to investigate factors affecting the development of CHD in growing dogs from the *main study*.

### 2.2. Inclusion of dogs

Puppies born in Norway between November 1998 and June 2001 were eligible for inclusion in the *main study*. All geographic areas of Norway were represented. The breeding stock consisted of dogs born in Norway as well as dogs that had been imported. Inclusion of a litter began when the bitch was mated. All puppies were registered in the Norwegian Kennel Club (NKC).

Each breeder, dog owner and veterinarian who participated in the project signed a written agreement of cooperation to comply with the project plan. Not all dogs enrolled in the study continued to completion. Reasons for dropouts included but were not limited to death of the dog, relocation of the owners during the study, and exportation of dogs abroad (Trangerud et al., 2007a).

Inclusion criteria for the present study were that the dogs were officially screened for CHD and that they had at least one recorded weight measurement during the growth period. The dogs were privately owned, and each dog had a housing and feeding regimen decided by its owner.

### 2.3. Screening for CHD

The signed written agreement of cooperation encouraged dog owners to have their dogs officially CHD screened. LR and IW were radiographed at 12 months of age, and LEO and NF at 18 months of age, which are the screening ages for these breeds in the NKC. More than 90% of the radiographs were scrutinized by the same radiologist at the NKC. All dogs were sedated or anaesthetized before the radiographic examination to achieve complete muscle relaxation. The identity of the dogs (NKC registration number, tattoo or microchip) was photographed onto the film before developing the radiographs. The radiographs were made at 100-cm film to focus distance.

The dogs were placed in dorsal recumbency with the hind limbs extended and abducted so that the patellae

were superimposed over the femora. The entire pelvis and femora including the patellae were included on the radiographs. The FCI five class grading scale was used to classify the hip status of the dogs: A (excellent), B (normal), C (mild dysplasia), D (moderate dysplasia) and E (severe dysplasia). The CHD grades are defined descriptively based on the size of the Norberg angle (NA), degree of subluxation, shape and depth of the acetabulum, and signs of secondary joint disease (Flückiger, 2007). Each hip joint was graded separately, and the final grading was based on the worst hip joint. Dogs graded C (mild dysplasia), D (moderate dysplasia), and E (severe dysplasia) were considered affected by CHD.

## 2.4. Questionnaires and clinical registrations

History, husbandry and clinical information for each included dog were obtained from three sources: (1) the breeder of the litter, (2) the owner of the puppy, and (3) the veterinarian examining the dog. All three sources completed questionnaires and recorded information in a booklet prepared for each included dog. All questionnaire sheets appeared in duplicates in the booklet, so that one sheet could be mailed to the researchers and a copy retained in the booklet.

The breeder was asked to record the bodyweight in grams for each puppy at birth and on days 3 and 7, and then weekly until 56 days of age. The breeder decided the feeding and housing regimen of the litter, and this information along with information about any medications given including routine antiparasite treatments were recorded in the booklet. The puppies stayed with the bitch until approximately 8 weeks of age when they were sold.

The owners reported information regarding feeding, housing, exercise, and any signs indicative of disease in their dogs. The owner completed the questionnaires and reported information regarding the dog at the scheduled ages 3, 4, 6, and 12 months (so-called observational ages).

The owners agreed to have their dogs examined at the scheduled veterinary visits. Clinical examination, blood sampling, measurement of bodyweight in kg, and measurement of the circumference of the distal radius and ulna (CDRU) in cm were done at each of the veterinary visits. Vaccinations and antiparasitic treatments were administered at the veterinary visits. Average daily gain (ADG) was generated from the weight measurements for specific intervals of the growth period until 12 months of age. Changes in CDRU between ages (CDRU change) were generated from the measurements of CDRU.

## 2.5. Laboratory analyses

Complete blood counts (CBCs) were carried out at ages 3, 4, 6, and 12 months, and the blood serum was analyzed for the following 20 biochemical variables: alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine kinase (CK), amylase, lipase, total protein, albumin, globulin, urea, creatinine, bile acids, total bilirubin, cholesterol, glucose, inorganic phosphate, potassium, calcium, sodium, and chloride. The

analyses were conducted at the Central Laboratory, Norwegian School of Veterinary Science, Oslo, Norway.

## 2.6. Statistical analyses

The software package Stata 11 (Stata Corporation, 4905 Lakeway Drive, College Station, TX 77845, USA) was used for all analyses.

### 2.6.1. Power calculation

The power of the present study to detect a difference of 15% and 20% in occurrence of CHD was calculated for the whole cohort with significance level 0.05 and a two-sided test.

### 2.6.2. Descriptive statistics

The incidence risks of CHD were calculated by relating the number of CHD-affected dogs to the total number of dogs in each breed in the cohort. Mean litter size in the four breeds was estimated, as well as sex distribution and mean litter size in dogs with CHD and without CHD. Bodyweight data separated by breed, sex, and the groups with and without CHD were fitted to a Gompertz function. A Gompertz function is a nonlinear, sigmoid function, with its point of inflection at 36.8% of mature bodyweight. Growth was modeled with the following equation (Helmink et al., 2000), using the NLIN procedure (SAS Inst. Inc., Cary, NC):

$$W_t = W_{\max} \exp\{-e^{-(t-c)/b}\},$$

where  $W_t$  is the bodyweight at time  $t$ ,  $W_{\max}$  the mature bodyweight,  $b$  the proportional to duration of growth,  $c$  the age at point of inflection, 36.8% of mature bodyweight is reached,  $t$  is the age in days.

Analyses were carried out separately for each breed and sex and groups with and without CHD. Duration of growth was estimated by  $(4b + c)$ , which describes 98% of the growth duration (Helmink et al., 2000). The derivative of the Gompertz function describes the growth rate.

### 2.6.3. Multivariable analyses

For the purpose of these analyses the hip status was reclassified into 2 categories: free (grades A and B) and affected (grades C, D, and E). Thus the dependent variable CHD is a dichotomous variable (CHD free or CHD affected), and a logistic regression model of the relationship between predictors and CHD was considered. The dogs in the study were clustered into litters which violate the assumption of independence between observations, and a random effect for litter was therefore included in a generalized linear mixed model. A random effect for breeder was considered, but since the breeder: litter ratio was close to one, the breeder level was omitted.

Due to the large number of variables, they were arranged in three groups of risk factors: signalment data, weight/growth/size measurements, and blood sample variables. Overview of the type of variables in these groups is presented in Table 1.

Associations between the dependent variable and the predictor variables were first screened with univariable random effects logistic regression. Variables with a  $P$ -value

**Table 1**

Overview of the variables used to investigate potential risk factors regarding weight/growth and Canine Hip Dysplasia (CHD) in four large breeds in Norway (1998–2001).

Variable	Definition	Abbreviated name
Signalment data (3 variables)		
Breed	Breed of the dog (categorical): Newfoundland Labrador retriever Leonberger Irish wolfhound	NF LR LEO IW
Sex	Sex of the dog (dichotomous): female, male	Sex
Litter size	Number of puppies in each litter (continuous)	Litter size
Weight, growth, and size measurements (34 variables)		
Bodyweight	Bodyweight in grams at birth, and at 3, 7, 14, 21, 28, 35, 42, 49, and 56 days of age (continuous) Bodyweight in kg at 3, 4, 6, and 12 months of age (continuous)	BW
Average daily gain	Average daily gain in kg/day in the following periods (continuous): Birth to 7 days Birth to 14 days Birth to 21 days Birth to 56 days Birth to 3 months Birth to 6 months Birth to 12 months 35 days to 3 months 56 days to 3 months 3–4 months 4–6 months 6–12 months	ADG
Circumference of distal radius and ulna	Measurement of this circumference in cm at 3, 4, 6, and 12 months of age (continuous) Changes in these measurements between the following ages (continuous): 3–4 months 3–12 months 4–6 months 6–12 months	CDRU
Blood sample variables (135 variables)		
Complete blood count	Counts of leucocytes, erythrocytes, thrombocytes, neutrophils, eosinophils, lymphocytes, monocytes, and basophils at 3, 4, 6, and 12 months of age (continuous)	CBC
Clinical chemistry parameters	Measurement of mean cell volume, hematocrit, hemoglobin, and mean cell hemoglobin concentration at 3, 4, 6, and 12 months of age (continuous) Measurement of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine kinase (CK), amylase, lipase, total protein, albumin, globulin, urea, creatinine, bile acids, total bilirubin, cholesterol, glucose, inorganic phosphate, potassium, calcium, sodium, chloride, and sodium/potassium ratio at 3, 4, 6, and 12 months of age (continuous) Changes in ALP between the following ages (continuous): 3–4 months 4–6 months 6–12 months	CCP

$\leq 0.20$ , provided that there was no colinearity ( $r < 0.70$ ) between variables, were then considered for further analysis in a multivariable random effects logistic regression model to assess the relationship with CHD. For screening of the blood parameters a more restrictive  $P$ -value of 0.10 was applied due to the large number of variables. When colinearity was detected between two predictors, the predictor with least missing data was selected. Lowess curves were used to assess the linear relationship between the continuous variables and the logit of the outcome. Variables with many missing values (>20% missing observations) were not used in the multivariable analysis.

The Stata command `xtmelogit` using adaptive quadrature was used for the multilevel modeling. A multivariable random effects logistic model for signalment and

weight/growth/size measurements was constructed using manual backward elimination. Predictor variables were retained in the models when the  $P$ -value was  $< 0.05$ . Potential confounding and intervening variables were considered after constructing a causal diagram. Changes of more than 20% in the coefficients in the model with the potential confounder present were used as additional indication of confounding. Interactions between significant predictors were tested by adding an interaction term to the final model, and the interaction term retained when  $P < 0.01$ . Following manual backward elimination, the model was built again by forward selection by offering the excluded variables one at a time to the final model. A variable was considered intervening if adding it removed the entire effect of another variable. Intervening

variables were excluded from the final model. The likelihood ratio test (LRT) was used to evaluate the significance of the random litter effect in the models with and without random litter effect, but containing the same fixed effects. The LRT was considered significant at  $P=0.05$  and one-sided test. Due to the large number of variables the blood sample variables were analyzed in a separate sub model, and the same model building procedure was applied to these variables. Significant and biologically explainable variables from this sub-model were entered to the weight model and retained if they were significant at the  $P<0.05$  level. The multiple Wald test and LRT were used to evaluate differences between categories of categorical predictors. The Stata command `lincom` was used to conduct contrasts among each category of categorical predictors.

From the final multivariable random effects logistic regression model the between litter variance ( $\sigma^2_{\text{litter}}$ ) was estimated. The intraclass correlation coefficient (ICC) calculated by the latent variable approach, assuming that dog level variance ( $\sigma^2$ ) is the constant  $\pi^2/3$ , was calculated using

$$\text{ICC} = \frac{\sigma^2_{\text{litter}}}{\sigma^2 + \sigma^2_{\text{litter}}}$$

To evaluate and assess the fit of the final multi-level model the residuals at the dog level were estimated and the residuals at the litter level were estimated and evaluated by plotting of residuals against both predicted values and against fitted values to evaluate homoscedasticity and normality.

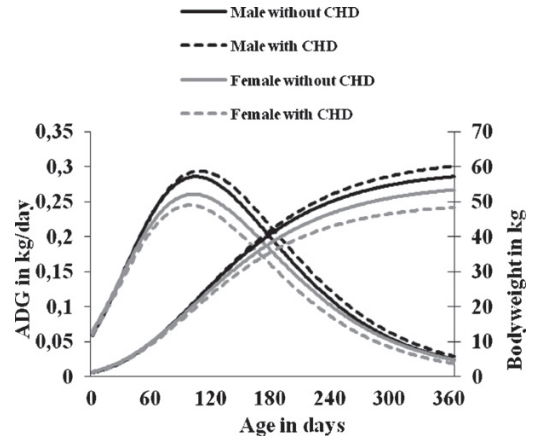
### 3. Results

#### 3.1. Study sample

In total, 647 dogs from 106 litters from the *main study* were eligible for inclusion in the present study, which is a convenience sample consisting of 23.2% of the total number of litters born in the included breeds in Norway. Of these dogs, 501 dogs from 103 litters were officially screened for CHD and thus included in the study sample. Among dogs eligible for inclusion, 146 were not officially screened and thus not included. Reasons for not screening were commonly due to death of the dog or unwillingness of the owner to take the cost of radiographing the dog. The 103 litters were the offspring of 94 dams from 86 different breeders.

The breed distribution was 180 LEO, 125 NF, 133 LR and 63 IW. The vast majority of the CHD radiographs were scrutinized by the radiologist in NKC ( $N=491$ ), and 10 dogs had their radiographs scrutinized by the radiologists in the Swedish and Danish Kennel Club. The power of the present study was 0.85 for the cohort of 501 dogs.

Not all breeders, owners, and veterinarians that completed the questionnaires answered all questions at all observational ages, hence there was a varying number of missing values.

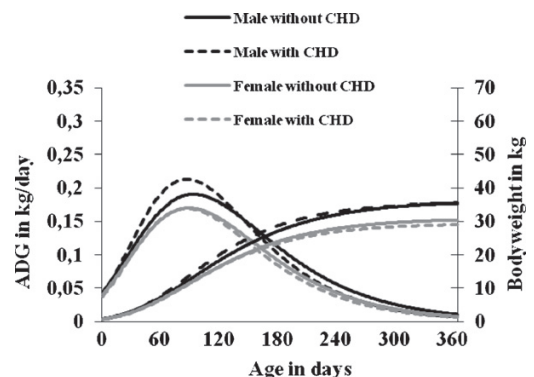


**Fig. 1.** Gompertz derivative (left axis) and average growth (ADG) curves (right axis) estimated with Gompertz function for male and female Newfoundland in Norway with and without Canine Hip Dysplasia (CHD) (1998–2001).

#### 3.2. Descriptive statistics

Among the 501 dogs that were officially screened for CHD there were 260 females (51.9%) and 241 males (48.1%). There were 123 dogs (24.6%) affected by CHD. NF was the breed with the highest occurrence of CHD with an 18 months incidence risk of 36%. In LEO, the 18 months incidence risk was 25%, and in LR and IW the 12 months incidence risks were 20%, and 10%, respectively. The mean litter size was smallest in NF with a mean of 7.5 puppies followed in increasing order by IW (8.0 puppies), LR (8.6 puppies), and LEO (9.0 puppies). Distribution of CHD among sexes and mean litter size in dogs with and without CHD in the four breeds are outlined in Table 2.

ADG and bodyweight are shown in Figs. 1–4, and least squares means for the variables of the Gompertz func-



**Fig. 2.** Gompertz derivative (left axis) and average growth (ADG) curves (right axis) estimated with Gompertz function for male and female Labrador retriever in Norway with and without Canine Hip Dysplasia (CHD) (1998–2001).



**Table 2**

Descriptive statistics of the variables sex and litter size in dogs with and without Canine Hip Dysplasia (CHD) in four large breeds in Norway (1998–2001).

	CHD <sup>b</sup>	Breed <sup>a</sup>			
		NF N (%)	LR N (%)	LEO N (%)	IW N (%)
Female	0	38 (60.3)	50 (75.8)	70 (72.2)	31 (86.1)
	1	25 (39.7)	16 (24.2)	27 (27.8)	5 (13.9)
Male	0	42 (67.7)	56 (83.6)	63 (78.3)	26 (96.3)
	1	20 (32.3)	11 (16.4)	18 (21.7)	1 (3.7)
Litter size <sup>c</sup>	0	7.8 (1, 12)	8.7 (6, 12)	9.0 (2, 13)	8.2 (4, 12)
	1	6.8 (1, 12)	8.1 (6, 12)	8.7 (3, 13)	6.8 (4, 11)

<sup>a</sup> Newfoundland (NF), Labrador retriever (LR), Leonberger (LEO), and Irish wolfhound (IW).<sup>b</sup> 0 = without CHD, 1 = with CHD.<sup>c</sup> Mean litter size (minimum, maximum).

tion by CHD status and mean birth weight are outlined in Table 3. Estimated bodyweight increased rapidly during the first 100 days and then plateaued reaching maturity between 422 days (male NF with CHD) and 335 days (male LR with CHD) (Figs. 1–4, Table 3). ADG expressed by the derivative of the Gompertz function, reached its maximum value between 87 days (female LR with CHD) and 109 days (male NF with CHD), after which is gradually declined as mature bodyweight was reached (Figs. 1–4, Table 3). The growth curves for the groups with and without CHD, diverged slightly after approximately 60 days, and females with CHD being less heavy than females without CHD, most pronounced in NF (Figs. 1–4). For the males the divergence of the curves was most obvious for male NF and LEO where the males with CHD appeared to be heavier (Figs. 1–4). In male LR with CHD there seemed to be a peak in ADG between approximately 50 and 120 days (Fig. 2).

### 3.3. Multivariable analyses

Weight variables which did not meet the criteria for further model building were weight at birth, 3, 7, 21–56 days, 4 months, 6 months, 12 months, ADG variables in the period from birth to 4 months, from 6 to 12 months, and birth to 12 months. All of the CDRU measurements had *P*-values greater than 0.20 in univariable screening, and they were not considered in further analysis. Colinearity ( $r > 0.7$ ) was detected between the following variables: body weight at 3 days, 42 days, 49 days, and 3 months, and ADG from birth to 3 months, from 2 to 3 months, from birth to 14 days, and from birth to 56 days. The variables with least missing data were selected for further analyses.

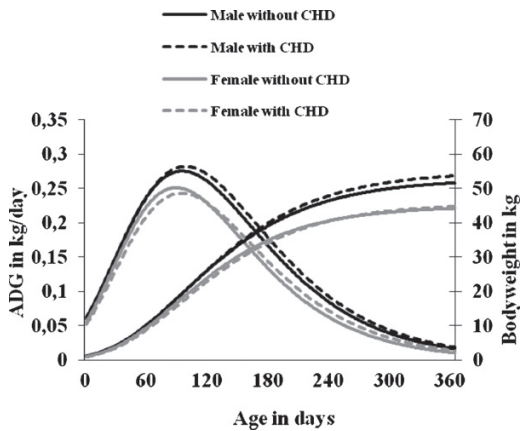
From unconditional screening ( $P < 0.10$ ) of the blood parameters, the following variables were selected for building of a sub-model: ALP change from 6 to 12 months (OR 1.74), chloride 3 months (OR 0.90), sodium/potassium

**Table 3**Least squares means for the variables<sup>a</sup> of the Gompertz function and mean birth weight by status on Canine Hip Dysplasia (CHD) and sex in four large breeds in Norway (1998–2001).

