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2007

[Link to publication](#)

Citation for published version (APA):

Jönsson, T. (2007). *Healthy Satiety Effects of Paleolithic diet on Satiety and Risk factors for Cardiovascular disease*. [Doctoral Thesis (compilation), Medicine/Emergency Medicine, Lund]. Division of Family Medicine Department of Clinical Sciences Lund University Sweden.

Total number of authors:

1

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Healthy Satiety

Effects of Paleolithic diet on Satiety and
Risk factors for Cardiovascular disease

TOMMY JÖNSSON, M.D.

Doctoral Thesis

The public defense of this thesis for the degree Doctor of Philosophy in
Medicine will, with due permission from the Faculty of Medicine at Lund
University, take place in Föreläsningssal 3, Lund University Hospital, Lund,
Sweden, on Friday, November 23, 2007, at 13.00.

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Organization LUND UNIVERSITY Division of Family Medicine Department of Clinical Sciences, Lund Lund University, Sweden	Document name DOCTORAL DISSERTATION	
	Date of issue 2007-11-23	
	Sponsoring organization	
Author(s) Tommy Jönsson		
Title and subtitle Healthy Satiety. Effects of Paleolithic diet on Satiety and Risk factors for Cardiovascular disease.		
Abstract The prevalence of cardiovascular diseases (CVD) exhibits considerable variation across agrarian societies, whereas they are virtually absent in non-agrarian societies such as hunter-gatherer and horticultural societies. When looking for lifestyle factors which could promote CVD, it therefore seems logical to focus on the agrarian lifestyle and ways in which it departs from a non-agrarian lifestyle. The aim of this thesis was to evaluate the effect of a hunter-gatherer diet (also called Paleolithic diet) compared to agrarian diet in prevention of CVD. In paper I we reviewed evidence from epidemiology and evolutionary biology for a possible maladaptation of human satiety hormone leptin to agrarian diet. We found that leptin resistance hypothetically may be a sign of maladaptation to dietary cereals as staple food, and that lectins could be a cereal constituent with sufficient properties to cause leptin resistance. In paper II we experimentally studied the long-term effect of a Paleolithic diet compared to a cereal-based diet on risk factors for cardiovascular disease in domestic pigs. We found that a Paleolithic diet conferred higher insulin sensitivity, lower C-reactive protein and lower blood pressure than a cereal-based diet. In paper III and IV we experimentally studied the effect of a Paleolithic diet compared to a Mediterranean-like diet on risk factors for cardiovascular disease, satiety and leptin in individuals with ischaemic heart disease and either glucose intolerance or type 2 diabetes. Paper III showed that a Paleolithic diet improved glucose tolerance more than a Mediterranean-like diet, and paper IV showed that a Paleolithic diet was more satiating per calorie than a Mediterranean-like diet. In conclusion, we have found beneficial effects of a Paleolithic diet on risk factors for cardiovascular disease and satiety.		
Key words: Paleolithic diet, Cardiovascular disease, Risk factors, Satiety, Leptin		
Classification system and/or index terms (if any):		
Supplementary bibliographical information:		Language English
ISSN and key title: 1652-8220		ISBN 978-91-85897-23-0
Recipient's notes	Number of pages 122	Price
	Security classification	

Distribution by (name and address) Tommy Jönsson, Division of Family Medicine, Department of Clinical Sciences, Lund University, Box 117, 221 00 Lund, Sweden

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”There is grandeur in this view of life, with its several powers, having been originally breathed into a few forms or into one; and that, whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved.”

Charles Darwin

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Printed by KFS AB, Lund

ISSN 1652-8220

ISBN 978-91-85897-23-0

Contents

List of Papers	6
Summary	7
Populärvetenskaplig sammanfattning	8
Abbreviations	9
Introduction	11
Cardiovascular disease and the ‘diseases of affluence’	11
Hunter-gatherer versus agrarian lifestyle	14
The role of excess and restricted energy intake	16
The concept of satiety and its determinants	16
Leptin, the leptin receptor and free leptin index	17
Molecular evolution and leptin.....	19
The domestic pig as a model for cardiovascular disease	19
Dietary lectins	20
Aims	22
Materials and Methods	23
Study populations	23
Interventions	25
Outcomes.....	27
Statistical analysis	29
Results and Comments	31
Conclusions	43
Future aspects and recommendations	44
Acknowledgements	45
Bibliography	47
Papers I-IV	61

List of Papers

The thesis is based on the following papers, which will be referred to by their Roman numerals in the text:

- I. **Jönsson T**, Olsson S, Ahrén B, Bøg-Hansen TC, Dole A, Lindeberg S: Agrarian diet and diseases of affluence - Do evolutionary novel dietary lectins cause leptin resistance? *BMC Endocr Disord* 2005, 5:10.
- II. **Jönsson T**, Ahrén B, Pacini G, Sundler F, Wierup N, Steen S, Sjöberg T, Ugander M, Frostegård J, Göransson L, Lindeberg S: A Paleolithic diet confers higher insulin sensitivity, lower C-reactive protein and lower blood pressure than a cereal-based diet in domestic pigs. *Nutr Metab (Lond)* 2006, 3:39.
- III. Lindeberg S, **Jönsson T**, Granfeldt Y, Borgstrand E, Soffman J, Sjöström K, Ahrén B: A Paleolithic diet improves glucose tolerance more than a Mediterranean-like diet in individuals with ischemic heart disease. *Diabetologia* 2007, Sep;50(9):1795-807. Epub 2007 June 22.
- IV. **Jönsson T**, Granfeldt Y, Erlanson-Albertsson C, Ahrén B, Lindeberg S: A Paleolithic diet is more satiating per calorie than a Mediterranean-like diet in individuals with ischemic heart disease. Submitted.

Summary

The prevalence of cardiovascular diseases (CVD) exhibits considerable variation across agrarian societies, whereas they are virtually absent in non-agrarian societies such as hunter-gatherer and horticultural societies. When looking for lifestyle factors which could promote CVD, it therefore seems logical to focus on the agrarian lifestyle and ways in which it departs from a non-agrarian lifestyle. The aim of this thesis was to evaluate the effect of a hunter-gatherer diet (also called Paleolithic diet) compared to agrarian diet in prevention of CVD.