Breed	CHD <sup>b</sup>	$V_{\max}$ (kg) Estimate (SE)	$c$ (days) Estimate (SE)	$b$ (days) Estimate (SE)	$4b + c$ (days) Estimate (SE)	Birth weight (g) Mean (SE)
Newfoundland						
Male	0	59.04 (0.35)	105.20 (1.07)	76.05 (1.32)	409.40 (6.24)	611 (14.3)
	1	62.44 (0.89)	109.10 (2.29)	78.17 (2.78)	421.78 (18.27)	637 (28.9)
Female	0	55.22 (0.42)	102.80 (1.47)	77.99 (1.94)	414.76 (9.90)	584 (17.3)
	1	49.77 (0.53)	99.23 (1.74)	74.79 (2.15)	398.39 (12.20)	579 (20.3)
Labrador retriever						
Male	0	36.21 (0.20)	94.11 (0.90)	70.07 (1.18)	374.40 (5.27)	406 (12.6)
	1	35.80 (0.55)	87.26 (2.30)	61.97 (2.93)	335.14 (16.67)	417 (38.2)
Female	0	30.95 (0.21)	89.61 (1.06)	66.90 (1.41)	357.19 (6.45)	390 (11.6)
	1	29.44 (0.26)	87.12 (1.29)	63.87 (1.63)	342.60 (8.01)	374 (15.4)
Leonberger						
Male	0	55.76 (0.28)	95.68 (0.91)	70.55 (1.16)	377.95 (5.22)	510 (12.5)
	1	55.00 (0.53)	98.96 (1.71)	71.77 (2.27)	386.05 (12.01)	513 (23.9)
Female	0	44.84 (0.23)	89.80 (0.84)	65.75 (1.09)	352.83 (4.84)	464 (10.9)
	1	45.70 (0.46)	95.09 (1.71)	69.20 (2.27)	371.90 (11.79)	469 (31.1)
Irish wolfhound						
Male <sup>c</sup>	0	66.64 (0.43)	106 (1.18)	76.26 (1.50)	411.10 (7.23)	603 (26.3)
	1	63.31 (1.15)	103.00 (2.91)	69.62 (3.62)	381.49 (27.14)	580 (-)
Female	0	55.82 (0.52)	93.80 (1.50)	67.93 (1.89)	365.51 (9.81)	614 (21.3)
	1	54.66 (1.16)	95.30 (3.14)	69.07 (4.18)	371.56 (30.89)	680 (10.0)

<sup>a</sup>  $W_{\max}$  = mature weight,  $c$  = age at point of inflection (36.8% of mature bodyweight),  $b$  = proportional to duration of growth,  $4b + c$  = duration of growth (98% of the growth duration).<sup>b</sup> 0 = without CHD, 1 = with CHD.<sup>c</sup> Only one male IW with CHD.



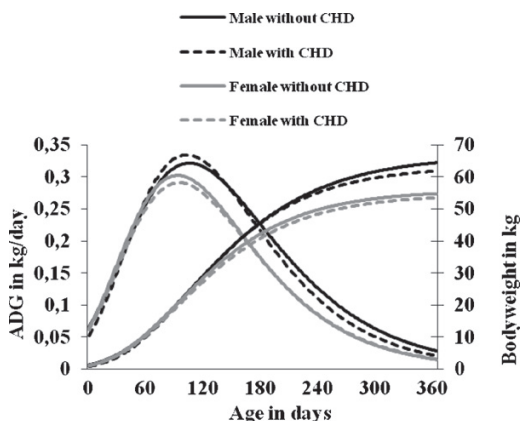


**Fig. 3.** Gompertz derivative (left axis) and average growth (ADG) curves (right axis) estimated with Gompertz function for male and female Leonberger in Norway with and without Canine Hip Dysplasia (CHD) (1998–2001).

ratio 3 months (OR 0.88), leucocytes 6 months (OR 0.83), and neutrophils 6 months (OR 0.77). When building this sub-model ( $P < 0.05$ ), ALP change from 6 to 12 months and sodium/potassium ratio 3 months were retained, but only the change in ALP from 6 to 12 months was considered both significant and biologically plausible. No collinearity was detected among these variables.

Therefore the variables breed, sex, litter size, bodyweight at 14 days and 3 months, ADG from 4 to 6 months, and change in ALP from 6 to 12 months were variables considered for inclusion in the multilevel model. The unconditional OR,  $P$ -value, and 95% confidence interval (CI) for these variables are outlined in Table 4.

Breed and bodyweight at 3 months were the only significant variables in the final model. Litter size was forced in the model as a confounder. The following interactions



**Fig. 4.** Gompertz derivative (left axis) and average growth (ADG) curves (right axis) estimated with Gompertz function for male and female Irish wolfhound in Norway with and without Canine Hip Dysplasia (CHD) (1998–2001).

**Table 4**

Unconditional associations (with  $P \leq 0.20$ ) from univariable mixed logistic regression models between risk factors related to signalment and weight/growth and Canine Hip Dysplasia (CHD) in four large breeds in Norway (1998–2001).

Variable	Odds ratio	$P$ -value	95% confidence interval
Breed <sup>a</sup>			
NF	1.00	–	–
LR	0.38	0.03	0.15–0.91
LEO	0.44	0.05	0.20–1.00
IW	0.13	0.001	0.04–0.45
Sex			
Female	1.00	–	–
Male	0.72	0.173	0.44–1.16
Litter size	0.86	0.021	0.75–0.98
BW <sup>b</sup>			
Day 14	1.83	0.137	0.82–4.07
3 months	0.94	0.205	0.86–1.03
ADG <sup>c</sup>			
4–6 months	22.37	0.175	0.25–1998.93
ALP <sup>d</sup>			
6–12 months	1.74	0.049	1.00–3.01

<sup>a</sup> Newfoundland (NF), Labrador retriever (LR), Leonberger (LEO), and Irish wolfhound (IW).

<sup>b</sup> Bodyweight (BW).

<sup>c</sup> Average daily gain (ADG) from 4 to 6 months.

<sup>d</sup> Serum alkaline phosphatase (ALP), change from 6 to 12 months.

were tested: breed  $\times$  litter size, breed  $\times$  bodyweight at 3 months, and litter size  $\times$  bodyweight at 3 months. None of the tested interactions were significant. Manual backward elimination and forward selection procedures both resulted in the same model. Regression coefficients ( $\beta$ ), OR,  $P$ -values, and 95% CI for the variables included in the final model and the ICC for the model are presented in Table 5. The OR for bodyweight at 3 months decreases by 0.10 for an increase across the interquartile range (IQR 13.6–18.7 kg). The OR for litter size decreases by 0.13 with an increase across the IQR (7–10 puppies). The multiple Wald test and LRT for comparing models with and without the categorical variable breed were both significant with  $P = 0.0062$  and  $P = 0.0063$ , respectively. The differences between LR and LEO (OR 0.37,  $P = 0.052$ ), LR and IW (OR 1.00,  $P = 0.990$ ), and LEO and IW (OR 2.72,  $P = 0.105$ ) were not significant.

### 3.4. Model evaluation

The litter level residuals from the random part of the final model showed no evidence of heteroscedasticity or lack of normality. No extreme values were found in the dog level residuals.

## 4. Discussion

The main findings in this cohort study of growth related risk factors for CHD were that the incidence risk varied by breed, that there appeared to be an inverse relationship between body weight at 3 months of age and odds of CHD, that CDRU and certain blood parameters were not associated with the odds of CHD, and that there was a strong clustering of the log odds of CHD within litters. Based on the findings we rejected our hypothesis that odds of CHD would be greater in heavy and fast growing dogs from large

**Table 5**

Results from the final multivariable random effects logistic regression model for weight/growth related risk factors for Canine Hip Dysplasia (CHD) in four large breeds in Norway (1998–2001).

Variable	$\beta$	Odds ratio	P-value	95% confidence interval
Breed <sup>a</sup>				
NF	–	1.00	–	–
LR	–1.498	0.22	0.005	0.08–0.63
LEO	–0.507	0.60	0.229	0.26–1.37
IW	–1.507	0.22	0.016	0.07–0.75
BW <sup>b</sup>				
3 months	–0.112	0.89	0.044	0.80–0.99
Litter size <sup>c</sup>	–0.131	0.88	0.068	0.76–1.01
Overall P (final model) <sup>d</sup>			0.002	
ICC <sup>e</sup> (estimate)	0.226		<0.001 <sup>f</sup>	

<sup>a</sup> Newfoundland (NF), Labrador retriever (LR), Leonberger (LEO), and Irish wolfhound (IW).

<sup>b</sup> Bodyweight (BW).

<sup>c</sup> Included as confounder.

<sup>d</sup>  $\beta_0$  was 2.252 for the final model.

<sup>e</sup> Intraclass correlation coefficient.

<sup>f</sup> Likelihood ratio test of the random litter effect.

sized breeds. Each finding will be discussed in more detail in the following sections.

#### 4.1. Breed and sex differences

Incidence risk of CHD differed between the four breeds and the effect of breed on the odds of CHD was statistically significant, as judged by the LRT of the breed variable in the random effects model. The largest breeds NF and LEO had the highest incidence of CHD, whereas the smaller LR had a lower incidence. The OR for categories of breed from the final multilevel model indicates that both LR and IW have decreased odds of CHD when compared to NF (baseline breed) (Table 5). Based upon the final model it appears that the odds of CHD among both LR and IW are about one-fifth (OR 0.22) of the odds for NF, while the odds for LEO (OR 0.60) are not significantly different from NF. The differences in the odds of CHD comparing LR and LEO, LR and IW, and LEO and IW were however not significant either. Interpretations of all coefficients in the model are conditional upon the random effect.

The findings regarding breed differences are in accordance with other studies showing differences between breeds (Martin et al., 1980; LaFond et al., 2002). In general, large and giant breeds seem more susceptible to CHD. Some exceptions are breeds like the Collie, Borzoi, and IW which are large breeds with low risk (Priester and Mulvihill, 1972; Corley and Hogan, 1985; LaFond et al., 2002). In our study, IW had a low incidence when compared to the other breeds, but after controlling for litter, weight at 3 months and litter size the odds of CHD in IW and LR were identical when compared to NF (Table 5). Small and miniature breeds are to a much lesser extent studied with regard to occurrence of CHD. However, the disease might be just as common in these breeds as in the large ones; Martin et al. (1980) found that 25% of radiographed miniature poodles had CHD. A recent Swedish study has found a marked increased hazard of having clinical signs related to CHD with deteriorating hip status and with breed differences; the largest breeds having the greatest hazard (Malm et al., 2010). Thus clinical signs could be more common and the

disease more detrimental in large dog breeds. The clinical signs and prognosis in dogs affected by CHD is to be studied further in our cohort.

The variable sex was considered a possible confounder in the way that sex affects bodyweight and growth and that other studies of large breeds have found a significant effect of sex on the risk of CHD (Hedhammar et al., 1979; Martin et al., 1980; Lingaas and Heim, 1987; Swenson et al., 1997; Wood et al., 2002). The difference between males and females was not significant in our study, and no evidence of confounding was found. Hence the variable sex was excluded from the final multivariable model.

#### 4.2. Bodyweight and ADG

In the present study, dogs diagnosed with CHD had slightly higher mean birth weight compared to those not diagnosed with CHD, except for female NF and LR. During the growth period the differences in body weight and ADG were only modest between dogs with and without CHD, the difference being most obvious in female NF with CHD which were less heavy and had smaller ADG. Vanden Berg-Foels et al. (2006) found in LR that increased birth weight and increased early postnatal body weight increased the probability of degenerative changes in the hip joint later in life. In our study male LR with CHD seemed to have a peak in ADG although somewhat later in the growth period. In a previous study Trangerud et al. (2007a) modeled the growth pattern in dogs from the *main study* with the Gompertz function. In this model, as in ours, NF was the slowest growing breed and LR fastest growing breed. Interestingly, NF is the breed with the highest incidence of CHD.

The only weight variable in this study which was statistically significant ( $P=0.044$ ) in the final model was body weight at 3 months of age. There was an apparent protective effect of greater body weight at 3 months of age on the odds of CHD. The upper limit of the 95% CI approached one and the effect seems modest (Table 5), but the change in OR for this variable over the IQR is quite large. The result is in conflict with some studies, but in line with others (Kasstrom, 1975; Kealy et al., 1992; Ohlert et al., 1998;

Lopez et al., 2006). When growth is modeled with the Gompertz function, the point of inflection has been found to be at approximately 3 months of age (Trangerud et al., 2007a) and between 87 and 109 days in the present study (Figs. 1–4, Table 3). This may indicate that the body weight at, and growth rate around, 3 months of age might be important predictors of growth and possibly explain why body weight at 3 months was the only significant weight variable found in the present study.

Several studies have reported a correlation between early rapid growth and both frequency and severity of CHD. In these studies dogs fed *ad libitum* have been found to have a higher incidence of CHD than dogs on a restricted diet (Riser et al., 1964; Hedhammar et al., 1974; Kasstrom, 1975; Kealy et al., 1992). Lust et al. (1973) found that only abnormally slow growing and hand reared puppies seemed to a certain extent to be protected against development of CHD. Manipulating growth rate by restricted or *ad libitum* feeding might not cause CHD, but perhaps maximize the expression of the CHD phenotype (Todhunter and Lust, 2003). A recent study on a cohort of “mixed breed hounds” fed *ad libitum* revealed no significant effect of growth on hip distraction index. However, there was a trend for negative correlations between high distraction index and growth from 14 to 15 weeks of age (Lopez et al., 2006).

In a study of CHD in a colony of LR there was no significant association between weight measurements and risk for development of CHD (Ohlerth et al., 1998). Two recent cross-sectional studies have compared prevalence of CHD from official registries with the breed standards of weight, height, and body mass index (BMI). There seems to be a significant correlation between high prevalence of CHD and weight, BMI, and relative body length (Comhaire and Snaps, 2008; Roberts and McGreevy, 2010). Selection of body shapes that are longer than they are tall, in combination with high BMI can act together giving too much load on the coxofemoral joint thus exacerbating some characteristics of CHD such as subluxation and osteoarthritis (Roberts and McGreevy, 2010).

Most studies regarding rapid growth as risk factor for CHD have been done in dogs where either one or both parents have CHD, thus rendering the offspring more likely to be affected. Commonly, the studies include dogs pre-selected for certain purposes (e.g. guide dogs) and with housing and management controlled and standardized in a manner that will reduce the environmental variance (Lust et al., 1973; Kasstrom, 1975; Kealy et al., 1992; Ohlerth et al., 1998; Lopez et al., 2006). By reducing the environmental variance the effect of genetic variation will become relatively more important and estimates of heritability need to be interpreted with this in mind.

The present study differs from most other studies on CHD in several ways. In our study population of dogs, which are registered in the NKC, the occurrence of dysplastic parents is low as breeding restrictions exist on dogs with CHD in Norway. Furthermore, our study sample is heterogeneous consisting of dogs from 4 different breeds living in private homes. Hence they are under influence of maternal, genetic, and environmental factors which most probably influence growth simultaneously. Many studies regarding

growth on the risk of CHD were done 20–30 years ago and have provided the basis for recommendations on feeding of growing dogs, and especially the large breeds. There has been extensive research on dog feeds. The feeding of the dogs in our study is therefore likely to be different from what were common 20–30 years ago. Commonly puppies and young dogs of large breeds are fed diets designed for heavy fast-growing dogs and these diets intend to regulate growth thus reduce the risk of skeletal disorders. Size and body shape of the breeds might also have changed over this time period. These are all factors that might provide some explanation for the findings in the present study. Although weight and growth might be influenced by the diet, the incidence of CHD is high and genetic and other environmental factors are factors also influencing both weight and growth and the occurrence of CHD.

#### 4.3. CDRU and blood parameters

The measure CDRU was originally included as a measure of skeletal growth and thought to be a potential predictor of skeletal diseases affecting the metaphyses. CDRU has been investigated previously in growing dogs (Trangerud et al., 2007a), and as a clinical measure to identify pathological metaphyses (Trangerud et al., 2007b). Disturbances in endochondral ossification in several joints have been found in dogs affected by CHD (Todhunter et al., 1997). In the present study it was investigated whether CDRU was different between dogs affected by CHD and those not affected. However, there was no statistically significant difference between the two groups of dogs.

Hip joint laxity and abnormalities in endochondral ossification are both proposed as important factors in the etiology of CHD, and the two most probably act together (Todhunter and Lust, 2003). Differences in collagen types in dysplastic hip joint capsules and delayed chondroepiphyseal ossification and growth plate closure in dysplastic hips are found. The disturbances in endochondral ossification are not confined to the hip joint (Madsen, 1997; Todhunter et al., 1997). These disturbances might be reflected in the blood, and factors related to modeling and remodeling of bone during growth were thought of as biologically plausible when analyzing the data from the blood samples; serum ALP, calcium, and phosphorus. Serum ALP has been found to be lower in a population of NF affected by metaphyseal irregularities (Trangerud et al., 2007b), but not found significantly different among German shepherds with and without CHD (Szilagyi and Sagi, 1976). In our study none of the mentioned blood parameters turned out significant in the final model. There could be other factors and hormones that are measurable in blood which might be more suitable biomarkers of CHD.

#### 4.4. Clustering at litter level

In this study, a high and significant amount of clustering at the litter level was present; the ICC representing the proportion of variance at litter level was 22.6%. The variation in the log odds of CHD within a litter is smaller than between litters. Intraherd correlations for infectious diseases have been observed to range from 4% to 42%, but mostly below

20% (Otte and Gumm, 1997). In a study relating preweaning mortality in puppies to additive genetic, common-litter, and within-litter factors, the litter factors explained most of the variation (van der Beek et al., 1999).