In paper I we reviewed evidence from epidemiology and evolutionary biology for a possible maladaptation of human satiety hormone leptin to agrarian diet. We found that leptin resistance hypothetically may be a sign of maladaptation to dietary cereals as staple food, and that lectins could be a cereal constituent with sufficient properties to cause leptin resistance.

In paper II we experimentally studied the long-term effect of a Paleolithic diet compared to a cereal-based diet on risk factors for cardiovascular disease in domestic pigs. We found that a Paleolithic diet conferred higher insulin sensitivity, lower C-reactive protein and lower blood pressure than a cereal-based diet.

In paper III and IV we experimentally studied the effect of a Paleolithic diet compared to a Mediterranean-like diet on risk factors for cardiovascular disease, satiety and leptin in individuals with ischaemic heart disease and either glucose intolerance or type 2 diabetes. Paper III showed that a Paleolithic diet improved glucose tolerance more than a Mediterranean-like diet, and paper IV showed that a Paleolithic diet was more satiating per calorie than a Mediterranean-like diet.

In conclusion, we have found beneficial effects of a Paleolithic diet on risk factors for cardiovascular disease and satiety.

Populärvetenskaplig sammanfattning

Förekomsten av hjärt-kärlsjukdomar skiljer sig mycket mellan olika jordbruksbaserade samhällen, medan de praktiskt taget saknas helt bland samhällen som inte är baserade på jordbruk, såsom jägar- och samlarsamhällen. När man letar efter livsstilsfaktorer som skulle kunna gynna uppkomsten av kardiovaskulära sjukdomar verkar det därför logiskt att fokusera på livsstilen i samhällen baserade på jordbruk och hur denna skiljer sig från livsstilen i samhällen utan jordbruk. Målet med denna avhandling var att jämföra effekten av jägar- och samlarkost (även kallad paleolitisk kost) med kost baserad på jordbruk avseende förebyggande av hjärt-kärlsjukdomar.

Delarbete I granskar belägg från epidemiologi och evolutionsbiologi för en möjlig otillräcklig anpassning hos människan av mättnadshormonet leptin till en kost baserad på jordbruk. Vi fann att leptinresistens hypotetiskt kan vara ett tecken på otillräcklig anpassning till spannmål som stapelföda, och att lektiner skulle kunna vara en beståndsdel i spannmål med tillräckliga egenskaper för att orsaka leptinresistens. Leptinresistens är kopplat till hjärt-kärlsjukdom och flertalet feta människor med höga leptinnivåer och dålig effekt av leptinbehandling antas vara leptinresistenta.

I delarbete II studerades långtidseffekter av paleolitisk kost jämförd med en spannmålsbaserad kost avseende risk faktorer för hjärt-kärlsjukdom hos tamsvin. Vi fann att paleolitisk kost medförde högre insulinkänslighet, lägre C-reaktivt protein (CRP) och lägre blodtryck jämfört med en spannmålsbaserad kost.

Delarbete III och IV studerade effekter av paleolitisk kost i jämförelse med en Medelhavsliknande kost avseende risk faktorer för hjärt-kärlsjukdom, mättnad och leptin hos individer med kranskärlssjukdom och som dessutom hade antingen nedsatt glukostolerans (nedsatt förmåga att omsätta kostens kolhydrater) eller diabetes typ 2. Delarbete III visade att en paleolitisk kost förbättrade glukostoleransen mer än en Medelhavsliknande kost, och delarbete IV visade att en paleolitisk kost var mer mättande per kalori än en Medelhavsliknande kost.

Sammanfattningsvis visade studierna gynnsamma effekter av en paleolitisk kost på riskfaktorer för kardiovaskulär sjukdom och mättnad.

Abbreviations

ANOVA	analysis of variance
AUC	area under the curve
BIA	bioelectrical impedance analysis
BMI	body mass index= body weight (kg) / body length squared (m ²)
CRP	C-reactive protein
HLA	human leukocyte antigen system
HOMA-IR	homeostasis model assessment of insulin resistance
IVGTT	intravenous glucose tolerance test
OGTT	oral glucose tolerance test (75 gram glucose)
QUICKI	quantitative insulin sensitivity check index
SLR	soluble leptin receptor
WGA	wheat germ agglutinin

Introduction

Cardiovascular disease and the 'diseases of affluence'

Cardiovascular diseases, such as coronary heart disease and stroke, and their lifestyle-related risk factors, such as obesity and diabetes type 2, are among the leading causes of mortality and morbidity globally, and have been predicted to rise over the next few decades [1]. A key driver of the expected increase is aging of the world's population, since cardiovascular disease rates tend to increase with age. However, in addition to this basically positive demographic change, an epidemiological change that involves increases in age-specific rates of cardiovascular diseases in developing countries has also been predicted [2]. This epidemiological change is a corollary of a predicted population-wide rise in cardiovascular disease risk factors including obesity, high blood pressure, dyslipidemia, and diabetes type 2 with increasing income; the “diseases of affluence” paradigm [3].

Thomas McKeown, professor of Social Medicine, gives a thorough introduction to the diseases of affluence paradigm in his book 'The Origin of Human Disease' [3]. McKeown concludes, like others before and after him ([4] *and references therein*), that cardiovascular diseases were probably rare or absent among hunting and gathering human ancestors during the Paleolithic (i.e. the Old Stone Age 2.6 million-10,000 years ago) and are also rare among contemporary hunter-gatherers and animals other than humans [3]. The main causes of death among hunter-gatherers were instead starvation, malnutrition and parasitic disease. The rapid expansion of populations after the advent of agriculture some 10,000 years ago [5] led to the creation of densely populated and unhygienic urban areas in which infectious diseases acquired from domesticated animals became the predominant causes of illness and death. Cardiovascular diseases continued to be rare until the advent of industrialization, when death rates from infections dropped while life expectancy rose and cardiovascular diseases became a dominant cause of death. Diseases such as cardiovascular diseases, which became common first with the advent of industrialization and ensuing affluence, were thus referred to as 'diseases of affluence', although other terms such as 'diseases of civilization' and 'Western diseases' also have been used [3]. McKeown concluded that the rise of cardiovascular mortality

Tommy Jönsson

after the advent of industrialization was not mainly explained by increased life expectancy, but rather by maladaptation to novel environmental and behavioral influences. This conclusion was based on two lines of evidence: firstly, that there has been little change in the genetic constitution of the human population since the end of the Paleolithic; and, secondly, that studies on human twins and animals suggest that common causes of death such as cardiovascular diseases are not merely genetic disorders established at fertilization, but are also largely determined by environmental factors.

However, there are some problems with the diseases of affluence paradigm. The paradigm has been interpreted to predict a population-wide rise in cardiovascular disease and accompanying risk factors including obesity, high blood pressure, dyslipidemia, and tobacco use with increasing income [6]. Accordingly, many Western countries documented a rise in mortality from cardiovascular disease in the 1960s and 1970s with increasing affluence. However, these peaks were followed by substantial declines in cardiovascular disease mortality despite continuing increases in affluence [7]. Instead, cardiovascular disease and accompanying risk factors are now beginning to shift to low and middle income countries [6]. On the other hand, these declines in high-income countries were mainly due to improved medical therapies and medically induced reductions in major risk factors [8], without which much of the paradigm's predictions probably hold true. This weakness of the paradigm is thus more of a problem in its modern interpretation than in the paradigm itself. However, perhaps a bigger weakness in the paradigm is McKeown's conclusion that diseases of affluence were due to maladaptation to changed living conditions during industrialization. This conclusion would put an emphasis on changes in living conditions during the last centuries. However, many diseases of affluence seem to have affected humans long before industrialization. Evidence for this comes from descriptions of diseases resembling coronary artery disease, myocardial infarction and cerebrovascular accidents in ancient Egyptian hieroglyphs, diseases which are also well substantiated by severe aortic and coronary atherosclerosis and myocardial fibrosis in ancient Egyptian, Chinese and medieval Italian mummies [9-11]. Similarly, stroke and obesity have been described and discussed in etiologic terms at least since late antiquity by various Greek and Latin sources [12-15]. Also, Indian texts mentioned already in the 5th century BC cases suggestive of diabetes with excessive urine, coupled with thirst, emaciation and sweet urine which primarily affected rich people who consumed large quantities of rice, cereals,

and sweets [16]. Thus, humans seem to have been affected by cardiovascular diseases and associated risk factors at least since antique times, although they did not become common until the advent of industrialization and ensuing affluence. Also, affluence is a relative term, and the individuals afflicted by cardiovascular diseases and associated risk factors in antique times were possibly regarded as affluent by their contemporaries. From this second objection to the paradigm, it stands to reason that the putative lifestyle to which McKeown concluded that humans were maladapted was already in place by antique times. When looking for alternative places in time for the introduction of a lifestyle to which humans are not fully adapted, it could therefore be required to go all the way back to the invention of agriculture. This is supported by an observed global epidemiologic pattern of cardiovascular diseases and associated risk factors, whose prevalence exhibits considerable variation both in time and place across agrarian societies [17-19], whereas they are virtually absent in non-agrarian societies [4, 20, 21]. Some of the variation in cardiovascular diseases among agrarian societies may be explained by variation in known risk factors [22, 23], but some variation is puzzling with no consistent association with westernization, urbanization or rise in known risk factors [18, 24]. Also, when people migrate from non-agrarian to agrarian societies, or when their own society becomes agrarian, the prevalence of cardiovascular diseases increases [4, 20]. This illustrates the general rule that there is no genetic protection against cardiovascular disease, only genetic variation in degree of susceptibility [20]. Thus, when looking for lifestyle factors to which humans could be maladapted and which could promote cardiovascular diseases, it seems logical to focus on the agrarian lifestyle and ways in which it departs from one that existed during much of human evolution: the hunter-gatherer lifestyle.

Hunter-gatherer versus agrarian lifestyle

To our knowledge, few interventional studies have previously been performed on the effects of a hunter-gatherer lifestyle on cardiovascular diseases and associated risk factors, as compared to an agrarian lifestyle. In a non-controlled study of ten overweight Australian Aborigines with diabetes, O'Dea et al found that reversion to a hunter-gatherer lifestyle during seven weeks led to 10% weight loss and reductions in fasting and 2-hour glucose of 45% and 36% during an oral glucose tolerance test (OGTT, $p < 0.0001$ for all) [25]. Fasting insulin decreased by 48% ($p < 0.0001$), while 2-hour insulin did not change (+20%, not significant). In contrast, in a similar study on healthy Australian Aborigines by the same authors, the insulin response to 70 gram of starch from white bread (and butter) was reduced, while the glucose response was not, after 10-12 weeks of reversion to a traditional lifestyle [26]. Both diet and physical activity changed markedly in these studies, which precludes evaluation about the isolated role of either intervention. The average physical activity is typically higher with a hunter-gatherer lifestyle than with a sedentary Western lifestyle, although overlapping between the two appears considerable [4]. Estimated expenditure of energy by male adults in a non-agrarian society was reportedly similar to adult males with moderate activity at work and during non-occupational activities in Western societies [4]. Thus, although regular physical activity undoubtedly exerts beneficial effects on cardiovascular disease, such as delaying the development of atherosclerosis and reducing the incidence of coronary heart disease events [27], lower physical activity is probably not an environmental factor which is unique to agrarian societies. Another prominent risk factor for cardiovascular disease is tobacco [28]. As previously noted, cardiovascular diseases are known in antique societies long before their acquisition of tobacco [10], and heavy smoking in non-agrarian societies does not appear to induce cardiovascular disease [4]. Thus, although smoking tobacco is undoubtedly deleterious to health and strongly associated with cardiovascular disease [28], it is an environmental factor which is not required for the development of cardiovascular disease and which also is not unique to agrarian societies.