The high level of clustering in the present study is most probably a result of common genetic background and common environment within a litter both before and after birth. This litter effect probably continues to have an influence after the puppies are delivered to their owners because the genetic background still is the same and many breeders have recommendations regarding management which the dog owners usually follow, at least to a certain extent. The environmental variance, i.e. all variation of non-genetic origin, is most probably greater in our study population than in dogs living in kennels under standardized conditions. Genotype by environment interactions have been described for several traits in several species (Lillehammer, 2008). Genotype–environment interaction is very important when individuals of a population are reared under different conditions (Falconer and Mackay, 1996) as the dogs in our cohort and might contribute to the observed litter effect. The high level of clustering at the litter level in this study makes estimation of how much the additive genetic variation contributes to the observed cluster effect important, and separation of additive genetic and litter variance would be desirable. Environmental influences on the CHD phenotype and development of clinical signs are to be further investigated in this cohort of large breed dogs.

#### 4.5. Validity

In this cohort study all risk factors are measured prior to the outcome, which is necessary for claims of causation. Cohort studies generally have high relevance to real-world situations and a relatively high external validity (Dohoo et al., 2009). All kinds of dogs, from highly rewarded breeding dogs to privately owned pet dogs from the four breeds, were included in our cohort, which increases the external validity. The present study is a large scale prospective study of large sized breeds, and the results might be valid for other populations of large sized breeds, but perhaps not for small and miniature breeds. Some of the dogs eligible for inclusion in the present study were not officially screened for CHD and therefore not included a fact that could lead to selection bias. Reasons for not screening the dogs were, however, most commonly due to death of the dog and owner unwillingness to have the radiographs taken because of the cost. The vast majority of the CHD radiographs in the present study were scrutinized by a single radiologist, which reduces inter-observer variation.

A potential shortcoming of the study is the use of conventional hip radiography to diagnose CHD. False negative diagnoses occur more often when the conventional radiographic projection is used, but false positive results are more common with other methods like distraction index radiography (Zhu et al., 2009). Age of the dog at screening has been found affect the screening result. Older dogs tend get worse grades than younger because secondary changes in the hip joint are more common with increasing age (Swenson et al., 1997; Maki et al., 2000; Leppanen et al.,

2000; Wood and Lakhani, 2003) The fact that NF and LEO in our study were 6 months older than LR and IW at screening might be a source of differential misclassification. Reclassification of the hip status from five grades to two categories in the analyses in this study might reduce the variation and result in some loss of information. The fact that a very large number of predictors were investigated also warrants some caution in the interpretation of the final model. The effect of a predictor might appear significant by chance alone and strictly, a lower cut-off for statistical significance than the conventional  $P < 0.05$  level should be applied to account for multiple comparisons. This is particularly relevant for the factor bodyweight at 3 months, which is only borderline significant ( $P = 0.044$ ).

#### 5. Conclusion

The aim of this study was to measure the effect of weight and growth related parameters on the risk of development of CHD in privately owned dogs followed from birth and throughout the growth period until the diagnosis of CHD was made. The findings failed to support the hypothesis that when comparing large sized breeds, large and fast growing dogs were at risk for development of CHD when compared to less heavy and slower growing dogs. On the contrary, it was found that higher body weight at 3 months of age was associated with lower odds of CHD, although the estimated effect was modest. A high level of clustering at the litter-level was observed and might indicate that environmental effects influence the development of the CHD phenotype, although the contribution of the genetic variance to the clustering needs to be further investigated.

#### Conflict of interest statement

Neither of the authors of this paper has financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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**A prospective study of the effect of housing and exercise conditions on radiographically diagnosed Canine Hip Dysplasia in a cohort of four large breeds in Norway (1998-2001)**

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## **Abstract**

**Objective**—To measure the effect of parameters related to housing and exercise on the risk of Canine Hip Dysplasia (CHD) at radiographic screening in a prospective cohort study of privately owned dogs.

**Sample population**—501 dogs from 103 litters of the breeds Newfoundland (NF), Labrador Retriever (LR), Leonberger (LEO), and Irish Wolfhound (IW) in Norway.

**Procedures**—Pre-weaning housing conditions and post-weaning housing and exercise conditions were registered by use of questionnaires. The dogs had individualized nutrition and environment and were followed from birth until the official screening for CHD at 12 (LR, IW) or 18 (NF, LEO) months of age. Multivariable random effects logistic regression models were used to assess potential housing and exercise related risk factors for CHD.

**Results**— Identified risk factors were walking in stairs, which increased the risk of CHD, and off-leash exercise which decreased the risk of CHD. These effects were significant in the period from birth to 3 months of age. Being born during spring and summer and breeder house type being farm decreased the risk of CHD. Clustering at the litter-level was high and significant.

**Conclusions and clinical relevance**— If the effect of breeder house type and season of birth reflects amount of outdoor activity, varied physical activity appeared to have a beneficial effect while the use of stairs on a daily basis had negative effect on the risk of CHD. These findings might form a basis for practical recommendations and preventive efforts for privately owned dogs such as to avoid the use of stairs for puppies younger than 3 months of age and to give the puppies access to outdoor exercise on soft ground in moderately rough terrain.

## **Abbreviations**

NF Newfoundland

LR Labrador Retriever

LEO Leonberger

IW Irish Wolfhound

CHD Canine Hip Dysplasia

NKK Norwegian Kennel Club

SKK Swedish Kennel Club

DKK Danish Kennel Club

NKU Nordic Kennel Union

FCI Fédération Cynologique Internationale

ICC Intraclass Correlation Coefficient

## **Introduction**

Hip dysplasia is an inherited developmental orthopedic disease in the dog.<sup>1</sup> Major genes are identified by segregation analysis, and quantitative trait loci have been identified by molecular genetic analysis.<sup>2,3</sup> The expression of these genes might be influenced by a number of environmental factors.<sup>4</sup> Although not completely understood, two broad etiological categories have been proposed for CHD, either hip joint laxity causing hip instability or abnormal progression of the endochondral ossification affecting different joints including the

hip joints.<sup>1,5,6</sup> Hip instability in young puppies alters the concentration of forces on the growing femoral head and acetabulum and affects growth and remodeling which can result in joint deformity and osteoarthritis.<sup>7,8</sup> Oedema and torn ligament fibers in the teres ligaments have been reported as the first observed changes of the coxofemoral joints in puppies, and detected at 14 to 30 days of age.<sup>9,10</sup>

The true occurrence of CHD is not known, although there are several reports on the prevalence of CHD from official registries in different countries.<sup>11-15</sup> Reported breed prevalences vary from 2% to 67%, and large and giant breeds are commonly reported to have the highest prevalences, although exceptions exist.<sup>16-20</sup> Recently estimated incidence risks for CHD in a cohort of privately owned dogs from four large breeds in Norway ranged from 10% in IW to 36% in NF.<sup>21</sup>

The NKK has a screening program for CHD in certain breeds, which includes standardized radiographic evaluation of hip joints at 12 or 18 months of age and recording of the hip status in a central register. Studies on heritability of CHD based on radiographic diagnosis from official registries have yielded moderate to high heritability estimates.<sup>22-28</sup> Efforts to reduce the occurrence of CHD have been made by radiographic screening of dogs at a certain age and subsequently imposing breeding restrictions on dogs with a radiographic diagnosis of CHD.<sup>13,17,28-35</sup> Several studies have been undertaken to evaluate the effectiveness of this selective breeding in reducing the occurrence of CHD, and although the results are mixed they are in some breeds disappointing.<sup>12,13,19,20,36</sup> Information about the phenotype of relatives incorporated in mixed linear models to predict breeding values have been introduced to enable a more accurate genetic evaluation in selection of breeding animals, and this might improve the effectiveness of selective breeding in the future.<sup>23,27,37</sup>

The most extensively studied risk factors regarding occurrence of CHD are factors related to growth rate, body size and conformation, food consumption, and composition of the diet.<sup>21,38-44</sup> Hormonal influences have also been studied as possible risk factors for development of CHD.<sup>45-47</sup> Confinement of puppies predisposed to hip dysplasia in small areas where they stay seated for long periods have been reported to prevent development of CHD.<sup>10</sup> Development of CHD was not found to be influenced by restricted exercise in another experimental study.<sup>40</sup> On the other hand, the effect of housing and exercise conditions have not been intensively studied in dogs living under domestic conditions, but certain types of housing and exercise conditions have been associated with either occurrence of radiological CHD or development of clinical signs related to CHD.<sup>48,49</sup>

The present prospective cohort study followed dogs from the breeds NF, LR, LEO, and IW from birth until 12 or 18 months of age, an age at which they were screened for CHD and given an official radiological CHD diagnosis based on the FCI standard. In these privately owned dogs living under domestic conditions, the housing and exercise conditions during the first 12 months of life were extensively recorded by breeder and owner questionnaires at specific ages. The aim was to investigate if these registrations could identify risk factors for a radiographic diagnosis of CHD which in turn could be used to suggest housing and exercise conditions during the first 12 months of life for the prevention of CHD in large breed dogs. The large number of variables recorded at each age made it necessary to analyze data from each age separately in order to identify key variables.<sup>50,51</sup> These variables were subsequently combined in a final model explaining housing and exercise related risk factors for a radiological CHD diagnosis. The hypothesis was that housing and exercise conditions in growing dogs will influence the risk of a radiological CHD diagnosis at screening.

## Materials and Methods

The study was carried out in agreement with the provisions enforced by the National Animal Research Authority of Norway.

**Study design**—The present study is part of a larger study (the so called *main study*) aimed at investigating the effects of risk factors on the occurrence of skeletal diseases: CHD, elbow dysplasia, panosteitis, and osteosarcoma. The *main study* included dogs from four large breeds: NF, LR, LEO, and IW. A prospective single cohort study was conducted to investigate factors affecting the radiological occurrence of CHD in growing dogs from the *main study*.

**Inclusion of dogs**—The sampling procedure and inclusion of dogs in this cohort have previously been described.<sup>21</sup> Puppies born in Norway between November 1998 and June 2001 were eligible for inclusion in the *main study*. All geographic areas of Norway were represented. The breeding stock consisted of dogs born in Norway as well as dogs that had been imported. Inclusion of a litter began when the bitch was mated. All puppies were registered in the NKK.

Each breeder, dog owner and veterinarian who participated in the project signed a written agreement of cooperation to comply with the project plan. Not all dogs enrolled in the study continued to completion. Reasons for dropouts included but were not limited to death of the dog, relocation of the owners during the study, and exportation of dogs abroad.<sup>21,a</sup> In total, 647 dogs from 106 litters from the *main study* were eligible for inclusion in the present

study, which is a convenience sample consisting of 23.2% of the total numbers of litters born in the included breeds in Norway.

The inclusion criteria for the present study were that the dogs were officially screened for CHD and that they had housing and exercise conditions recorded at least once during the observation period which was from birth to 12 months of age. The dogs were privately owned, and each dog had a housing, exercise, and feeding regimen decided by its owner.

**Radiographic screening for CHD**—The signed written agreement of cooperation and consent encouraged dog owners to have their dogs officially CHD screened. LR and IW were radiographed at 12 months of age, and LEO and NF at 18 months of age, which are the recommended screening ages for these breeds in the NKK. Hip radiographs were taken by a veterinarian and subsequently sent to NKK for judging irrespective of whether the dogs showed any clinical signs or not. More than 90% of the radiographs were scrutinized by the same experienced veterinary radiologist at the NKK. In the present study some of the dogs were living in Sweden and Denmark. The hip radiographs of these dogs were therefore scrutinized by the radiologists in the SKK and DKK, respectively. The radiographic panel of the NKU consists of panelists from the Nordic countries and ensures that the hips are graded according to the same protocol, to minimize differences in grading between the Nordic countries. All dogs were sedated before the radiographic examination to achieve complete muscle relaxation. The identity of the dogs (NKK registration number, tattoo or microchip) was photographed onto the film before developing the radiographs. The radiographs were made at 100-cm film to focus distance.

The dogs were placed in dorsal recumbency with the hind limbs extended and abducted so that the patellae were superimposed over the femora. The entire pelvis and

femora including the patellae were included on the radiographs. The FCI five class grading scale was used to classify the hip status of the dogs: A (excellent), B (normal), C (mild dysplasia), D (moderate dysplasia) and E (severe dysplasia). The CHD grades are defined descriptively based on the size of the Norberg angle (NA), degree of subluxation, shape and depth of the acetabulum, and signs of secondary joint disease.<sup>52</sup> Each hip joint was graded separately, and the final grading was based on the most severely affected hip joint. Dogs graded C (mild dysplasia), D (moderate dysplasia), and E (severe dysplasia) were considered affected by CHD.

**Questionnaires and risk factors**—Housing and exercise information for each included dog was obtained from questionnaires originating from two sources: 1) the breeder of the litter and 2) the owner of the puppy. Both sources completed questionnaires and recorded information in a booklet prepared for each included dog. All questionnaire sheets appeared in duplicates in the booklet, so that one sheet could be mailed to the researchers and a copy retained in the booklet. A Norwegian version of the booklet including all questionnaires is available from the first author upon request.

Three types of data from the questionnaires were studied as potential CHD risk factors: pre-weaning data regarding housing conditions (*breeder data*), post-weaning data regarding housing conditions (*owner housing data*), and post-weaning data regarding exercise conditions (*owner exercise data*). In addition, individual dog characteristics such as breed, sex, and litter size were explored as potential explanatory, confounding, or intervening variables. Season of birth of the litter was investigated as a variable possibly reflecting amount of outdoor exercise for the litter in the breeder period. All variables are defined in Table 1.



The breeder decided the housing regimen of the litter in the pre-weaning period from birth to 8 weeks of age and these *breeder data* were recorded in the booklet. The puppies stayed with the bitch until approximately 8 weeks of age when they were sold to the owner. *Breeder data* consisted of information about breed, season of birth, litter size, region, type of house, padding in the whelping box, indoor flooring, and environment in the outside run. The padding, flooring, and outdoor environment were commonly of several different types simultaneously for each litter and categorization of these variables was not deemed feasible. Padding was therefore studied as three separate dichotomous variables and flooring as four separate dichotomous variables categorized according to quality, as presented in table 1. Environment in the outside run was categorized according to softness into two separate dichotomous variables, with presence of snow and ice as a third separate outdoor environment variable. The month of birth was initially included as a continuous variable, but due to lack of linearity the calendar year was instead categorized into four seasons. In temperate regions like Norway there are large climatic variations between the seasons, and the calendar year was divided according to the climatic conditions in Norway. The meteorological definition of spring is that spring starts when night temperatures are above 0 degrees centigrade in 7 consecutive nights,<sup>53,54</sup> thus March is a winter month in most parts of Norway. Therefore the winter is longer than summer and fall, and spring is shorter (Table 1).

The *owner housing and exercise data* were recorded at the scheduled ages 3, 4, 6, and 12 months. *Owner housing data* consisted of: region, type of house, presence of children and other dogs, and type of padding/surface on indoor (soft or hard) and outdoor (soft or hard) resting area. Again, the presence of snow and/or ice was studied as a separate outdoor variable (Table 1).

Data on exercise conditions were recorded by the owners in detail, with duration in minutes of the different kinds of exercise on an average day. Preliminary analyses revealed

bias in the owners recording, and to minimize this bias the different kinds of exercise were studied as separate dichotomous variables. The *owner exercise data* consisted of the use of stairs and different types of outdoor exercise as walks on leash, exercise in run, running off-leash, running alongside a bike, and other activities (Table 1).

**Risk factor analyses**—The software package Stata 11 was used for all analyses.<sup>b</sup>

For the purpose of these analyses the hip status was reclassified into 2 categories: free (grades A and B) and affected (grades C, D, and E). The dependent variable CHD is dichotomous (CHD free or CHD affected), and a logistic regression model of the relationship between predictors and CHD was considered appropriate. The dogs in the study were clustered into litters. The assumption of independence between observations was therefore violated; hence a random intercept for litter was included in a generalized linear mixed model. A random intercept for breeder was also considered, but because the breeder: litter ratio was close to one, the breeder level was omitted.

The data consisted of a very large number of predictors from five different ages; 0-8 weeks of age (*breeder data*) and 3, 4, 6, and 12 months of age (*owner data*). As an attempt to reduce the number of predictors to be considered for multivariable model building two approaches were applied as described in the literature:<sup>50,51</sup> 1) screening of variables from each age based on unconditional associations with a liberal p-value and 2) building multivariable sub-models for each age applying a restrictive p-value with the variables selected from unconditional screening. The important predictors from each of the sub-models were then evaluated in an overall final model.

Associations between the dependent variable and the predictor variables from each age were therefore first screened with univariable random effects logistic regression. Collinearity were evaluated by Goodman and Kruskal's gamma for ordinal and dichotomous variables and

the phi coefficient for nominal variables. The variables with a P-value  $\leq 0.20$ , provided that there was no collinearity ( $r < 0.70$ ,  $r > -0.70$ ) between them, were then considered for further analysis in the multivariable sub-models for each age to assess the relationship with the dependent variable. When collinearity was detected between two predictors, the predictor with least missing data was selected for further analyses. Lowess curves were used to assess the linearity of the relationship between the continuous variables and the logit of the outcome. Variables with many missing values ( $>20\%$  missing observations) were not used in the multivariable analysis.

The Stata command `xtmelogit` using adaptive quadrature was used for all multilevel modeling. A sub-model for *breeder data* (*breeder data model*) was constructed using manual backward elimination. Predictor variables were retained in the model when the P-value was  $< 0.05$ . Potential confounding and intervening variables were evaluated after initially constructing a causal diagram. Changes of more than 20% in the coefficients in the model with the potential confounder present were also used as indication of confounding. Interactions between significant predictors were tested by adding an interaction term to the *breeder data model*, and the interaction term was retained if  $P < 0.01$ , as judged by the likelihood ratio test (LRT) comparing models with and without the interaction term. Following manual backward elimination, the model was build again by forward selection by offering the excluded variables one at a time. A variable was considered to be intervening if adding it removed the entire effect of another variable and if the intervening variable lay on the causal path between the factor and the outcome. Intervening variables were excluded. The LRT was used to evaluate the significance of the random litter effect in models with and without random litter effect, but containing the same fixed effects. The LRT was considered significant at  $P=0.05$  and one-sided test. The multiple Wald test and LRT were used to

evaluate differences between categories of categorical predictors. The Stata command `lincom` was used to conduct contrasts among each category of categorical predictors.