Diet, however, is one important and defining difference between life as a hunter-gatherer and life as a farmer, as noted by McKeown. Diet affects many important risk factors for cardiovascular disease, such as hypertension,

diabetes type 2, insulin resistance and obesity [21]. The diet of an agrarian society is based on large amount of seeds from grasses such as cereals (e.g. wheat, rice, maize). Non-agrarian societies can be further divided into hunter-gatherer and horticultural societies. The diet of a hunter-gatherer society is based on hunting, fishing and gathering wild plants and insects. Hunting and gathering is thought to represent the original mode of life common to all prehistoric humans during the Paleolithic (i.e. Old Stone Age 2,6 million-10,000 years ago), and a hunter-gatherer diet is sometimes also referred to as a Paleolithic diet [21]. Horticultural societies obtain the bulk of their food from gardening, which sometimes implies heavy dependence on a single starchy cultivar such as a root crop (e.g. manioc). Cereals (from Ceres, roman goddess of agriculture, ultimately derived from the Proto-Indo-European root "ker", meaning "to grow") seem to be a very recent addition to the human diet. Considering that the last common ancestor of living primates, including humans, emerged some 90 to 65 million years ago [29, 30], before the appearance of grass some 65 to 55 million years ago [31], it cannot have had a diet consisting of seeds from grass. The diets of our subsequently evolving ancestors in the trees [31-36] and the savannah [37] also seem to have been largely devoid of cereals. *Homo sapiens*, fully modern humans, emerged about 200,000 years ago, and cereals were probably not key dietary staples of *Homo sapiens* hunter-gatherers either [5]. Starting about 10,000 years ago (i.e. 500 generations) some hunter-gatherer populations invented agriculture and switched to an agrarian lifestyle with a diet based on large amount of cereals [5]. Their descendents possibly have some genetic adaptation to an agrarian diet such as lower prevalence of celiac disease and related HLA genotypes [38, 39], as well as a higher expression of the salivary amylase gene [40]. However, many populations shifted to agrarian diet more recently (e.g. 300 generations in southern Scandinavia), which from an evolutionary perspective is a very short time to enable any significant adaptation [41]. Thus, humans could be maladapted to an agrarian diet, and the general aim of the research presented in this thesis was to experimentally examine the effects of a Paleolithic diet compared to a cereal-based agrarian diet on risk factors for cardiovascular diseases. Unavoidably, this research has come across some subjects, such as energy intake, satiety, leptin, molecular evolution and dietary lectins, which require a few additional explanatory passages.

Tommy Jönsson

The role of excess and restricted energy intake

Most adult humans maintain a stable body weight for many years. To have a constant weight, there must be an energy balance (energy homeostasis); energy intake (diet) has to be equal to energy expenditure (the product of basal metabolic rate, thermogenesis and physical activity). The contribution of each factor in the regulation of body weight remains unclear [42]. However, when average energy intake exceeds average energy expenditure, this eventually leads to overweight or obesity. The connection between obesity and diet and physical activity is known since antiquity, although its connection with cardiovascular diseases became clear more recently [15]. The importance of energy intake for health is underlined by known effects of caloric restriction, which in most studies has been a 20–40% restriction of dietary energy compared to free feeding [43, 44]. Such caloric restriction slows aging and increases maximum lifespan in many species, including yeasts, flies, worms, fishes and dogs, and in rodents it has been shown to increase longevity by preventing or delaying the occurrence of chronic diseases such as diabetes, cancer and cardiovascular disease [44]. Ongoing studies indicate that many of the metabolic changes noted in calorie restricted rodents also occur in rhesus monkeys, including improvements in blood pressure, serum lipid profile, serum glucose and insulin concentrations, and insulin sensitivity [44]. Similar improvements in cardiovascular risk factors have also been found in observational studies on calorie restricted humans [44].

The concept of satiety and its determinants

Our understanding of the physiological systems that regulate food intake and body weight has increased immensely over the past decade [45, 46]. Several hormones, such as insulin and leptin, have a substantial influence on appetite behavior through their actions on brain centers, such as the hypothalamus, the brainstem, reward centers and afferent autonomic nerves [45, 46]. These brain centers are critical for the regulation of body weight and energy homeostasis [45, 46]. Adipocyte-derived hormones, such as leptin, provide long-term information to the brain about the state of nutrient stores, whereas a variety of gut-derived signals triggered by ingestive

status have important roles in influencing meal initiation and termination [45, 46].

Foods differ in their satiating efficiency, partly due to their nutritional composition [47, 48]. The concept of satiating efficiency may be defined as the capacity of a consumed food to suppress hunger and decrease subsequent food intake [49]. The impact of ingested foods on subjectively perceived measures of motivation to eat (e.g. hunger, fullness) can be quantified in a dietary trial, where participants assess their motivation to eat and mark this on fixed point (category) scales or visual analogue scales [47, 50]. Such subjective ratings of appetite usually show positive correlations with the amount of food consumed, and can be considered a valid indicator of the strength of appetite [47, 48, 50]. The satiating effect of different foods has been frequently assessed by use of the Satiety Quotient, which gives a measure of the extent to which the food eaten reduces subjective appetite per unit of intake (e.g., per kg or MJ) and is predictive of energy intake [47]. The Satiety Quotient is calculated by the following formula:

$$\text{Satiety Quotient} = \frac{\text{satiety rating pre-eating episode} - \text{satiety rating post-eating episode}}{\text{food intake during eating episode}}$$

Leptin, the leptin receptor and free leptin index

Leptin (from Greek *Leptos*, meaning *thin*) is a peptide hormone, mainly secreted from adipose tissue, which influences appetite, reproduction, hematopoiesis, angiogenesis, blood pressure, bone mass, energy homeostasis and immune and neuroendocrine function (*for full review, see [51]*). Production of leptin correlates positively with adipose tissue mass [52]. Restriction of food intake, over a period of days, results in a suppression of leptin levels, which can be reversed by refeeding [52]. Circulating leptin levels are thus both determined by recent food intake and by current energy stores. Increased leptin levels are sensed by rodent and human brains and result in a decrease in food intake and an increase in energy expenditure [53]. Temporary involuntary over-feeding in rodents, non-human primates and humans results in a transient increase in weight, and a return to the initial weight after over-feeding has ended [54]. This homeostatic response to involuntary overfeeding clearly demonstrates that a potent defense mechanism against excessive energy storage exists. As obese humans show

elevated levels of leptin in serum and adipocytes, and experience limited weight loss with leptin treatment, many researchers suggest obese humans to be leptin resistant [51, 53]. Sometimes such hormone resistance can be caused by mutations in hormone receptors, as described for several hormones including leptin [45]. The pathophysiology of acquired forms of hormone resistance, which would be the case for leptin in most obese humans, has however been elusive [55]. It is also still not clear if leptin resistance are a cause or a consequence of obesity, although the homeostatic response to involuntary overfeeding suggests that leptin resistance could be causal [54].

Leptin circulates in both free and protein-bound forms. The soluble leptin receptor (SLR) is identified as the major binding component of leptin in plasma [56]. The leptin receptor is also expressed in the central nervous system, as well as in a wide spectrum of peripheral tissues, including the skeletal muscle, heart, adrenals, kidneys, pancreatic β -cells and the haematopoietic and immune systems [51, 57]. SLR functions to delay leptin clearance and increase the available leptin in circulation, and is crucial for leptin action [58]. Leptin correlates significantly with BMI, while SLR is inversely correlated with BMI [59]. In lean subjects, there is a molar equivalence of free leptin to SLR [59]. In morbidly obese subjects, SLR is significantly decreased, whereas leptin is significantly increased, such that a 25-fold excess of free hormone has been reported [59]. It is suggested that hyperleptinemia, low SLR levels as well as a low fraction of leptin bound to SLR are markers of leptin resistance, which is independently associated with impaired glucose metabolism, hypertension and a pro-atherogenic state in obesity and the metabolic syndrome [60-62]. Increased leptin concentrations are significantly associated with cardiovascular disease in men and women independently of traditional cardiovascular risk factors and obesity status in observational studies [63]. Although total circulating leptin levels was not prospectively associated with cardiovascular disease in women with diabetes [64], other prospective studies have shown leptin to be an independent predictor of future cardiovascular disease [65, 66].

Free leptin index was introduced as a surrogate for the laborious direct measurement of the free fraction of leptin, and is calculated as the ratio between leptin and SLR [67]. Hitherto, no studies on the actual correlation between free leptin index and the free fraction of leptin appear to have been done, which makes the interpretation of other correlations with free leptin index uncertain. Free leptin index in healthy humans correlates positively

with body fat mass and insulin, but negatively with waist to hip ratio [68, 69]. Furthermore, free leptin index was positively associated with energy intake from fat and negatively associated with energy intake from carbohydrates [69]. Free leptin index has also been associated with non-alcoholic fatty liver disease in children [70].

Molecular evolution and leptin

Molecular evolution pertains to evolutionary change at the molecular level (e.g. the genes, *for further background see* [41]). In molecular evolution, negative (or purifying) selection means that most changes in the genes are deleterious to the individual carrying the gene and are therefore eliminated through natural selection, causing the genes to remain unchanged over time [41]. Positive selection means that one or more changes in the genes are beneficial to the individual who carries the gene and are therefore selected for by natural selection, causing the genes to change over time in response to some selective agent in the environment [41]. Studies on molecular evolution suggest that the human leptin gene has changed very little since the emergence of hominoids (superfamily of humans and great apes) 25-30 million years ago [71], which implies negative selection [41]. Other studies on molecular evolution have shown high similarity of the leptin genes in such distantly related species as mouse, rat, chicken and turkey, which was ascribed to convergent evolution [72]. Convergent evolution implies positive selection through a selective agent shared by these rodents and birds [41], but the responsible selective agent was unknown to the authors [72].

The domestic pig as a model for cardiovascular disease

The extensively studied domestic pig is nearly ideal as an animal model of human cardiovascular disease [73]. Atherogenic diets thus easily induce atherosclerosis with a remarkable similarity of lesion distribution, pathogenesis, morphology and cholesterol levels to that of humans [73]. In addition, domestic pigs are one of the few animal models to develop atherosclerosis naturally in intracranial arteries and to have a significant incidence of end-organ lesions resulting from atherosclerosis [74]. Cerebral

Tommy Jönsson

infarction has also been reported to be relatively common and both myocardial infarction and aneurysmal dilation of the abdominal aorta have been seen [74]. Further similarities with humans in nutrition, gastrointestinal tract, pancreas development, clinical chemistry and metabolism, including the leptin system, also make the pig useful as a model of cardiovascular risk factors such as diabetes [75, 76]. Furthermore, domestic pigs readily accept a diet similar to that consumed by humans [74], with the additional advantages of low genetic variance, absence of individual feeding regimes and habits (such as smoking and alcohol consumption), and the availability of body fluids and tissues [77].

Dietary lectins

Lectins are proteins which bind reversibly to specific sugar structures (*for most references and background see [78, 79]*). Lectins are abundant among most living things, including viruses, bacteria, animals and plants. Different classes of plants have different lectins with differing biochemical properties, and there is a subclass of lectins only found in grasses like cereals. The intensively studied wheat germ agglutinin (WGA) is a lectin present in wheat seed in both the germ and the gluten-rich part of the endosperm [80]. Peptides behaving in a lectin-like manner have also been obtained upon cleavage of gliadin in gluten [81]. White flour consumed by humans contains a high proportion of gluten and has agglutinating activity suggestive of lectins or peptides behaving in a lectin-like manner [82-85]. Plant lectins are heat-stable and resistant to breakdown in the gastrointestinal tract, and they can bind to the surface epithelium of the digestive tract and cause anti-nutritional, mild allergic or other subclinical effects in humans and animals [78, 79]. Dietary plant lectins can be transported through the gut wall into the blood circulation, where they can directly influence peripheral tissues and body metabolism through the binding to glycosylated structures, such as the insulin receptor, the epidermal growth factor receptor and the interleukin 2 receptor [85-91]. Dietary plant lectins can also bind to several types of mammalian cells including pancreatic duct epithelial cells [92], prostatic cancer cells [93], arterial macrophages and smooth muscle cells [94, 95], glomerular capillary walls, mesangial cells and tubules of human kidney [85]. Human serum

contains antibodies against WGA and lectins of soybean and peanut [96]. Lectins bound to sugar structures of a membrane receptor can mimic or block the effect of the physiological ligand [78, 87, 88, 91, 97-102]. The binding of WGA to the insulin receptor is strong and long-lasting with high molecular efficiency, suggesting that it may affect insulin signaling for many hours [89, 90, 98]. The possible binding of lectin to the leptin receptor has not been evaluated.

Aims

The general aim of this thesis was to evaluate the effect of Paleolithic diet in prevention of cardiovascular disease by conducting randomized controlled clinical trials.