The same model building procedures were used for the *owner housing and exercise data*, and sub-models were built for the different observational ages (3, 4, 6, and 12 months of age). Sub-models from the different observational ages were then combined with each other to a *owner data model* where predictor variables were retained when the P-value was  $< 0.05$ . Finally the *breeder* and *owner data models* were combined, and predictor variables were retained in this overall *final model* when the P-value was  $< 0.05$ . Interactions between the significant predictors in the *final model* were tested by adding an interaction term and the interaction term was retained if  $P < 0.01$ .

The between litter variance ( $\sigma^2_{\text{litter}}$ ) was estimated from the *final model*. The intraclass correlation coefficient (ICC) was calculated using the latent variable approach, assuming that dog level variance ( $\sigma^2$ ) is constant at  $\pi^2/3$ .<sup>50</sup>

$$\text{ICC} = \sigma^2_{\text{litter}} / (\sigma^2 + \sigma^2_{\text{litter}})$$

To evaluate and assess the fit of the final multi-level model residuals were estimated at the individual dog level, as well as at the litter level. Litter level residuals were plotted against both predicted and fitted values to assess the assumptions of homoscedasticity and normality.<sup>50</sup>

## Results

**Study sample**—A total of 501 dogs from 103 litters in the *main study* (n=647) were officially screened for CHD, and had housing and exercise conditions recorded at least once during the observation period and were thus included in the study sample. The 103 litters

were the offspring of 94 dams from 86 breeders. The most common reason for not screening (n=146) was death of the dog or unwillingness of the owner to cover the cost of the radiographs. The breed distribution was 180 LEO, 125 NF, 133 LR and 63 IW. The total number of dogs considered affected by CHD at radiological screening was 123 (24.5 %). Distribution of affected and non-affected dogs by breed is presented in Table 2. The vast majority of the CHD radiographs were scrutinized by the NKK (n=491) radiologist, although 2 dogs were scrutinized by the DKK radiologists and 8 dogs were scrutinized by the SKK radiologists.

Not all breeders and owners who completed the questionnaires regarding housing and exercise conditions answered all questions at all observational ages, hence there was a variable number of missing values. The number of retrieved questionnaires at 3 months were 478, and at 4, 6, and 12 months 460, 443, and 397, respectively. The number of retrieved questionnaires from the breeder period were 457.

**Risk factors**—The variables breed, sex, and litter size were tested in all multivariable modeling as possible explanatory, confounding, or intervening variables. Breed and litter size were retained in all models in order to control for breed differences and litter size differences between the breeds. No explanatory, confounding, or intervening effect was detected for the variable sex; hence this variable was not retained in the models.

Out of the 16 potential *breeder data* risk factor variables, four risk factors were retained for the multivariable modeling: “breed”, “litter size”, “season” and “breeder house”. Collinearity was not detected. In the *breeder data model* three factors were significantly ( $P < 0.05$ ) associated with a radiological diagnosis of CHD: “breed”, “season”, and “breeder house”. The following interactions were tested: breed x litter size, breed x breeder house, breed x season, and season x litter size. None of the tested interactions were significant.

*Owner housing data and exercise data* consisted of 22 variables at each observational age (3, 4, 6, and 12 months of age). From the unconditional screening ( $p \leq 0.20$ ) nine risk factors were retained for the multivariable modeling at the age 3 months and the age 4 months. At 6 and 12 months of age, six risk factors were retained ( $p \leq 0.20$ ) at each of the two ages. Collinearity was not detected. When building sub-models from the ages 3, 4, 6, and 12 months, only four factors at 3 months of age were significantly ( $P < 0.05$ ) associated with a radiological diagnosis of CHD resulting in the *owner data model*; “breed”, “soft run 3 months”, “stairs 3 months”, and “free park 3 months”. The following interactions were tested: breed x litter size, breed x stairs, breed x soft run, breed x free park, stairs x soft run, stairs x free park, soft run x free park. None of the tested interactions were significant.

Combination of the *breeder data model* with the *owner data model* resulted in an overall *final model* with five factors significantly associated ( $P < 0.05$ ) with a radiological diagnosis of CHD, and with a significant amount of clustering at the litter level ( $ICC=0.183$ ) (Table 3). The significant exercise variables stairs and free park was registered at 3 months of age (Table 3). The following interactions were tested: breed x litter size, breed x season, breed x house breeder, breed x free park, breed x stairs, season x litter size, season x free park, season x stairs, breeder house x free park, breeder house x stairs, and free park x stairs. None of the tested interactions were significant.

The multiple Wald test and LRT for comparing the *final model* with and without the categorical variables breed and season were significant with  $P < 0.05$ . Significant differences were found between season spring and fall and between summer and fall ( $P < 0.05$ ). For comparison the effect of season was also analyzed where season was split by 3-month interval, thus the seasons were winter December-February, spring March-May, summer June-August, and fall September-November. The multiple Wald test and LRT had  $P=0.05$  and the

effect of spring and summer were slightly reduced (OR 1.3 and 0.9 respectively) when compared to winter, while the effect of fall was slightly increased (OR 3.2).

**Model evaluation**— The assumptions of homoscedasticity and normality were met as evaluated by the litter level residuals. No extreme values were found in the dog level residuals.

## **Discussion**

The main findings in this cohort study of housing and exercise related risk factors for a radiographic diagnosis of CHD at screening were that some of the examined housing and exercise conditions affected the risk of CHD. The identified risk factors were walking in stairs, which increased the risk of CHD, and off-leash exercise which decreased the risk of CHD, i.e. had a protective effect. These effects were significant in the period from birth to 3 months of age. Furthermore, there appeared to be an effect of season of birth and type of house at the breeders on the risk of CHD. There was also a strong clustering of the log odds of CHD within litters. The findings supported our hypothesis that housing and exercise conditions in growing dogs of large breeds influence the occurrence of CHD at radiographic screening. The different risk factors will be discussed in more detail below, except for the effects of breed and litter size which were forced into the model to control for breed and litter size differences.

Among the exercise-related variables in our analysis, walking in stairs daily and off-leash exercise in park terrain were significantly associated with the risk of CHD, but only up to the age of 3 months. Daily use of stairs nearly doubled (OR 1.96) the risk of CHD whereas off-leash exercise in park terrain appeared to be protective (OR 0.31). Restricted exercise by confining puppies predisposed to CHD development in a cage has been reported to prevent

the condition.<sup>10</sup> By confinement in small areas where the puppies stay seated for long periods, an abduction-flexion position is maintained, thus supporting a forced hip congruence.<sup>10</sup> This treatment is not recommended as puppies will not develop socially,<sup>4</sup> and it is not practicable in a breeder setting. Development of CHD was not found to be influenced by restricted exercise in another experimental study of growing puppies.<sup>40</sup> A retrospective case-control study in adult dogs (12 to 24 months of age) with known radiographic CHD status, indicated that regular exercise by running after a ball or stick thrown by the owner increased the risk for CHD.<sup>48</sup> The beneficial effect of off-leash exercise in the current study might be due to increased muscle development and strength. Dogs with high pelvic muscle mass have been found to be more likely to have a normal development of the hip joints.<sup>55,56</sup> Kinematic analysis studies in adult healthy dogs have shown that controlled stair descent and uphill walking improves flexion and range of motion of the hip joints, and thus are useful as therapeutic exercises for dogs with musculoskeletal diseases of the pelvic limb.<sup>57,58</sup> Gait inconsistencies have been observed when evaluating gait kinetics in puppies probably because neuromuscular function and coordination have not been fully developed,<sup>59</sup> which might provide explanation for the observed negative effect of the use of stairs in the current study. However, also the frequency of stair climbing and descent might influence the effect of this activity and should be studied further. Some of the other exercise related variables in the current study like running alongside a bike and running after balls or sticks thrown by the owner, were activities not commonly performed, a fact that might provide some explanation for why these variables did not show any significant association with the risk of CHD.

None of the risk factors regarding exercise found to affect the risk of CHD were significant after 3 months of age in the present analysis. Studies in foals have found that it is during the first months of life that the musculoskeletal system is most vulnerable to adverse influences that may result in developmental orthopedic disease.<sup>60,61</sup> Rapid growth and



development might make the musculoskeletal apparatus susceptible to environmental influences. In the current cohort of large breed dogs, average daily weight gain has been estimated to reach its maximum at approximately 3 months of age.<sup>21,62</sup> In the dog the hip joints are normal at birth, and the most critical time in the development and stability is from birth to 60 days of age.<sup>9,10</sup> The beneficial and negative effects of different kinds of exercise might be most pronounced during this time. The effect of different exercise conditions early in life on the development of clinical signs and long term survival is to be studied further in this cohort of large breed dogs.

The only variable regarding housing conditions that was significant in the final model was the type of house of the breeder. If housing conditions at the breeders were on a farm or small farm/holding (rural area), the risk of CHD were decreased (OR 0.34). Taken together with the other findings in this study, the most probable explanation for the effect of breeder house-type is related to outdoor environment and exercise conditions. The opportunity of the puppies to get off-leash exercise outdoors on a regular basis might be greater when they are reared in rural area on a farm or small farm/holding compared to in a single family house in more urban areas. Box rest and irregular exercise has been found to increase severity of osteochondrosis lesions in foals.<sup>60</sup> Housing conditions of dogs have not been extensively studied with respect to CHD, but slippery floor cover in the pre-weaning period have been found to increase the hazard of developing clinical signs related to CHD in a cohort of Boxers.<sup>49</sup> Neither of the variables related to flooring, padding in the resting area, or outdoor environment in the current study showed any significant association with CHD, but in the current study the outcome was radiographic diagnosis of CHD and not clinical signs related to CHD.

In the current study, dogs born in the fall had twice the risk of CHD compared to dogs born in the winter. There was apparently a protective effect of being born during spring and

summer with the risk of CHD being about one half (OR 0.54 and 0.62 respectively) of the risk of CHD when born in winter. Previous studies have found similar effects.<sup>37,63-65</sup> A possible explanation for the observed seasonal effect might be that puppies born during fall and winter in cold temperate climates are reared in a different manner than those born in spring and summer. Housing and exercise conditions might change according to season, and puppies born during spring and summer might get more free exercise on soft ground than puppies born in the fall and winter. This can be hypothesized to have a beneficial impact on muscle development and strength, although other unmeasured factors might be present possibly biasing the result. In the analysis where March was included in winter, the protective effect of spring was less obvious (OR close to 1), while the risk of CHD among dogs born during fall was increased (OR 3.2). March is a winter month in most parts of Norway (by meteorological definition),<sup>53,54</sup> and merging March into spring will therefore probably diminish the differences between winter and spring and is probably a support for the hypothesis that the seasonal effect observed are mediated through outdoor climate and less outdoor exercise during winter months.

A high and significant amount of clustering at the litter level was present in this analysis; the ICC representing the proportion of variance at litter level was 18.3% representing common genetic background and unmeasured litter effects. In a previous study regarding growth related risk factors for CHD in this cohort, a similar amount of clustering (22.6%) was found.<sup>21</sup> In a study relating pre-weaning mortality in puppies to additive genetic, common-litter, and within-litter factors, the litter factors explained most of the variation.<sup>66</sup>

A potential shortcoming of the study is the use of hip radiography to diagnose CHD. False negative diagnoses occur more often when the conventional radiographic projection is used, while false positive results are more commonly encountered with other methods like distraction index radiography.<sup>67</sup> The vast majority of the CHD radiographs in the present

study were scrutinized by a single radiologist, which reduces inter-observer variation and thus misclassification bias.<sup>68</sup> Furthermore, reclassification of the hip status from five grades to two categories in the analyses in this study might reduce variation and result in some loss of information. Age of the dog at screening has been found to affect the screening result. Older dogs tend get more severe grades than younger dogs because secondary changes in the hip joint increase with increasing age.<sup>22,28,37,69</sup> The fact that NF and LEO in our study were six months older at screening might be a source of differential misclassification. Some of the dogs eligible for inclusion in the present study were not officially screened for CHD and therefore not included, a fact that could lead to selection bias if these dogs were more or less likely to have CHD than the included sample. Reasons for not screening the dogs were, however, most commonly due to death of the dog or owner unwillingness to have the radiographs taken because of the cost. Another shortcoming of the study is the number of missing observations reducing the power to detect potential associations, possibly explaining the lack of associations at the older ages. Reclassification of the exercise variables from categorical to dichotomous variables also result in some loss of information. However, it might reduce potential information bias from the questionnaires. When large numbers of predictors are investigated, a predictor might appear significant by chance alone. In this study, efforts to reduce the number of predictors was made by selecting variables based on the causal diagram, unconditional screening of predictors at a liberal P-value ( $\leq 0.20$ ), and then building multivariable models using logical subsets of predictors (from the pre-weaning period and the different observational ages) to identify key predictors from each period.<sup>50,51</sup> These key predictors were then retained for consideration in the final multivariable model.

Cohort studies are generally considered to have high relevance to real-world situations and a high external validity.<sup>50</sup> Most studies regarding the development of CHD have been done in populations of dogs preselected for certain purposes (e.g. guide dogs) or as controlled

studies. In such experimental studies, dogs are often offspring of hip dysplastic parents and are raised in kennels where feeding, exercise, and housing regimens are controlled and standardized and will therefore not necessarily be representative of privately owned dogs with a more diverse husbandry. The present study is a large scale prospective study. All kinds of privately owned dogs, both those used for breeding and those only held as pets from four different large breeds, were included in our cohort, which increases the external validity. The results might be extrapolated to the Norwegian population of large breed dogs, but perhaps also to large breed dogs in other countries with similar dog management.

In conclusion, our results showed that type of housing, exercise conditions and season of birth were significantly associated with the risk of a CHD diagnosis at radiographic screening in this cohort of large breed dogs. The effects of these risk factors were, however, limited to the first 3 months of life. This could indicate that any preventive efforts regarding housing and exercise should be initiated early in the puppy's life. If the effect of breeder house type and season of birth reflects amount of outdoor activity, varied physical activity appeared to have a beneficial effect while the use of stairs on a daily basis had negative effect on the risk of CHD. These findings might form a basis for practical recommendations and preventive efforts for privately owned dogs such as to avoid the use of stairs for puppies younger than 3 months of age and to give the puppies access to outdoor exercise on soft ground in moderately rough terrain. The study cannot, however, give recommendations specifying amount of time spent on these activities as this was not analyzed.

## **Footnotes**

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Table 1—Definition of potential risk factors considered in a study on Canine Hip Dysplasia (CHD) in four large breeds in Norway (1998-2001).

Variable	Definition	Abbreviated name
<b>Descriptive data</b>		
Breed	Breed of the dog (categorical): Newfoundland, Labrador retriever, Leonberger, Irish wolfhound	Breed
Sex	Sex of the dog (dichotomous): female, male	Sex
Litter size	Number of puppies in each litter (continuous)	Litter size
Season of birth	Season of birth of the litter (categorical): winter (December- March), spring (April-May), summer (June-August), fall (September-November)	Season
<b>Pre-weaning housing conditions (breeder data)</b>		
Living conditions	Area of living (categorical): countryside, suburban, city	Breeder region

Type of house	Type of building in which the dogs live (categorical): single family house or farm/small farm(holding)	Breeder house
Whelping box padding	Type of padding used in the whelping box (3 separate dichotomous variables): newspaper (0/1), sawdust (0/1), carpet (0/1)	Whelping box
Flooring	Type of floor cover on the indoor area (4 separate dichotomous variables): parquet/wooden (0/1), linoleum (0/1), carpet (0/1), tiles (0/1)	Flooring
Outside run	Outdoor environment if the puppies were allowed to be in an outside run (3 separate dichotomous variables): soft (0/1) (grass, ground, gravel), hard (0/1) (wooden, asphalt, concrete), snow/ice (0/1)	Outside run

### **Post-weaning housing conditions**

*(owner housing data)* at 3, 4, 6,

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**and 12 months of age**

Living conditions	Area of living (categorical): countryside, suburban, city	Owner region
Type of house	Type of building in which the dogs live (categorical): single family house, farm/small farm(holding), or apartment building	Owner house
Children	If the household included children (dichotomous)	Children
Other dogs	Presence of other dogs in the household (dichotomous)	Other dogs
Indoor environment	Type of padding/surface on indoor resting area (dichotomous): soft (bed, madras, pillow, quilt, sofa) or hard (wooden floor, parquet, linoleum, tiles/concrete, thin carpet)	Indoor padding
Outdoor environment	Type of padding/surface on the outdoor resting area (dichotomous): soft (grass, ground, sand, madras) or hard (wooden, asphalt/concrete, gravel)	Outdoor padding

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Snow and ice outdoor	Presence of snow and ice outdoor (dichotomous)	Snow/ice
<b>Post-weaning exercise conditions</b> <b>(owner exercise data) at 3, 4, 6,</b> <b>and 12 months of age</b>		
Use of stairs	Daily use of inside stairs (dichotomous)	Stairs
Leash on asphalt paving	Daily walks in leash on asphalt paving (dichotomous)	Leash asphalt
Leash on graveled road	Daily walks in leash on graveled road (dichotomous)	Leash gravel
Leash in rough terrain	Daily walks in leash in forest, mountain, or other rough terrain (dichotomous)	Leash rough
Exercise run graveled	Daily exercise in graveled run (dichotomous)	Graveled run
Exercise run ground/grass	Daily exercise in run with soft covering, ground or grass (dichotomous)	Soft run
Exercise run concrete	Daily exercise in run with hard covering, concrete or asphalt	Hard run

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	(dichotomous)	
Off-leash in garden	Daily off-leash exercise in garden or yard (dichotomous)	Free garden
Off-leash in park	Daily off-leash exercise in park terrain (dichotomous)	Free park
Off-leash in rough terrain	Daily off-leash exercise in forest, mountain, or other rough terrain (dichotomous)	Free rough
Bicycling	Daily running alongside a bike (dichotomous)	Bicycling
Other activities	Other training, playing with other dogs or people (dichotomous)	Activities

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**Table 2**—Descriptive statistics of radiological Canine Hip Dysplasia (CHD) status in a study of four large breeds in Norway (1998-2001).

<b>Breed</b>	<b>Radiological CHD grade</b>	
	<b>Free (A+B) N (%)</b>	<b>Affected (C+D+E) N (%)</b>
Newfoundland	80 (64.0)	45 (36.0)
Labrador retriever	106 (79.7)	27 (20.3)
Leonberger	135 (75.0)	45 (25.0)
Irish wolfhound	57 (90.5)	6 (9.5)

**Table 3**—Final multivariable random effects logistic regression model of pre-weaning (*breeder data*) and post-weaning housing and exercise (*owner data*) related risk factors for a radiological diagnosis of Canine Hip Dysplasia (CHD) at official screening in four large breeds in Norway (1998-2001).

Variable	Estimate	SE	OR	95% CI of OR	P value
Breed					
Newfoundland	Baseline	-	1.00	-	-
Labrador retriever	-1.340	0.472	0.26	0.10, 0.66	0.005
Leonberger	-0.531	0.426	0.59	0.26, 1.36	0.213
Irish wolfhound	-1.820	0.647	0.16	0.05, 0.58	0.005
Litter size	-0.099	0.072	0.91	0.79, 1.04	0.170
Season					
Winter	Baseline	-	1.00	-	-
Spring	-0.621	0.472	0.54	0.21, 1.36	0.188
Summer	-0.480	0.466	0.62	0.25, 1.54	0.303
Fall	0.758	0.431	2.13	0.92, 4.96	0.078
Breeder house					

House	Baseline	-	1.00	-	-
Farm/small farm	-1.074	0.419	0.34	0.15, 0.78	0.010
Stairs 3 months	0.670	0.275	1.96	1.14, 3.35	0.015
Free park 3 months	-1.160	0.372	0.31	0.15, 0.65	0.002
Intraclass correlation coefficient	0.183				0.006 <sup>a</sup>
Overall P (final model) <sup>b</sup>					<0.001

<sup>a</sup>Likelihood ratio test of the random litter effect. <sup>b</sup> $\beta_0$  was 0.810 for the final model











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# The effect of radiological hip dysplasia and breed on survival in a prospective cohort study of four large dog breeds followed over a 10 year period

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## ABSTRACT

The aim of the study was to measure the effect of radiological hip and elbow dysplasia status and breed on overall survival in a cohort of four large dog breeds in Norway. Privately owned dogs of the Newfoundland (NF), Labrador Retriever (LR), Leonberger (LEO), and Irish Wolfhound (IW) breeds were followed prospectively from birth to 10 years of age. The age of death/euthanasia was registered. A total of 501 dogs from 103 litters were enrolled. Kaplan–Meier survival curves were used to describe breed differences in survival times. The effects of radiological hip and elbow dysplasia status as well as breed were assessed using a Cox proportional hazards model. The variables 'sex' and 'living region' were explored as potential confounders.

Among LRs, 60.2% of the dogs were still alive at 10 years of age, and the corresponding figures for NFs, LEOs, and IWs were 28.8%, 16.11%, and 6.4%, respectively. Radiological hip dysplasia status and breed were found to influence overall survival. Two different time-varying effects were observed in that with the IW the hazard of death increased linearly through time, while the effect of severe radiological hip dysplasia decreased logarithmically with time. Location influenced the death hazard and dogs living in suburban areas or cities had longer mean time to death and a lower hazard compared to dogs living in the countryside. Radiological elbow dysplasia status was not found to have an effect on overall survival.

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## Introduction

Age pattern of survival differs between dog breeds, and large and giant breeds are often reported to have shorter life span than small and miniature breeds indicating that different breeds of dogs age at different speeds (Michell, 1999; Egenvall et al., 2000, 2005; Proschowsky et al., 2003; Adams et al., 2010; Fleming et al., 2011). A Norwegian dog census of Bernese Mountain Dogs, Boxers, and Bichon Frisés found similar aging patterns (Moe et al., 2001).

Canine hip dysplasia (HD) is a frequently occurring skeletal developmental disease, which is probably more prevalent in large breeds, and the occurrence is also influenced by environmental factors (see review by Ginja et al. (2010)). Musculoskeletal diseases are common reasons for euthanasia in large dog breeds (Bonnett et al., 1997; Moore et al., 2001; Fleming et al., 2011) and euthanasia due to HD has been associated with several breeds of dogs (Bonnett et al., 1997; Proschowsky et al., 2003; Malm et al., 2010; Adams et al., 2010).

Many studies on life span and causes of death in dogs have been retrospective and used veterinary teaching hospital populations,

records from pet insurance companies, or surveys based on membership of kennel or breed clubs (Bonnett et al., 1997; Michell, 1999; Proschowsky et al., 2003; Egenvall et al., 2005; Fleming et al., 2011). These studies do not address the longevity and cause of death in a general dog population as they have inherent sampling and recall biases (van Hagen et al., 2005; Adams et al., 2010). In the current study, a cohort of privately owned dogs from four large breeds was followed from birth until death or 10 years of age. The aim was to measure if radiological HD and elbow dysplasia (ED) status and breed influenced time to death irrespective of the reported main cause of death.

## Materials and methods

## Study design

The study was carried out in agreement with the provisions enforced by the National Animal Research Authority (Protocol approval number 05/591-GC).

The work reported here is part of a larger prospective cohort study (the 'main study') aimed at investigating the effects of risk factors on the occurrence of four skeletal diseases (osteosarcoma, panosteitis, HD and ED). The main study included privately owned dogs from four large breeds: Newfoundland (NF), Labrador Retriever (LR), Leonberger (LEO) and Irish Wolfhound (IW) followed from birth and throughout their lives (Trangerud, 2008). A prospective single cohort study was conducted to study to which extent certain factors influence overall survival among dogs from the main study.

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## Inclusion

The initial sampling procedure and inclusion of dogs has been previously described (Krontveit et al., 2010). Puppies born in Norway between November 1998 and June 2001 were eligible for inclusion in the main study. All geographical areas of Norway were represented. The breeding stock consisted of dogs born in Norway as well as dogs that had been imported, and inclusion of a litter began when the bitch was mated. All puppies were registered with the Norwegian Kennel Club (NKK).

Not all enrolled dogs continued to completion (Trangerud et al., 2007). In total, 647 dogs from 106 litters from the main study were potentially eligible for inclusion in the present study, which was a convenience sample consisting of 23.2% of the total number of litters born in the included breeds during the period 1998–2001.

The owners of the dogs in the main study were encouraged to have their dog radiologically screened for HD and ED. Radiological HD and ED status were graded by and registered in NKK or in the Swedish ( $n = 8$ ) or Danish ( $n = 2$ ) Kennel Clubs. The inclusion criteria for the single-cohort study were that the dogs were radiologically screened for HD at the official NKK ages, namely 12 (LR, IW) or 18 months of age (NF, LEO). Dogs that were radiologically examined before ages 12 or 18 months (due to clinical signs of hip disease) were included if their hip radiographs were assessed by the NKK panelist.

## Questionnaires

Information about the dogs was obtained from the breeder of the litter, the owner of the dog, and the veterinarian examining the dog. Each breeder, dog owner, and veterinarian participating in the project signed a written agreement confirming cooperation and consent. Only information from owners and veterinarians was used in this study and both sources completed questionnaires and recorded information in a specially prepared booklet at specific ages, called 'the observational ages', namely, 3, 4, 6, 12, 18, and 24 months of age. Thereafter, annual questionnaires were mailed to the dog owners until death of the dog or the end of the present study period which was when the dog was 10 years of age.

Not all dogs enrolled continued to completion or filled in all questionnaires, and a few owners did not receive the booklet. Reasons for dropouts included (but were not limited to) the death of the dog, relocation of the owners during the study, and export of dogs abroad (Trangerud et al., 2007).

## Outcome variable

Dog owners and veterinarians reported information on the health of the dogs at the observational ages and dates of death/euthanasia were recorded. The owners continued to report on the annual questionnaires at the approximate ages of 3, 4, 5, 6, 7, 8, 9, and 10 years. Participants who did not respond for various reasons were contacted by telephone, and the age of death/euthanasia was registered. Factors related to the dog and the owner as well as the veterinarian examining the dog influence decisions regarding euthanasia (Yeates and Main, 2011) so the endpoint in the analysis was defined as euthanasia or death irrespective of the reported main cause and the outcome is simply referred to as 'death'.

Cases lost to follow-up or still alive at the end of the study were included in the survival analysis up to the last time point at which they were known to be alive and were thereafter censored. Dogs that died before the age of 3 months were excluded and preliminary analyses showed that no dogs died between the ages of 3 and 6 months. For technical reasons, 6 months were therefore subtracted from the time at risk, and the outcome variable 'time from 6 months to death until 10 years of age' was obtained.

## Risk factors

The variables breed, sex (male or female), location (living region), and radiological HD and ED statuses were examined for potential influence on overall survival. The screening procedure for HD for the dogs has been previously described (Krontveit et al., 2010). The Fédération Cynologique Internationale (FCI) grading scale was used with the following measures: A (excellent), B (normal), C (mild dysplasia), D (moderate dysplasia) and E (severe dysplasia) (Flückiger, 2007). In the analyses the HD status was reclassified into free (A and B), mild (C), moderate (D), and severe (E) and this variable was termed radiological HD status. The International Elbow Working Group (IEWG) elbow protocol<sup>1</sup> was used for grading of elbow radiographs into the grades free, mild, moderate, and severe. This variable was termed radiological ED status.

The location (or living region) of the owner was categorized as countryside, suburban, or city, based on the owner questionnaires. Preliminary analysis showed very few observations in the city category, so city and suburban were merged into a single category.

## Statistical analyses

Stata 11 (Stata Corporation) was used for all analyses. The distribution of dogs by breed, sex, radiological HD and ED status, and living region was calculated. The number and percentage of deaths, mean time to death or censoring, and total number of subjects in each category of the variables were calculated. Kaplan–Meier survival curves were used to describe breed differences in survival times and to estimate median survival time. Separate Kaplan–Meier curves for each breed and by radiological HD status were used to evaluate the effect of radiological HD status on survival in each of the breeds.

A Cox proportional hazards model was applied to estimate the effect of possible risk factors on 'time to death', and 'time at risk' was measured in months from 6 months to death or censoring or 10 years (120 months). The dogs were clustered into litters. The assumption of independence between observations was therefore violated and a shared frailty term for 'litter' was included in the model. The Efron approximate method was used to handle ties (Dohoo et al., 2009). Collinearity between all variables was evaluated by Goodman and Kruskal's gamma ( $\gamma$ ) for ordinal or dichotomous variables and by the phi ( $\phi$ ) coefficient for nominal variables.

A multivariable Cox proportional hazard model with shared frailty for litter was constructed using manual forward selection by offering the variables one at a time. Predictors were retained in the model when the probability ( $P$ ) of the likelihood ratio test (LRT) was  $<0.05$ . Potential confounding and intervening variables were evaluated after initially constructing a causal diagram. Changes of  $>20\%$  in the coefficients in the model with the potential confounder present were also used as indication of confounding. A variable was considered to be intervening if adding it substantially altered the effect of a factor and if the intervening variable lay on the causal path between the factor and the outcome. Interactions between significant predictors in the model were tested by adding an interaction term. To specifically evaluate the relationship between radiological HD and ED status an interaction term between these two variables was also added to the model. Interaction terms were retained if  $P < 0.01$  as judged by the LRT. Following forward selection the model was built again using backward elimination. The LRT was used to evaluate the significance of the categorical predictors in the final model. The significance of the shared frailty term was evaluated through a LRT. An estimate of the baseline hazard was derived conditional upon the set of coefficients in the model.

## Validation of the model

The assumption of proportional hazards was evaluated using the test for proportional hazards based on the Schoenfeld residuals for each variable in the model. If there was violation of the proportional hazards assumption and graphical assessment indicated a time-varying effect (TVE) of a variable, an interaction term between the variable and time (on either a linear or logarithmic scale) was included in the model. The assumption of independent censoring was evaluated by sensitivity analyses based on both complete positive and complete negative correlation between censoring and outcome. The amount of explained variation was evaluated by an overall  $r^2$  statistic for proportional hazard models. Plots of the deviance residuals, score residuals, and scaled score residuals against time at risk were used to identify outlying observations with influence on the model, and the model was fit with and without any outlying observations (Dohoo et al., 2009).

## Results

## Descriptive statistics

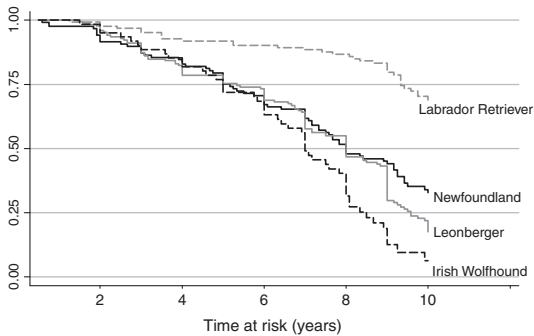
The number of deaths, mean time to death or censoring, and total number of subjects in each category of the variables investigated are provided in Table 1. Of the 501 dogs included, 73 were lost to follow up some time during the observation period, 149 were still alive at the end of the study, and 279 dogs died. Fig. 1 shows the Kaplan–Meier survival curves by breed and indicates that  $>50\%$  of LRs were alive at 10 years of age. The median survival time was 7.3 years for NF and 7 years for both LEO and IW. Among LR, 60.2% were still alive at 10 years of age, and corresponding figures for NF, LEO, and IW were 28.8%, 16.11% and 6.4%, respectively.

Fig. 2a and c indicates that severe radiological HD status had the greatest effect on survival before 2 years of age in NF and LEO, while moderate radiological HD status had a great effect in IW (Fig. 2d).

## Survival analysis

Collinearity was not detected between any of the variables. Both forward selection and backward elimination resulted in the

<sup>1</sup> International Elbow Working Group, 2001. International Elbow Protocol (Vancouver). <http://www.iewg-vet.org/archive/protocol.html> (accessed 15 March 2011).



**Fig. 1.** Kaplan-Meier survival curves by breed describing survival in a prospective cohort of four large dog breeds. Time at risk is from 6 months to 10 years of age.

same model with the variables breed, sex, radiological HD status, and living region retained ( $P < 0.05$ ). None of the tested interactions was significant.

The assumption of proportional hazards was violated for the IW breed and for severe HD status, indicating a TVE for these variables. The graphical assessment indicated that the effect of belonging to the IW breed increased on a linear time scale and that the effect of having severe radiological HD status decreased on a logarithmic time scale (log-time). Thus, TVE interactions between IW and time and between severe radiological HD and log-time were included in the model.

The overall survival model including the TVE for IW and severe radiological HD is presented in Table 2. Breed, radiological HD

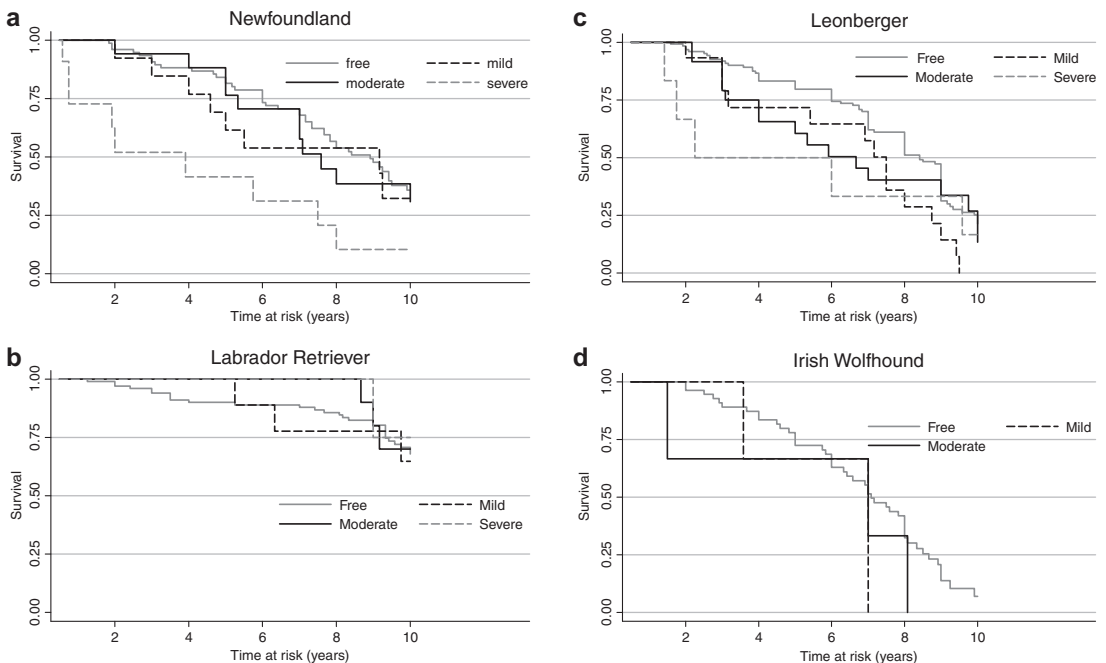
status, and living region had significant effect on survival, while sex was retained in the model as a potential confounder.

The effects of the interaction terms between IW and time and between severe radiological HD and log-time at different time points are presented in Table 3. LR was the breed with the lowest hazard of dying and the longest mean time to death compared to NF (the baseline), whereas LEO had almost the same hazard and equal mean time to death as NF (Tables 1 and 2). IW had the same hazard of dying and equal mean time to death as NF (Tables 1 and 2). However, the IW effect on the hazard of death increased over time as indicated by the TVE for IW: by 4 years of age the hazard for IW dogs was almost twice that of NF, and by 8 years the hazard was three times that of NF (Table 3).

The hazard of death for dogs with mild or moderate radiological HD was not significantly different from dogs free of HD (Table 2) with almost equal mean time to death (Table 1). For severe radiological HD the hazard was dramatically increased at the beginning of the study period (hazard ratio 270) and the mean time to death was shorter (Table 1). The effect of severe radiological HD on the hazard of death decreased over time and the hazard of death was almost equal to the hazard of dogs free of radiological HD by 8 years of age (hazard ratio 0.94) (Table 3).

Radiological ED status did not have an effect on the hazard of dying. The hazard ratio for dogs living in suburban areas or cities was significantly lower than for dogs living at the countryside (Table 2). The variable sex was retained in the model as a potential confounder although not significant (Table 2). The shared frailty variance for litter was small and not significant (Table 2).