The specific aim for each paper was:

- I. To review evidence from epidemiology and evolutionary biology for a possible maladaptation of human leptin to a cereal based diet as a cause of leptin resistance and cardiovascular disease.
- II. To experimentally study the long-term effect of a Paleolithic diet compared to a cereal-based diet on risk factors for cardiovascular disease in domestic pigs.
- III. To experimentally study the effect of a Paleolithic diet compared to a Mediterranean-like diet on risk factors for cardiovascular disease in individuals with ischaemic heart disease and either glucose intolerance or type 2 diabetes.
- IV. To experimentally study the effect of a Paleolithic diet compared to a Mediterranean-like diet on satiety and the leptin system in individuals with ischaemic heart disease and either glucose intolerance or type 2 diabetes.

Materials and Methods

Study populations

Paper II (animal experiment)

Twenty-four cross-bred (dam (Swedish Landrace × Yorkshire) × sire Hampshire) piglets from four different litters were eligible for the study. In the Paleolithic group one pig, an apparent runt, failed to thrive from early post weaning and was culled at 3.5 months of age. The Ethical Committee for Animal Experiments at Lund University approved of the study, and the study animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health publication 85-23, revised 1985).

Papers III-IV (human trials)

The study participants consisted of 29 (out of 38 eligible) male patients with ischemic heart disease with waist circumference >94 cm and increased blood glucose at screening OGTT or known diabetes type 2, recruited from the Coronary Care Unit at Lund University Hospital, Sweden. Standard methods were used for glucose testing and definitions of glucose tolerance [103]. We included patients with any of the following conditions: an ongoing acute coronary syndrome, a history of myocardial infarction diagnosed by creatinine kinase MB isoenzyme or troponin elevation, percutaneous coronary intervention or coronary artery bypass graft surgery or angiographically diagnosed coronary stenosis $\geq 30\%$. Exclusion criteria were BMI <20 kg/m², serum creatinine >130 $\mu\text{mol/L}$, poor general condition, dementia, unwillingness/inability to prepare food at home, participation in another medical trial, chronic inflammatory bowel disease, type 1 diabetes and treatment with hypoglycemic agents, warfarin or oral steroids. Other drugs were not restricted, and treatment with statins and beta blockers were usually initiated and/or changed during the trial. In

Tommy Jönsson

In addition to the 29 patients who completed the trial, nine randomized subjects were excluded for the following reasons: worsening general condition (two in each group), non-willingness to continue (n=3, all in the Paleolithic group) or missing OGTT data (one in each group). The trial was approved by the regional Medical Ethics Committee for human research at Lund University, and all individuals gave written informed consent to participate in the study.

Interventions

Paper II

Upon weaning, the piglets were randomly allocated either to a group fed a standard cereal based swine feed (hereafter referred to as Cereal group) supplemented with rapeseed oil in order to match fat intake in the two groups, or to a group fed a cereal free Paleolithic diet (hereafter referred to as Paleolithic group) consisting of vegetables, fruit, meat and a small amount of tubers. Average intake during the study of protein, fat and carbohydrates were 17%, 18% and 65% respectively in the Cereal group, and 27%, 16% and 57% respectively in the Paleolithic group. Both diets were thus high in carbohydrate and low in fat compared to the spectrum of macronutrient intake estimated for contemporary hunter-gatherers [104]. For

more detailed account of provisions during the last three months of the study, see Table 1. Both groups were fed their respective diet from 2 to 17 months of age by an experienced experimental pig farmer who allocated rations on a group basis judged sufficient to achieve healthy animals.

Table 1: Provisions during last three months in study

Paleolithic group	kg/pig/day	kJ/pig/day
Cabbage	1	915
Turnip	1	703
Cauliflower	0.7	661
Green pepper	0.05	34
Red pepper	0.05	55
Yellow pepper	0.05	47
Broccoli	0.15	179
Apple	1	2053
Pear	0.7	1286
Kiwi fruit	0.1	192
Water melon	0.1	149
Grape	0.03	88
Pineapple	0.03	60
Cherimoya	0.03	115
Potato	0.3	878
Carrot	0.3	474
Beetroot	0.1	160
Parsnip	0.05	106
Black radish	0.05	29
Beef	0.45	2995
Fish-meal	0.36	5882
Total	6.6	17063

Cereal group	kg/pig/day	kJ/pig/day
Cereal swine	1.5	18600
Rape-seed oil	0.06	2220
Total	1.56	20820

Energy intake at the end of the study was approximately 20% lower in the Paleolithic group as compared to the Cereal group despite much larger feed rations in terms of both volume and weight.

Paper III-IV

All eligible subjects were informed of the intention to compare two healthy diets and that it was unknown if any of them would be superior to the other with regard to weight reduction and improved glucose metabolism. Subjects were randomized to one of two healthy diets: a Consensus (Mediterranean-like) diet (n=15) or a Paleolithic diet (n=14). All subjects were informed individually during two one-hour sessions and were given written dietary advice and food recipes. The Consensus diet was based on whole-grain cereals, low-fat dairy products, potatoes, legumes, vegetables, fruit, fatty fish, and refined fats rich in monounsaturated fatty acids and alpha-linolenic acid. Only subjects in the Consensus group were informed of the possible benefits of Mediterranean-like diets rich in whole grains and about the Lyon Diet Heart Study [105]. The Consensus group was also educated by use of a dietary questionnaire for nutrition counseling ('20 questions') used in a successful health promotion program, 'Live For Life', which led to lowered cardiovascular and total mortality in the Habo municipality, Sweden [106]. Only subjects in the Paleolithic group were educated in the concept of evolutionary health promotion [107] and the potential benefits of a Paleolithic diet. They were advised to increase their intake of lean meat, fish, fruit and vegetables and to avoid all kinds of dairy products, cereals (including rice), beans, sugar, bakery products, soft drinks and beer. The following items were accepted in limited amounts for the Paleolithic group: eggs (one or fewer per day), nuts (preferentially walnuts), potatoes (two or fewer medium-sized per day), rapeseed or olive oil (one or fewer tablespoons per day). The intake of other foods was not restricted and no advice was given with regard to proportions of food categories (e.g. animal vs. plant foods). Advice about regular physical activity was given equally to the two groups. Both groups were advised not to consume more than one glass of wine per day.