The results of the sensitivity analyses with complete positive and complete negative correlation yielded approximately the same estimates and the assumption of independent censoring was met. The  $r^2$  for the model was 0.18 (18%) indicating that the predictors



**Fig. 2.** Kaplan-Meier curves for Newfoundland (a), Labrador Retriever (b), Leonberger (c), and Irish Wolfhound (d) by radiological hip dysplasia (HD) status in a prospective cohort study estimating the effect of dog breed and radiological hip dysplasia status on overall survival. The figures illustrate the effect of radiological HD status on survival in each breed. Time at risk is from 6 months to 10 years of age.

**Table 1**  
Descriptive statistics for variables investigated in a survival analysis measuring the effects of radiological hip dysplasia status and breed on survival in a prospective cohort of four large dog breeds followed over a 10 year period.

Variable and level	Number of deaths (%)	Mean time to death (years)	Mean time to censoring (years)	Total number of subjects (%)
<b>Breed</b>				
Newfoundland	72 (57.6)	5.3	7.4	125 (25.0)
Labrador Retriever	35 (26.3)	6.4	8.1	133 (26.5)
Leonberger	121 (67.2)	5.9	5.9	180 (35.9)
Irish Wolfhound	51 (81.0)	5.7	6.0	63 (12.6)
<b>Sex</b>				
Female	144 (55.0)	5.9	7.2	262 (52.3)
Male	135 (58.0)	5.6	7.2	239 (47.7)
<b>Radiological hip dysplasia status</b>				
Free	203 (53.7)	6.0	7.3	378 (75.4)
Mild	27 (70.4)	5.6	6.3	45 (9.0)
Moderate	34 (59.6)	5.5	7.1	57 (11.4)
Severe	15 (71.4)	3.6	8.1	21 (4.2)
<b>Radiological elbow dysplasia status<sup>a</sup></b>				
Free	219 (54.5)	5.9	7.3	402 (83.2)
Mild	33 (64.7)	5.8	7.6	51 (10.6)
Moderate	10 (45.5)	5.8	5.7	22 (4.5)
Severe	5 (62.5)	3.8	9.2	8 (1.7)
<b>Living region<sup>b</sup></b>				
Countryside	167 (63.5)	5.6	7.2	263 (55.1)
Suburban/city	104 (48.6)	6.1	7.8	214 (44.9)

<sup>a</sup> Radiological elbow dysplasia status was missing for 18 dogs.

<sup>b</sup> Information on living region was missing for 24 dogs.

**Table 2**  
Results from a multivariable Cox proportional hazards model estimating the effects of radiological hip dysplasia status and breed on survival in a prospective cohort of four large dog breeds followed over a 10 year period.

Variable and level	Estimate	Hazard ratio	P	95% confidence interval
<b>Main effects</b>				
<b>Breed</b>				
Newfoundland	Baseline	1.00	–	–
Labrador Retriever	–1.19	0.30	<0.001	(0.20, 0.47)
Leonberger	0.30	1.34	0.087	(0.96, 1.89)
Irish Wolfhound	0.04	– <sup>a</sup>	0.925	(0.43, 2.52)
<b>Sex</b>				
Female	Baseline	1.00	–	–
Male	0.24	1.27	0.063	(0.99, 1.62)
<b>Radiological hip dysplasia status</b>				
Free	Baseline	1.00	–	–
Mild	0.37	1.44	0.089	(0.95, 2.21)
Moderate	0.22	1.24	0.269	(0.85, 1.83)
Severe	5.60	– <sup>a</sup>	<0.001	(25.08, 2939.51)
<b>Living region</b>				
Countryside	Baseline	1.00	–	–
Suburban/city	–0.41	0.67	0.002	(0.52, 0.86)
<b>Time varying effect (TVE) interaction</b>				
Irish Wolfhound	0.012	–	0.033	(0.001, 0.02)
Severe hip status	–1.26	–	<0.001	(–1.92, –0.61)
<b>Frailty (litters)</b>				
Theta	0.072		0.079 <sup>b</sup>	

<sup>a</sup> Estimates of effect of factor varied over time – see Table 3 for estimates.

<sup>b</sup> Likelihood ratio test of frailty variance.

in the model explained approximately 18% of the variation in survival times. Outlying observations with influence on the model were not found.

## Discussion

Mean time to death was longest in LR and these dogs had the lowest hazard of dying. The mean time to death was lower in NFs, LEOs, and IWs, and these breeds had initial hazards that were not significantly different from each other. The breed effect for IW was, however, changing over time and by 4 years of age the hazard

for IW was almost twice the hazard for NF indicating an increasing effect on the hazard over time for IW. There was slight evidence that the negative effect on the hazard of death of being LEO also increased over time but this was only just significant so the TVE for LEO was omitted for simplicity.

The findings indicate that these breeds have different ageing patterns. The medium-sized LR had a longer life span than the larger breeds NF, LEO, and IW. Additionally our analyses indicated not only that IW, and to a lesser extent LEO, have shorter life spans, but also that the effect of these breeds on the hazard of dying increases as they get older, thus they age faster than NF and LR dogs. The reason for the difference in ageing between breeds is not well

**Table 3**

The effects of the interaction terms between Irish Wolfhound and time and between severe radiological hip dysplasia status and log-time at different time points estimated from the results of a survival model in a prospective cohort of four large dog breeds followed over a 10 year period.

Interaction term	Time (years)				
	0.5	2	4	6	8
Irish Wolfhound and time					
Estimate	0.04	0.26	0.54	0.83	1.12
Hazard ratio	1.04	1.29	1.72	2.30	3.06
Severe radiological hip dysplasia status and log time					
Estimate	5.60	1.96	0.89	0.32	−0.06
Hazard ratio	270.42	7.09	2.43	1.38	0.94

understood, but a shorter life span for large and giant breeds compared to smaller breeds has been reported (Michell, 1999; Egenvall et al., 2000, 2005; Moe et al., 2001; Proschowsky et al., 2003; Adams et al., 2010; Fleming et al., 2011). Within a breed, larger dogs did not die younger compared to smaller dogs (Galis et al., 2007). A dog's weight was more predictive of life span than either height or breed, and it seems that breeds smaller by weight live longer than heavier breeds (Greer et al., 2007). Diseases that arise because of a breed's morphological or genetic characteristics can affect survival and are widely reported (see review by Asher et al. (2009)). It has also been reported that metabolism changes with age and differs between small and large breeds (Speakman et al., 2003).

The aim of this study was to measure the effect of radiological HD and ED on survival without considering the cause of death. Mild and moderate radiological HD was shown to have no significant effect on the hazard of death. Dogs with severe radiological HD status had shorter mean time to death and initially a very high hazard (hazard ratio 270). The hazard decreased over time and by 2 years of age it had dropped to 7, and by 8 years the effect was equal to that of the other radiological HD grades. This indicated that dogs with severe radiological HD died at younger ages compared to dogs having less severe radiological HD status, but this effect of severe radiological HD was predominantly observed in NF and LEO. A similar effect of severe radiological HD has been found in insured German Shepherd dogs (Malm et al., 2010). In the IW, a breed with no severe radiological HD in this study, moderate radiological HD appeared to have a negative effect on survival before 2 years of age. In the current study, many of the dogs that were euthanased before 2 years of age had severe radiological HD and were reported to have clinical signs of hip disease such that euthanasia was recommended by the local veterinarian (R.I. Krøntveit, unpublished data).

Although dogs with severe radiological ED had a shorter mean time to death than dogs having less severe grades, no significant effects of radiological ED on overall survival was found. A low number of dogs in the severe radiological ED category might yield low power and partly explain this, but grading of radiological ED status is based on signs of degenerative joint disease caused by various conditions in the elbow joint. In dogs affected bilaterally there might be different conditions in right and left elbow, and these might develop differently and have different impact on a dog. Pathological lesions in radiographically normal canine elbows have been reported (Punke et al., 2009; Goldhammer et al., 2010). Accordingly, the radiological ED status at 1 year of age did not seem to influence overall survival in the four breeds. There was no indication of correlation or interaction between radiological HD and ED status.

Dogs living in suburban areas/cities had a longer mean time to death and a significantly lower hazard of dying compared to dogs living in the countryside. The explanation for the regional effects is not clear, but may be due to different availability of veterinary

services or different inclination to seek veterinary care by owners in different regions.

The measured effects in the survival analysis are conditional upon the frailty variance. The frailty variance was, however, low in this study and not significant indicating that clustering was low. Unmeasured owner and environmental effects are probably more important than the litter effects, because the dogs were followed until 10 years of age.

A limitation of our study is the decision to include only pure-bred dogs of medium and large/giant size and as a result the findings may not be relevant for smaller breeds. Dog management, veterinary services, and options for choosing treatment rather than euthanasia for conditions like HD and ED are probably different among countries and regions of the world (Yeates and Main, 2011). Our results may therefore not be representative for regions where, for example, surgical treatment of HD in juvenile dogs is more commonly performed than in Norway. Moreover, the inclusion criteria for radiological HD screening might be a source of bias, as dog owners who screen their dogs may have different attitudes towards their dogs than those who do not, and this may also influence longevity. However, this would only bias the estimates of the effects of the factors if the probability of a dog being screened also varied across the levels of the factor of interest (e.g. suburban/city dogs were more likely to be screened than rural dogs). Non-response bias might be important if the association between exposure and outcome differed between responders and non-responders. High socioeconomic status can increase willingness to participate in observational studies (Dohoo et al., 2009). If the socioeconomic status of the owners is associated with both the dogs' exposures (e.g. breed) and outcome (hazard of death), then a bias would occur.

A strength of the study is the design as a prospective cohort of privately owned dogs from all parts of Norway, where the dog's management and veterinary services reflects real life. The use of multivariable survival analysis allowed us to control for several effects simultaneously. By using survival analysis from inclusion to censoring or death, selective survival bias was less likely to occur. In addition, since the outcome is death, misclassification bias of the outcome was probably low. Due to the study design the amount of recall bias was also probably low.

## Conclusions

Radiological HD status and breed were found to be important determinants of overall survival in this prospective cohort study of privately owned dogs from four large breeds. The IW effect on the hazard of death increased linearly over time compared to the other breeds. Severe radiological HD initially resulted in a drastic increase in the hazard of death compared to the less severe grades, but the HD effect decreased logarithmically with time. Radiological ED status was not found to have an effect on overall survival.

## Conflict of interest statement

Neither of the authors of this paper has financial nor personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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# Risk factors for hip-related clinical signs in a prospective cohort study of four large dog breeds in Norway

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### ABSTRACT

We conducted a prospective cohort study including privately owned dogs from the breeds Newfoundland (NF), Labrador Retriever (LR), Leonberger (LEO), and Irish Wolfhound (IW) followed from birth until age 9 yrs. We wanted to investigate whether radiological hip dysplasia status given at approximately age 12–18 mos and other factors during growth influenced development of clinical signs due to hip-joint disease necessitating veterinary consultation. Whether or not such signs occurred due to hip dysplasia or due to secondary or primary DJD could not be distinguished, and we therefore used the term “owner-reported veterinary-diagnosed hip-related clinical signs” (“the event”). The included dogs were followed from birth to the event or until a maximum of 9 yrs of age. Our objectives were to describe breed differences in time to incidence and to evaluate potential risk factors for the time to event. We used Kaplan–Meier curves to describe time to incidence, and potential risk factors were assessed by use of a Cox proportional-hazards model. We enrolled 494 dogs from 103 litters, and 46 dogs were reported as having had the event during the observation period. We observed a significant time-varying effect (TVE): LR and LEO developed clinical signs later in life than NF. If the radiological hip status was either mild, moderate, or severe the hazard of experiencing the event was significantly increased. Access to off-leash exercise at age 12 mos decreased the hazard of the event, and the hazard varied by litter. The findings supported the hypothesis that radiological hip status at screening and exercise conditions during growth influenced the time to incidence of the event and that there were breed differences in time to the event.

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## 1. Introduction

Hip dysplasia and degenerative joint disease (DJD) in the hips are common causes of lameness and exercise intolerance in large-breed dogs (Vaughan, 1990; Roush, 2001). Canine hip dysplasia is a uni- or bilateral developmental disorder with a genetic predisposition, and the occurrence and severity of radiologically diagnosed hip dysplasia are influenced by environmental factors (Lust et al., 1973; Riser, 1974; Fry and Clark, 1992; Kealy et al., 1992; Zhu et al., 2009; Ginja et al., 2010; Krontveit

et al., 2010, in press). DJD of the hips often occurs secondary to canine hip dysplasia, and both hip-joint laxity and increased bodyweight/body-condition score are risk factors for development of DJD in dogs with hip dysplasia (Smith et al., 1995, 2001; Runge et al., 2010). Hip instability allows the femoral head to subluxate during weight bearing which in turn alters the concentration of forces on the femoral head and acetabulum and results in DJD (characterized by loss of articular cartilage, fibrosis, bone remodeling, and loss of normal function) (Alexander, 1992; Fries and Remedios, 1995; Todhunter and Lust, 2003).

Clinical signs related to hip dysplasia can range from mild or intermittent lameness and stiffness with difficulty rising after rest, to nonambulation in severely affected

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dogs (Fry and Clark, 1992). Commonly, a bimodal age distribution is observed with affected younger dogs developing signs between age 3 and 12 mos and mature dogs displaying gradually increasing severity of signs due to progression of DJD (Riser, 1975; Vaughan, 1990; Dassler, 2003). The onset of signs in mature dogs varies from 2 to 12 yrs (Fry and Clark, 1992; Dassler, 2003; Ginja et al., 2010).

When relating clinical signs to the diagnosis of canine hip dysplasia, other causes of lameness or gait abnormalities must be ruled out (Fry and Clark, 1992). High frequencies of cranial cruciate-ligament ruptures have been found in dogs referred for lameness attributed to hip dysplasia (Roush, 2001; Powers et al., 2005). Additionally, both immature and mature dogs with hip dysplasia sometimes also have DJD in both shoulder and stifle joints which could contribute to lameness (Olsewski et al., 1983). Clinical tests can give information about hip joint-laxity and detect signs of DJD (Ginja et al., 2010). Standard radiographic examination of the coxofemoral joints might confirm the diagnosis of hip dysplasia through evaluation of hip-joint congruence and DJD, while stress radiography provides information about hip-joint laxity (Smith, 1997; Fluckiger et al., 1999; Ginja et al., 2010). Standard radiographic examination is used for official screening for canine hip dysplasia in many countries, including Norway, and the results of the radiographic screening are commonly registered in databases of national kennel clubs (e.g. the Norwegian Kennel Club (NKK)).

Aging, high birth weight, slippery pre-weaning floor cover, and neutering are risk factors for development of clinical signs related to canine hip dysplasia (van Hagen et al., 2005). The effect of neutering might be mediated by an increase in bodyweight (van Hagen et al., 2005). An association exists between radiological hip-dysplasia status at screening and incidence of veterinary insurance claims related to canine hip dysplasia (Malm et al., 2010). Not all dogs with a radiographic hip-dysplasia diagnosis develop clinical signs, and the severity of clinical signs will not always correspond with the radiographic findings (Brass, 1989; Fry and Clark, 1992; Dassler, 2003; Ginja et al., 2010).

In our analysis, a cohort of privately owned dogs from four large breeds was followed from birth until age 9 yrs. We wanted to investigate whether radiological hip-dysplasia status given at approximately age 12–18 mos and other factors during growth influenced development of clinical signs due to hip-joint disease necessitating veterinary consultation later in life. Whether or not such signs occurred due to hip dysplasia or due to secondary or primary DJD could not be distinguished, and we therefore used the term “owner-reported veterinary-diagnosed hip-related clinical signs” (also referred to as “the event”). The incidences over time under various exposures were studied. Specifically, our aims of the study were to describe breed differences in time to event and to evaluate potential risk factors by applying a Cox proportional-hazards model. The hypotheses were that radiological hip status at screening, bodyweight, and housing and exercise conditions during growth would influence time to the event.

## 2. Materials and methods

Our study was carried out in agreement with the provisions enforced by the National Animal Research Authority in Norway.

### 2.1. Study design

The present study is part of a larger study (the so-called *main study*) including privately owned dogs from four large breeds: Newfoundland (NF), Labrador Retriever (LR), Leonberger (LEO) and Irish Wolfhound (IW) (Trangerud, 2008). We conducted a prospective single-cohort study to investigate factors affecting time to the event in dogs from the *main study*.

### 2.2. Study population, inclusion criteria

The initial sampling procedure and inclusion of dogs was previously described for dogs in this cohort (Krontveit et al., 2010). Puppies born in Norway between November 1998 and June 2001 were eligible for inclusion in the *main study*. All geographic areas of Norway were represented. Inclusion of a litter began when the bitch was mated. All puppies were registered in the NKK.

Each breeder, dog owner, and veterinarian who participated in the project signed a written agreement of cooperation to comply with the project plan. Not all enrolled dogs continued to completion. Reasons for dropouts included (but were not limited to) death of the dog, relocation of the owners during the study, and export of dogs abroad (Trangerud et al., 2007a). In total, 647 dogs from 106 litters from the *main study* were potentially eligible for inclusion in the present study, which is a convenience sample consisting of 23.2% of the total number of litters born of the included breeds in Norway during 1998–2001.

The inclusion criteria for the present study were that: the dogs were radiologically screened for hip dysplasia at the official NKK ages (12 mos for LR and IW, 18 mos for NF and LEO); they were still present in the cohort at age 3 mos; and that they had bodyweight, housing, and exercise conditions registered at least once during the first year of life. Dogs that were radiologically examined before ages 12 or 18 mos (due to clinical signs of hip disease) were included if their hip radiographs were judged by the panelist in the NKK. A few dogs had hip radiographs taken and registered in the Swedish ( $n = 7$ ) and Danish ( $n = 2$ ) Kennel Clubs.

### 2.3. Questionnaires and clinical registrations

History, husbandry, and clinical information for each included dog were obtained from three sources: (1) the breeder of the litter; (2) the owner of the puppy/dog; and (3) the veterinarian examining the puppy/dog. All three sources completed questionnaires and recorded information in a booklet prepared for each of them. The breeder recorded data during the period from birth until age 8 wks, and the owner and veterinarians at specific ages, called “the observational ages”: 3, 4, 6, 12, 18, and 24 mos. After age 24 mos, annual questionnaires were mailed to the dog

owners until death of the dog or the end of the observation period. Owners not responding for various reasons were contacted by telephone to regain contact.

### 2.3.1. Outcome variable

During the veterinary visits scheduled especially as a part of this project, at “the observational ages”, the dogs were examined by a veterinarian and any clinical signs and treatments were recorded. The dog owners could also contact their veterinarian for an examination if their dogs had any signs of disease between the scheduled visits. After age 24 mos, health status was recorded by the owner on the annual questionnaires including information about any diagnoses and treatments given by a veterinarian. The owners were encouraged to include copies of any veterinary records from the preceding year. Definition of the event was based on both of the following two criteria applied blindly (with no knowledge of exposure status like breed and radiological hip dysplasia status): (1) owner-reported clinical signs (e.g. difficulties in standing up after rest, stiffness, exercise intolerance, and lameness) and (2) clinical signs and findings on veterinary examination (e.g. hip-joint laxity, pain, and reduced movements of the hip joints) reported either on one of the scheduled veterinary visits on one of “the observational ages” or as stated by the owner in the annual questionnaires. The impact of any concurrent causes of hind-limb lameness (as diagnosed by the veterinarian), were also taken into consideration: if other musculoskeletal conditions were reported (by the veterinarian or stated by the owner) as the dog’s main problem, this dog was not included as having the event at that time. A few dogs were reported (by the owner) to have been euthanized due to hip dysplasia/hip-related problems without veterinary reports of clinical signs prior to euthanasia. These dogs were also included as events, and the time of incidence of clinical signs for such a dog was considered the midpoint between the last observation point and the time of euthanasia (Dohoo et al., 2009). Dogs that died or were euthanized due to other causes, or were lost to follow-up, or were still alive at the end of the study were included up until the last time at which they were known to be alive without the event (and were thereafter censored in the analysis). Based on these data and assumptions, the outcome variable “time from birth to owner-reported veterinary-diagnosed hip-related clinical signs” (the event) was obtained.

### 2.3.2. Risk factors

We evaluated four types of data from the questionnaires and clinical registrations as potential risk factors for the event: individual-dog characteristics (*dog data*), bodyweight measurements (*bodyweight data*), housing conditions during growth (*housing data*), and exercise conditions during growth (*exercise data*). All variables are defined in Table 1.

Of the *dog data*, breed, sex, and season of birth were explored as potential explanatory, confounding, or intervening variables; radiological hip and elbow status (graded by and registered in the NKK) were explored as potential explanatory variables. The radiological screening procedure for hip dysplasia was described previously (Krontveit

et al., 2010). The Fédération Cynologique Internationale (FCI) five-class grading scale was used for the hip status of the dogs: A (excellent), B (normal), C (mild dysplasia), D (moderate dysplasia), and E (severe dysplasia) (Flückiger, 2007). For our analysis, the official hip status was reclassified into free (A and B), mild (C), moderate (D), and severe (E) and this variable was termed “radiological hip status”. The International Elbow Working Group (IEWG) elbow protocol was used for grading elbow radiographs (International Elbow Working Group, 2001). Elbow status was dichotomized into a yes/no variable. The calendar year was divided into four seasons according to the temperature and climate conditions in Norway.

Previous studies regarding growth in dogs from this cohort and in dogs from the *main study* guided the selection of *bodyweight data* for our study (Trangerud et al., 2007a, 2007b; Krontveit et al., 2010).

The *housing data* consisted of variables thought to affect general exercise conditions for the dogs during growth: type of house at the breeder’s, environment in outside run at the breeder’s, general living conditions at the owner’s, and the presence of children and other dogs in the owner household. Environment in the outside run was categorized according to softness into two separate dichotomous variables, with the presence of snow and ice as a third separate outdoor environment variable; these variables were not mutually exclusive (Table 1).

*Exercise data* were recorded by the owners in detail, with duration of the different kinds of exercise on an average day. Preliminary analyses revealed bias in the owners’ recordings; to minimize this bias, the different kinds of exercise were studied as separate dichotomous variables. The *exercise data* studied were the use of stairs and different types of outdoor exercise (such as walks on-leash and running off-leash in different types of terrain) at the ages 3 and 12 mos (Table 1).

### 2.4. Statistical analyses

We used the software package Stata 11 (Stata Corporation, 4905 Lakeway Drive, College Station, TX 77845, USA) for all analyses.

#### 2.4.1. Descriptive statistics

The distribution of dogs by breed, sex, radiological hip and elbow status, and season of birth was calculated. The number and percentage of events, mean time to event or censoring, and total number of dogs in each category of these variables were calculated. The numbers of events by official hip status in each of the breeds were described. Incidence rate of the event per 1000 dog-years at risk was estimated for each breed by relating the number of events to the total time at risk for the breed. We used Kaplan–Meier survival curves by breed and by breed and simultaneously with radiological hip status to describe breed differences in time to event.

#### 2.4.2. Survival analyses

We used a Cox proportional-hazards model to evaluate potential risk factors for time to the event. Time at risk was defined as months from birth to the event or

**Table 1**

Definition of potential risk factors for owner reported veterinary-diagnosed hip-related clinical signs in a prospective cohort study of four large dog breeds in Norway (1998–2010).

Variable	Definition	Abbreviated name
<b>Dog data</b>		
Breed	Breed of the dog (categorical): Newfoundland, Labrador Retriever, Leonberger, and Irish Wolfhound	Breed
Sex	Sex of the dog (dichotomous): female and male	Sex
Season of birth	Season of birth (categorical): winter (December–March), spring (April–May), summer (June–August), and fall (September–November)	Season
Hip dysplasia grade	Grade of hip dysplasia at official radiological screening (categorical): free, mild, moderate, and severe	Radiological hip status
Elbow dysplasia	Presence of concomitant elbow dysplasia at official radiological screening (dichotomous)	Radiological elbow status
<b>Bodyweight data</b>		
Bodyweight at birth	Bodyweight in g at birth Birth BW	
Bodyweight at 3 mos	Bodyweight in kg at age 3 mos	3 mos BW
Mature bodyweight	Bodyweight in kg at age 24–36 mos	Mature BW
Aging/old bodyweight	Bodyweight in kg at the highest recorded age up to age 9 yrs (end of study period)	Aging BW
<b>Housing data</b>		
Type of house at the breeders	Type of building in which the litter lived at the breeders (categorical): single family house or farm/small farm (holding)	Breeder house
Outside run at the breeders	Outdoor environment if the puppies were allowed to be in an outside run (3 dichotomous variables): soft (grass, ground, gravel) and hard (wooden, asphalt, concrete), snow/ice	Outside run
Living conditions at owners	Area of living (categorical): countryside, suburban, and city	Owner region
Children	Presence of children in the owner household (dichotomous)	Children
Other dogs	Presence of other dogs in the owner household (dichotomous)	Other dogs
<b>Exercise data</b>		
Use of stairs	Daily use of indoor stairs at age 3 and 12 mos (dichotomous)	Stairs 3 mos and 12 mos
Leash on asphalt paving	Daily walks on leash on asphalt paving at age 3 and 12 mos (dichotomous)	Leash asphalt 3 mos and 12 mos
Leash on graveled road	Daily walks on leash on graveled road at age 3 and 12 mos (dichotomous)	Leash gravel 3 mos and 12 mos
Leash on rough terrain	Daily walks on leash in forest, mountain, or other rough terrain at age 3 and 12 mos (dichotomous)	Leash rough 3 mos and 12 mos
Off-leash in garden	Daily off-leash exercise in garden or yard at age 3 and 12 mos (dichotomous)	Off-leash garden 3 mos and 12 mos
Off-leash in park	Daily off-leash exercise in park terrain at age 3 and 12 mos (dichotomous)	Off-leash park 3 mos and 12 mos
Off-leash in rough terrain	Daily off-leash exercise in forest, mountain, or other rough terrain at age 3 and 12 mos (dichotomous)	Off-leash rough 3 mos and 12 mos

censoring. The end of the observation period was set to 9 yrs (108 mos). The dogs in the study were clustered into litters. The assumption of independence between observations was therefore violated and a shared frailty term for litter was included in the model (Dohoo et al., 2009).

All of the potential risk factors were tested alone applying univariable Cox proportional-hazards models while controlling for litter (the frailty term for litter included). The variables were tested for collinearity (by Goodman and Kruskal's gamma for ordinal and dichotomous variables, the *phi* coefficient for nominal variables, and pair-wise correlations for continuous variables). Associations  $>0.7$  or  $<-0.7$  were considered evidence of collinearity. The variables with a univariable *P*-value  $\leq 0.20$ , provided that there was no collinearity between them, were then considered for further multivariable analysis. When collinearity was detected between two predictors, the predictor with fewest missing data was selected for further analyses. To assess the functional form of continuous predictors, martingale residuals were plotted against the continuous predictor of interest.

A multivariable Cox proportional-hazards model with shared frailty for litter was constructed using manual forward selection by offering variables selected from the univariable analyses one-at-a-time to the model by ascending *P*-value. We retained variables in the model when the *P*-value of the likelihood-ratio test (LRT) was  $<0.05$ . We constructed a causal diagram to evaluate potential confounding and intervening variables. Changes of  $>20\%$  in the coefficients in the model with the potential confounder present were also used as an indication of confounding. A variable was considered to be “intervening” if adding it removed the entire effect of another variable and if the intervening variable lay on the causal path between the factor and the outcome. Intervening variables were excluded from the final model. All possible two-way interactions between the predictors in the final model were tested by adding interaction terms to the model, and an interaction term was retained if  $P < 0.01$ . The significance of the shared frailty term was evaluated through a LRT. The multiple Wald test and LRT were used to evaluate differences between categories of categorical predictors.

**Table 2**

Descriptive statistics for individual-dog characteristic (*dog data*) variables in a study investigating risk factors for owner reported veterinary-diagnosed hip-related clinical signs in a prospective cohort of 494 dogs from four large breeds in Norway (1998–2010).

Variable and level	Number of events	% events	Mean time to event (yr)	Mean time to censoring (yr)	Total number of dogs	% dogs of total
<b>Breed</b>						
Newfoundland	13	10.7	1.9	6.8	122	24.7
Labrador Retriever	10	7.6	6.5	7.6	131	26.5
Leonberger	19	10.7	3.9	6.3	178	36.0
Irish Wolfhound	4	6.3	3.1	6.2	63	12.8
<b>Sex</b>						
Female	30	11.5	4.0	6.8	260	52.6
Male	16	6.8	3.5	6.7	234	47.4
<b>Radiological hip status</b>						
Free	10	2.7	6.1	6.8	375	75.9
Mild	9	21.4	3.5	6.5	42	8.5
Moderate	15	26.8	3.4	6.5	56	11.3
Severe	12	57.1	2.8	6.6	21	4.3
<b>Radiological elbow status<sup>a</sup></b>						
Non-affected	33	8.3	4.4	6.9	398	83.3
Affected	8	10.0	3.2	6.6	80	16.7
<b>Season</b>						
Winter	16	8.5	4.1	7.0	189	38.3
Spring	7	6.6	3.3	6.6	106	21.5
Summer	9	8.9	4.3	6.6	101	20.4
Fall	14	14.3	3.6	6.4	98	19.8

<sup>a</sup> Radiological elbow status was missing for 16 dogs.

#### 2.4.3. Model evaluation

The assumption of proportional hazards was evaluated for the model using the test for proportional hazards based on the Schoenfeld residuals for each variable in the model. If the assumption of proportional hazards was violated and the graphical assessment indicated a time-varying effect (TVE) of a variable, an interaction term between the variable and time was included in the final model. The assumption of independent censoring, overall fit, concordance, and identification of any outliers were tested as described in the literature (Dohoo et al., 2009).

### 3. Results

#### 3.1. Descriptive statistics

The number of events, mean time to event or censoring, and total number of subjects in each category of *dog data* are provided in Table 2. Of the 494 dogs enrolled, 65 were lost to follow-up some time during the 9-yr observation period and 237 died or were euthanized. At the end of the study 46 NF, 85 LR, 53 LEO, and 8 IW were still alive.

Of the 46 dogs considered to have had the event (Table 3), 10 dogs were reported to have been euthanized

**Table 3**

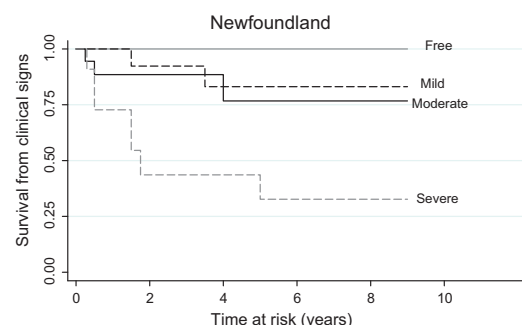
Number of dogs with owner reported veterinary-diagnosed hip-related clinical signs/total number within radiological hip status by breed in a prospective cohort study of four large breeds in Norway (1998–2010).

Breed	Radiological hip status			
	Free	Mild	Moderate	Severe
Newfoundland	0/79	2/14	4/18	7/11
Labrador Retriever	4/106	2/10	2/11	2/4
Leonberger	5/133	4/15	7/24	3/6
Irish Wolfhound	1/57	1/3	2/3	–
Total	10/375	9/42	15/56	12/21

due to hip dysplasia/hip-related problems without veterinary reports of clinical signs prior to euthanasia. We made the assumption that the time of occurrence of the event was placed at the midpoint between the last observation point and the time of euthanasia. In 5 of these dogs this time interval was <3 mos. Total amount of time at risk was 3197 dog-years. Estimated incidence rate of the event per 1000 dog-years at risk was 17 for NF, 10 for LR, 18 for LEO, and 11 for IW. Figs. 1–4 show Kaplan–Meier curves for NF, LEO, LR, and IW by official hip status. Kaplan–Meier curves for all four breeds combined are presented in Fig. 5.

#### 3.2. Risk factors

Collinearity was detected between the weight variables. The following variables were thus selected for multivariable modeling after unconditional screening ( $P < 0.20$ ): breed, sex, radiological hip status, 3-mo bodyweight,



**Fig. 1.** Kaplan–Meier curves for Newfoundland ( $n = 122$ ) in a prospective cohort of four large dog breeds describing time to occurrence of owner reported veterinary-diagnosed hip-related clinical signs. Time at risk is from birth to 9 yrs of age (Norway, 1998–2010).

**Table 4**

Results from a multivariable Cox proportional-hazards model estimating the effects of breed, radiological hip dysplasia status, and exercise conditions on time from birth to owner reported veterinary-diagnosed hip-related clinical signs ( $n = 46$ ) in a prospective cohort study of four large dog breeds ( $n = 494$ ) in Norway (1998–2010).

Variable and level	Estimate	SE	Hazard ratio	P value	95% confidence interval
<b>Main effects</b>					
<b>Breed</b>					
Newfoundland	–		1.00	–	–
Labrador Retriever	–3.24	1.47	0.04 <sup>a</sup>	0.03	0.00, 0.70
Leonberger	–1.19	0.81	0.30 <sup>a</sup>	0.14	0.06, 1.49
Irish Wolfhound	0.50	1.29	1.66	0.70	0.13, 20.60
<b>Radiological hip status</b>					
Free	–		1.00	–	–
Mild	2.48	0.54	12.00	<0.001	4.18, 34.40
Moderate	2.90	0.50	18.24	<0.001	6.84, 48.61
Severe	4.52	0.59	91.96	<0.001	28.77, 293.88
<b>Off-leash garden 12 mos</b>					
No	–		1.00	–	–
Yes	–0.75	0.35	0.47	0.03	0.24, 0.95
<b>Off-leash rough 12 mos</b>					
No	–		1.00	–	–
Yes	–1.04	0.37	0.35	0.005	0.17, 0.73
<b>Leash asphalt 12 mos</b>					
No	–		1.00	–	–
Yes	–1.12	0.38	0.33	0.003	0.16, 0.68
<b>Time-varying effect (TVE)</b>					
<b>Breed</b>					
Newfoundland					
Labrador Retriever	0.07	0.03	–	0.01	0.02, 0.12
Leonberger	0.04	0.02	–	0.03	0.01, 0.08
Irish Wolfhound	0.02	0.02	–	0.46	–0.04, 0.08
<b>Frailty (litter)</b>					
Frailty variance	0.74	0.54	–	0.03 <sup>b</sup>	–

<sup>a</sup> Estimates of effect of factor varied over time – see text for interpretation.

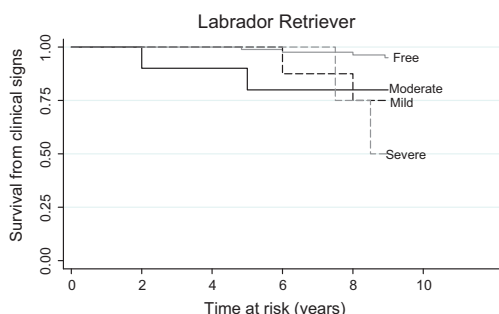
<sup>b</sup> Likelihood ratio test of frailty variance.

breeder house, outside run snow/ice, leash rough 3 mos, off-leash park 3 mos, leash asphalt 12 mos, off-leash rough 12 mos, and off-leash garden 12 mos. In the final model, radiological hip status and the exercise variables off-leash garden 12 mos, off-leash rough 12 mos, and leash asphalt 12 mos were significant ( $P < 0.05$ ) (Table 4). Breed was retained in the final model (Table 4) as a potential confounder to control for breed differences. The multiple Wald test and LRT for comparing models with and without the categorical variable radiological hip status were both significant at  $P < 0.001$ . No confounding or intervening effect

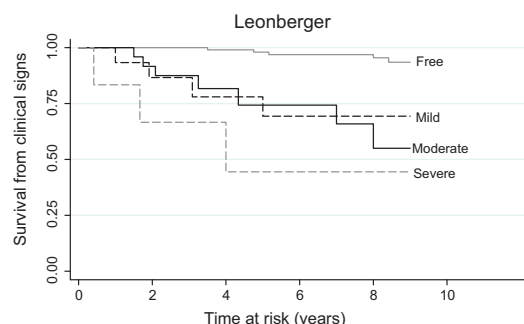
was detected for the variable sex; hence this variable was not retained in the model. None of the tested interactions was significant (all  $P \geq 0.10$ ). The shared frailty variance for litter was significant as judged by the LRT (Table 4).