Outcomes

Paper II

An intravenous glucose tolerance test (IVGTT) was performed during ventilated anesthesia in 20 pigs which had been fasted for at least 6 hours. A catheter was introduced into the external jugular vein and threaded into the superior vena cava for both glucose administration and blood sampling. At 5 min and 30 seconds before glucose administration, 2.5-ml blood samples were collected. At time 0, glucose (0.5 g/kg body weight) was infused over 20–30 seconds. Thereafter, 2.5 ml blood samples were frequently sampled during 120 min. The pigs were kept in a supine position during the IVGTT, and data on hemodynamic values, body temperature, body length and subcutaneous fat thickness mid sternum were recorded. Fasting insulin sensitivity, which mostly accounts for the processes in the liver [108], was calculated with the quantitative insulin sensitivity check index (QUICKI) [109]. For the dynamic part of the IVGTT, a surrogate of insulin sensitivity was calculated as glucose disappearance rate (expressed by K_G) divided by the prevailing insulin (expressed by $AUC_{Insulin_{0-120}}$). Dynamic insulin sensitivity accounts for the insulin action at the level of muscle and adipose tissues [108]. Insulin secretion was evaluated as the suprabasal $AUC_{Insulin_{0-120}}$, representing the glucose stimulated insulin response. Finally, acute insulin response was calculated as the average insulin concentration during the very early phase (2 to 4 min) after glucose injection. For immunohistochemistry of islet hormones and islet morphology, specimens were collected from mid- and tail portions of the pancreas from 5 pigs in each group.

Paper III

In the human study, changes of the AUC between 0 and 120 min during OGTT for plasma glucose ($AUC_{Glucose_{0-120}}$) and plasma insulin ($AUC_{Insulin_{0-120}}$) were predefined primary endpoints, along with changes in body weight and waist circumference. The computer-generated homeostasis model assessment of insulin resistance (HOMA-IR) index, which has been suggested to provide a reasonable estimate of insulin resistance, was derived

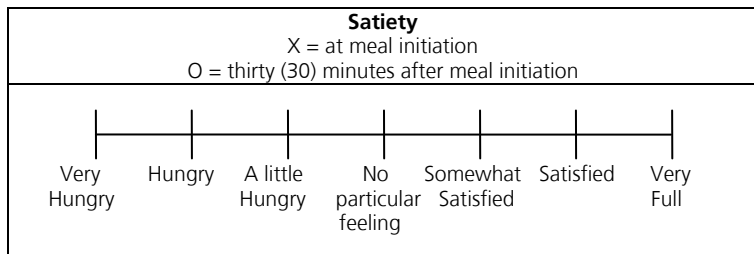
Tommy Jönsson

from fasting plasma glucose and insulin (www.dtu.ox.ac.uk). The early phase of post-challenge glucose and insulin responses were represented by the AUCs (Incremental AUC Glucose₀₋₃₀ and Incremental AUC Insulin₀₋₃₀) during the first 30 min of the OGTT, using levels at 0 min as the base of the area. A 4 day weighed food record on four consecutive days, including one weekend day, with weighing of each food item (including snacks) on a digital weighing scale (that could be set to zero), was completed by the participants, starting 15 ±5 days after initiating the dietary change. Glycemic load was calculated by multiplying the content of available carbohydrate in the serving of each food by the food's Glycemic Index (with glucose as the reference) as given by Foster-Powell et al. [110]. Under-reporting was checked for by comparing food records with baseline weight and achieved weight loss, and by evaluating distribution and amount of consumed food. Body composition was estimated in a subset of 15 patients by use of leg-to-leg bioelectrical impedance analysis (BIA).

Paper IV

During the 4 day weighed food record described in paper III, the participants also recorded the time of each meal (including snacks). Average time between meals was calculated by dividing total number of meals (including snacks, i.e. meals between breakfast, lunch or dinner) with total time between meals including night hours. Further, the participants also recorded their subjective rating of satiation sensation at meal initiation and 30 minutes after meal initiation on a 7-point equal interval, bipolar scale of hunger/fullness modified after Holt et al. 1992 [111] (Figure 1). The Satiety Quotient for energy and weight per meal was calculated as the individual mean change in satiety between meal initiation and 30 minutes after meal initiation divided by the individual mean intake of energy or weight (food and energy-containing drink) per meal. Fasting plasma samples were taken before 9.00 a.m. at baseline and after 6 weeks and 12 weeks, and were analyzed for leptin and leptin receptor. The free leptin index was calculated as the ratio of leptin to leptin receptor.

Figure 1: Rating scale used to assess subjective satiety (modified from Holt et al 1992).



Statistical analysis

Simple and multiple linear regression was used to analyze univariate and bivariate relationships. $P < 0.05$ was chosen for statistical significance. Data and results are expressed as mean \pm standard deviation.

Paper II

Two pigs, one in each group, were obvious outliers given the extremely elevated insulin response to injected glucose (AUC Insulin₀₋₁₂₀ 12 and 11 standard deviations above the group mean). These pigs were excluded from calculations regarding glucose and insulin (basal and dynamic variables) and their respective correlations with other variables. Group comparisons of continuous variables were made by use of the unpaired two-sided t test. Continuous variables showed reasonable normal distribution in normal plots, but in the case of C-reactive protein only after logarithmic transformation.

Paper III-IV

Assignment of patients to the two groups was made by use of minimization, a restricted randomization procedure which lowers the risk of group differences at baseline [112], using diabetes at screening (no/yes) and BMI (below or above 27 kg/m²) as restricting variables. A two-way paired t test

Tommy Jönsson

was used to analyze within-subject differences in absolute values, while a two-way unpaired t test and repeated-measures ANOVA were used to analyze between-subject differences in these changes. Fishers's exact test was used to analyze differences in proportions. Continuous variables showed reasonable normal distribution in normal plots.

Results and Comments

Paper I

Human leptin has changed very little since our ancestors first started eating cereals in larger quantities with the advent of agriculture [71]. It follows that, if dietary cereals in any way interfere with human leptin activity, we could expect some metabolic dysfunction as a sign of insufficient adaptation. Leptin resistance and its association with cardiovascular disease could be such a sign [113-118]. This is supported by the global pattern in the prevalence of cardiovascular diseases, with variation among agrarian societies, but virtual absence in non-agrarian societies.