### 3.3. Model evaluation

Because the assumption of proportional hazards was violated for LR and LEO, a TVE for breed was included, and it was significant for LR and LEO (Table 4). Relating the main effects coefficient for LR to the TVE coefficient indicated

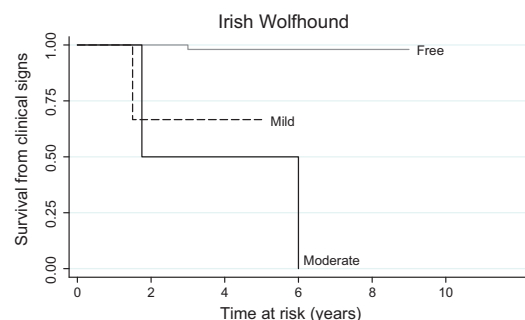


**Fig. 2.** Kaplan–Meier curves for Labrador Retriever ( $n = 131$ ) in a prospective cohort of four large dog breeds describing time to occurrence of owner reported veterinary-diagnosed hip-related clinical signs. Time at risk is from birth to 9 yrs of age (Norway, 1998–2010).

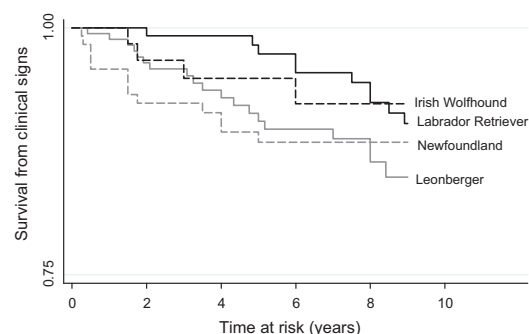


**Fig. 3.** Kaplan–Meier curves for Leonberger ( $n = 178$ ) in a prospective cohort of four large dog breeds describing time to occurrence of owner reported veterinary-diagnosed hip-related clinical signs. Time at risk is from birth to 9 yrs of age (Norway, 1998–2010).





**Fig. 4.** Kaplan–Meier curves for Irish Wolfhound ( $n = 63$ ) in a prospective cohort of four large dog breeds describing time to occurrence of owner reported veterinary-diagnosed hip-related clinical signs. Time at risk is from birth to 9 yrs of age (Norway, 1998–2010).



**Fig. 5.** Kaplan–Meier curves for 494 dogs in the breeds Newfoundland ( $n = 122$ ), Labrador Retriever ( $n = 131$ ), Leonberger ( $n = 178$ ), and Irish Wolfhound ( $n = 63$ ) in a prospective cohort study describing time to occurrence of owner reported veterinary-diagnosed hip-related clinical signs. Time at risk is from birth to 9 yrs of age (Norway, 1998–2010).

that the protective effect of being LR compared to NF was lost by ~4 yrs (age 47 mos). For LEO the protective effect was lost by ~2.5 yrs (age 28 mos). For IW the TVE was not significant (Table 4).

No major shortcomings of the model were detected through the model diagnostics.

#### 4. Discussion

Our main findings were that there were breed differences in time to event, that dogs with radiographic hip status mild, moderate or severe had significantly shorter time to event (increased hazard) compared with dogs with radiographic hip status free, and that dogs exercised off-leash at 12 mos of age in garden and rough terrain had longer time to event (decreased hazard) compared to dogs not exercised this way.

The protective effect of being LR rather than NF disappeared by 4 yrs and by 2.5 yrs for LEO as indicated by the TVE for breed. Primary DJD develops as a consequence of wear-and-tear and appears with advancing age; secondary DJD might be a result of conditions interfering with the normal mechanics of the joint (Vaughan, 1990; Roush, 2001).

Canine hip dysplasia is a developmental condition that can produce secondary DJD of the hips (Vaughan, 1990). DJD in the hips progresses throughout life (Kealy et al., 1997; Smith et al., 2006). Differentiating between primary and secondary DJD might be difficult when examining joints of old dogs (Vaughan, 1990; Roush, 2001). We followed dogs until age 9 yrs. It seems plausible that in the dogs experiencing the event late in life, these signs might be attributed to primary DJD and not necessarily secondary to hip dysplasia.

Factors significantly influencing time to event were radiological hip status and both on-leash and off-leash exercise at age 12 mos. Increasing severity of radiological hip status shortened the time to event. Association between radiological hip status at screening and subsequent veterinary insurance claims related to hip dysplasia was recently reported (Malm et al., 2010). Some of the dogs in our study were reported as having the event although they had an official radiological hip status “free”. These dogs might have had increased hip joint laxity not identified on conventional radiographs and developed DJD secondary to hip dysplasia with clinical signs increasing over time but the signs might also be due to primary DJD.

We had detailed information about exercise conditions at different ages. Specific exercise conditions at age 3 mos were selected for investigation as they might have influenced early occurring events. Exercise conditions at age 12 mos were selected because they could influence later occurring events but were not biased by the radiological hip dysplasia diagnosis assigned after screening at ages 12–18 mos. Furthermore, the exercise conditions at age 12 mos might be representative for how the dogs were exercised later in life. Among the exercise-related variables we studied, off-leash exercise in garden or yard and in rough terrain at age 12 mos combined with on-leash exercise on asphalt at age 12 mos delayed time to event (after controlling for breed and radiological hip status). Off-leash exercise in moderately rough terrain early in life was protective against radiographically diagnosed hip dysplasia at screening in a previous study in this cohort (Krøntveit et al., in press). These kinds of exercise might be beneficial by strengthening muscle mass and improving range of motion in the hip joints (Holler et al., 2010; Millard et al., 2010), thus possibly delaying time to event. Both off-leash and on-leash exercise is recommended for dogs with DJD, but excessive stress (such as that induced by playing with other dogs and chasing balls or sticks) should be avoided because these activities might result in flare-ups of clinical signs (Vaughan, 1990).

Neither of the bodyweight measurements was associated with time to event. Other studies have reported that restricted feeding prevented overweight and reduced both frequency and severity of the development of DJD of the hips in dogs (Kealy et al., 1997; Smith et al., 2006). Overweight can exacerbate clinical signs of DJD (Vaughan, 1990; Impellizzeri et al., 2000). Bodyweight does not take into account breed differences in size; body-condition score might be a better measure than bodyweight for assessment of “being overweight or obese” (Laflamme, 1997). In the present study, body-condition scores were not available.

Due to the clustering of the dogs in litters, a shared frailty term had to be included in all analyses. The shared

frailty term was significant, indicating that dogs from certain litters had higher hazard than others. The dogs lived most of their lives separated from their littermates under differing environmental conditions. The common genetic background of dogs in a litter and other unmeasured litter factors might be important parts of the frailty variance.

The outcome variable was defined as time to owner-reported veterinary-diagnosed hip-related clinical signs. Based on the breeds included and the reported clinical presentation, most of these clinical signs are assumed to be related to canine hip dysplasia although primary DJD or secondary DJD of other causes cannot be excluded. Radiographs are necessary to diagnose DJD and stress radiography or specific clinical tests are necessary to diagnose hip laxity (McLaughlin, 2001; Roush, 2001). These evaluations were not performed in all of the dogs. Also, concurrent musculoskeletal conditions in other body areas might have contributed to the signs observed and wrongly interpreted by the veterinarian as hip joint disease. In all cases, however, the reported signs were the main musculoskeletal problem considered by both the owner and the veterinarian. Some dogs ( $n=10$ ) were included as having the event because they had been euthanized by a veterinarian due to hip dysplasia/hip-related problems without preceding record of clinical signs, as also reported in another study (Malm et al., 2010). These dogs were considered likely to have had the event (Malm et al., 2010). The assumption that these dogs showed clinical signs prior to euthanasia might be associated with an uncertainty because reasons for euthanizing a dog are influenced by several factors. Additionally, the decision to set time of event at the midpoint between the last observation point and time of euthanasia for these dogs might cause a bias towards shorter time to event since the distribution of events might not be uniformly distributed. However, due to the relatively small number of dogs in this category and the long follow-up time, the amount of bias due to the inclusion of these dogs as events is expected to be low.

The event in our study is a reflection of an owner-perceived clinical problem necessitating veterinary consultation for these patients. Norwegian dog owners have great animal-directed empathy, and empathy was the best predictor of how the owners rated pain in dogs (Ellingsen et al., 2010). Such attitudes towards dogs could probably increase the number of reported events in the current study, because Norwegian dog owners could have a low threshold for consulting a veterinarian. Also, the dog owners' knowledge of the radiological hip dysplasia status of their dogs could increase the reporting because owners could be more aware of signs (such as exercise intolerance, lameness, and stiffness) thus having even lower thresholds for contacting veterinarian (especially in dogs with the more-severe grades of hip dysplasia). Among insured Swedish dogs, German Shepherds had very high risk of being euthanized shortly after hip dysplasia screening if graded with the more-severe dysplasia-grades (possibly because this is a breed commonly used as working dogs) (Malm et al., 2010). Norwegian dog owners have higher empathy and pain perception towards their dog when the dogs are kept for companionship than when kept primarily for working purposes (hunting) (Ellingsen et al., 2010).

However, most of the dogs in the current study were kept as companions and not as working dogs.

Misclassification of the outcome is a possible bias in this study. Non-differential misclassification of the outcome during follow-up in a cohort study will bias the measure of association towards the null and this potential bias will have a conservative effect on the results of the study and could possibly lead to associations between exposure and outcome being underestimated (Dohoo et al., 2009). We tried to reduce the amount of non-differential misclassification bias by including dogs from birth (free of clinical disease), by having criteria for being considered as an event, and by evaluating all information about the individual dogs "blindly" without information about breed and radiological hip dysplasia status. Differential misclassification (i.e. dog owners or veterinarians knowing the radiological hip dysplasia status affect the reporting of clinical signs and diagnosis) could bias the measure of association in any direction (Dohoo et al., 2009). Some of the dogs ( $n=10$ ) having the event were graded as free of hip dysplasia at the radiological screening. This could indicate that the bias caused by owner (and veterinarian) knowing of the official radiological hip dysplasia status might be relatively low.

Differential loss to follow-up related to exposure and outcome can bias the measure of association (Dohoo et al., 2009), and such bias can be an important problem in cohort studies of long duration as in our study (Pfeiffer, 2010). The number of events could be underestimated, especially after age 24 mos. Strategies aimed to reduce this bias were signed owner contracts for participation in the project, regular follow-up during the first 2 yrs of the study, and then by sending annual questionnaires accompanied by a cover letter encouraging owners to answer. Non-response bias might be important if there are dog-related differences between responders and non-responders (Dohoo et al., 2009), but the socio-economic status of the owners could also be a source of bias if this is associated with both exposure and outcome (Dohoo et al., 2009).

On the other hand, a strength of our data set is that all dogs were free of the clinical disease at inclusion because the observation period started at birth. The dogs were further followed until event or censoring; thus, selective survival bias was less likely to occur. Non-differential misclassification of health-status at inclusion can lead to either under- or overestimation of associations, and is a more serious bias (Dohoo et al., 2009). Other potential shortcomings of this cohort are described in detail previously (Krontveit et al., 2010).

## 5. Conclusion

Our findings supported our hypothesis that less severe radiological hip status at screening and provision of exercise during growth delayed the time to event. The LR and LEO breeds developed the event later than the NF. However, no effect of bodyweight was detected.

## Conflict of interest statement

None of the authors of this paper has financial or personal relationship with other people or organizations that



could inappropriately influence or bias the content of the paper.

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## Appendix 1



**Table 1.** Definition and description of individual dog data variables (Part a), weight, growth, and size measurement variables (Part b), blood sample variables (Part c), and housing and exercise variables (Part d) used in a study investigating epidemiology of canine hip dysplasia in a prospective cohort study of four large breeds

**Part a.** Dog data variables

<b>Dog data variables</b>	<b>Definition</b>	<b>Paper</b>
Breed	Breed of the dog (categorical): Newfoundland, Labrador retriever, Leonberger, Irish wolfhound	I, II, III, IV
Sex	Sex of the dog (dichotomous): female, male	I, II, III, IV
Litter size	Number of puppies in each litter (continuous)	I, II
Season of birth	Season of birth of the litter (categorical): winter (December-March), spring (April-May), summer (June-August), fall (September-November)	II, IV
Radiological hip dysplasia grade	Grade of hip dysplasia at official radiological screening (categorical): free, mild, moderate, severe	III, IV
Radiological elbow dysplasia grade	Presence of concomitant elbow dysplasia at official radiological screening; dichotomous or categorical: free, mild, moderate, severe	III, IV

**Part b. Weight, growth, and size measurement variables**

<b>Weight, growth, size measurement variables</b>	<b>Definition</b>	<b>Paper</b>
Body weight	Body weight in grams at birth, and at 3, 7, 14, 21, 28, 35, 42, 49, and 56 days of age (continuous) Body weight in kg at 3, 4, 6, and 12 months of age (continuous)	I
Body weight at birth	Bodyweight in g at birth (continuous)	IV
Body weight at 3 months	Body weight in kg at age 3 months (continuous)	IV
Mature body weight	Body weight in kg at age 24 – 36 months (continuous)	IV
Aging/old body weight	Body weight in kg at the highest recorded age up to age 9 years (end of study period) (continuous)	IV
Average daily gain	Average daily gain in kg/day in the following periods (continuous): Birth to 7 days Birth to 14 days Birth to 21 days Birth to 56 days Birth to 3 months	I

	Birth to 6 months	
	Birth to 12 months	
	35 days to 3 months	
	56 days to 3 months	
	3 to 4 months	
	4 to 6 months	
	6 to 12 months	
Circumference of distal radius and ulna	Measurement of this circumference in cm at 3, 4, 6, and 12 months of age (continuous)	I
	Changes in these measurements between the following ages (continuous):	
	3 to 4 months	
	3 to 12 months	
	4 to 6 months	
	6 to 12 months	

**Part c.** Blood sample variables

<b>Blood sample variables</b>	<b>Definition</b>	<b>Paper</b>
Hematology	Counts of leucocytes, erythrocytes, thrombocytes, neutrophils, eosinophils, lymphocytes, monocytes, and basophils at 3, 4, 6, and 12 months of age (continuous)  Measurement of mean cell volume, hematocrit, hemoglobin, and mean cell hemoglobin concentration at 3, 4, 6, and 12 months of age (continuous)	I
Clinical chemistry parameters	Measurement of alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, creatinine kinase, amylase, lipase, total protein, albumin, globulin, urea, creatinine, bile acids, total bilirubin, cholesterol, glucose, inorganic phosphate, potassium, calcium, sodium, chloride, and sodium/potassium ratio at 3, 4, 6, and 12 months of age (continuous)  Changes in ALP between the following ages (continuous):  3 to 4 months  4 to 6 months  6 to 12 months	I



**Part d. Housing and exercise variables**

<b>Housing and exercise variables</b>	<b>Definition</b>	<b>Paper</b>
<b>Pre-weaning housing conditions (<i>breeder data</i>)</b>		
Living conditions/living region	Area of living (categorical): countryside, suburban, city	II
Type of house	Type of building in which the dogs live (categorical): single family house or farm/small farm(holding)	II, IV
Whelping box padding	Type of padding used in the whelping box (3 separate dichotomous variables): newspaper (0/1), sawdust (0/1), carpet (0/1)	II
Flooring	Type of floor cover on the indoor area (4 separate dichotomous variables): parquet/wooden (0/1), linoleum (0/1), carpet (0/1), tiles (0/1)	II
Outside run	Outdoor environment if the puppies were allowed to be in an outside run (3 separate dichotomous variables): soft (0/1) (grass, ground, gravel), hard (0/1) (wooden, asphalt, concrete), snow/ice (0/1)	II, IV
<b>Post-weaning housing conditions (<i>owner housing data</i>) at 3, 4, 6, and 12 months of age</b>		
Living conditions/living region	Area of living (categorical): countryside, suburban, city	II, III

Type of house	Type of building in which the dogs live (categorical): single family house, farm/small farm(holding), or apartment building	II
Children	If the household included children (dichotomous)	II, IV
Other dogs	Presence of other dogs in the household (dichotomous)	II, IV
Indoor environment	Type of padding/surface on indoor resting area (dichotomous): soft (bed, madras, pillow, quilt, sofa) or hard (wooden floor, parquet, linoleum, tiles/concrete, thin carpet)	II
Outdoor environment	Type of padding/surface on the outdoor resting area (dichotomous): soft (grass, ground, sand, madras) or hard (wooden, asphalt/concrete, gravel)	II
Snow and ice outdoor	Presence of snow and ice outdoor (dichotomous)	II
<b>Post-weaning exercise conditions (<i>owner exercise data</i>) at 3, 4, 6, and 12 months of age<sup>1</sup></b>		
Use of stairs	Daily use of inside stairs (dichotomous)	II, IV
Leash on asphalt paving	Daily walks in leash on asphalt paving (dichotomous)	II, IV
Leash on graveled road	Daily walks in leash on graveled road (dichotomous)	II, IV
Leash in rough terrain	Daily walks in leash in forest, mountain, or other	II, IV

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	rough terrain (dichotomous)	
Exercise run graveled	Daily exercise in graveled run (dichotomous)	II
Exercise run ground/grass	Daily exercise in run with soft covering, ground or grass (dichotomous)	II
Exercise run concrete	Daily exercise in run with hard covering, concrete or asphalt (dichotomous)	II
Off-leash in garden	Daily off-leash exercise in garden or yard (dichotomous)	II, IV
Off-leash in park	Daily off-leash exercise in park terrain (dichotomous)	II, IV
Off-leash in rough terrain	Daily off-leash exercise in forest, mountain, or other rough terrain (dichotomous)	II, IV
Bicycling	Daily running alongside a bike (dichotomous)	II
Other activities	Other training, playing with other dogs or people (dichotomous)	II

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<sup>1</sup> In Paper IV the owner exercise data from 3 and 12 months of age were used