Highly relevant, in this context, is the unknown selective agent shared by mouse, rat, chicken and turkey that is responsible for their highly similar leptin genes through convergent evolution [72]. Except for a diet containing seeds from grass (e.g. cereals), we find it hard to discern an environmental characteristic shared by these rodent and bird species that is sufficient to explain their highly similar leptin genes [119-121]. If a diet containing seeds from grass is the selective agent with effects on rodent and bird leptin genes, it stands to reason that dietary cereals also could have effects on human leptin.

Lectins are cereal constituents with sufficient properties to cause leptin resistance. Lectins specific to cereals are present in human food, enter human systemic circulation and can bind to receptors where they mimic or block the effects of the physiological ligands. Cereal lectins could cause leptin resistance indirectly, through effects on metabolism central to the proper functions of leptin, or directly, through binding to human leptin or leptin receptor. Further studies are clearly warranted.

Paper II

This long-term study in pigs showed highly beneficial effects of a Paleolithic diet on risk factors for cardiovascular disease when compared to a cereal based control diet. The Paleolithic diet thus conferred significantly lower insulin response to injected glucose (AUC Insulin₀₋₁₂₀), higher dynamic insulin sensitivity, lower diastolic blood pressure and lower C-reactive protein (Table 2 and 3). Diet emerged as the strongest explanatory variable for group differences in these variables. At the end of the study the Paleolithic group weighed 22% less and had approximately 20% lower energy intake despite much larger feed rations in terms of both volume and weight (Table 3).

Table 2: Final glucometabolic characteristics (mean \pm standard deviation)

	Paleolithic group (n=9)	Cereal group (n=9)	P
fP-glucose (mmol/l)	5.6 \pm 1.4	6.0 \pm 1.9	0.6
AUC* glucose 0-120 min (mmol/lxmin)	1076 \pm 113	1199 \pm 212	0.14
K _c (%min ⁻¹)	0.58 \pm 0.12	0.67 \pm 0.17	0.20
fP-insulin (pmol/l)	8.3 \pm 4.6	9.2 \pm 4.6	0.7
QUICKI	0.66 \pm 0.15	0.70 \pm 0.36	0.7
Dynamic insulin sensitivity (%min ⁻¹ /(pmol/l))	2.35 \pm 0.76	1.41 \pm 0.39	0.004
AUC* insulin 0-120 min (pmol/lxmin)	2613 \pm 863	4973 \pm 1476	0.001
AUC* insulin 0-120 min ^a (pmol/lxmin)	1620 \pm 1074	3872 \pm 1112	0.0005
Acute insulin response 2-4 min (pmol/l)	14.9 \pm 8.3	15.8 \pm 10.7	0.8

*area under curve during intravenous glucose tolerance test; ^a stimulated secretion.
Final glucometabolic characteristics in pigs fed paleolithic or cereal diet from 2 to 17 months of age excluding cases fasting less than 6 hours.

This difference in energy intake could indicate caloric restriction in the Paleolithic group. Our study was not designed to analyze the isolated role of energy intake. However, caloric restriction typically lowers mean body temperature by 1–2°C [122], and we found no significant difference in mean body temperature during general anesthesia between the two groups (Table 3). In addition, the mean weight in the Paleolithic group is well within the normal range of pigs [120]. The beneficial effects on risk factors for cardiovascular disease and difference in weight between groups are thus not necessarily caused by caloric restriction in the Paleolithic group, but

could instead be due to obesity conferred by excess energy intake in the control group. Support for this latter conclusion comes from the high correlation between weight and subcutaneous fat thickness (adjusted $R^2=0.77$, $p<0.0001$), which was 43% lower in the Paleolithic group (Table 3).

Interestingly, immunohistochemical analysis suggested a diffuse and low-grade pancreatic inflammation in the control group, as evidenced by clearly more frequent leukocytes scattered throughout the exocrine pancreatic parenchyma or clustered around pancreatic ducts and blood vessels. This *post hoc* finding points to a novel approach in the research on the association between inflammation and type 2 diabetes [123].

Table 3: Final clinical characteristics (mean \pm standard deviation)

	Paleolithic group (n=11)	Cereal group (n=12)	P
Weight (kg)	129 \pm 16	166 \pm 28	0.0009
Length (cm)	159 \pm 6	170 \pm 9	0.003
Subcutaneous fat (cm)	1.9 \pm 0.4	3.3 \pm 0.9	0.0003
Body temperature ($^{\circ}$ C)	37.7 \pm 1.5	37.6 \pm 0.5	0.8
CRP (μ g/mL)*	4.0	21.7	0.0007
Systolic BP (mm Hg)	140 \pm 18	150 \pm 9	0.12
Diastolic BP (mm Hg)	108 \pm 12	123 \pm 12	0.007

*geometric mean, t-test on logarithmic CRP.

Final clinical characteristics in pigs fed paleolithic or cereal diet from 2 to 17 months of age.

Table 4: Primary outcome variables (mean ± standard deviation)

	Paleolithic group (n=14)	Consensus group (n=15)	P for difference between groups
Weight (kg)			
Baseline	91.7 ± 11.2	96.1 ± 12.4	0.3
6 weeks	88.0 ± 10.7	93.6 ± 12.8	0.2
Change 0-6 weeks	-3.7 ± 2.2	-2.5 ± 2.3	0.2
95% confidence interval	-4.9 to -2.4	-3.8 to -1.2	
P for change within group	0.0001	0.0009	
12 weeks	86.7 ± 11.3	92.2 ± 12.9	0.2
Change 6-12 weeks	-1.4 ± 2.1	-1.3 ± 1.1	0.9
95% confidence interval	-2.6 to -0.1	-1.9 to -0.7	
P for change within group	0.03	0.0003	
Change 0-12 weeks	-5.0 ± 3.3	-3.8 ± 2.4	0.3
95% confidence interval	-6.9 to -3.1	-5.2 to -2.5	
P for change within group	0.0001	0.0001	
Waist circumference (cm)			
Baseline	105.8 ± 7.6	106.6 ± 8.0	0.8
6 weeks	102.8 ± 7.8	105.2 ± 8.8	0.5
Change 0-6 weeks	-3.0 ± 1.8	-1.5 ± 2.0	0.04
95% confidence interval	-4.0 to -2.0	-2.7 to -0.2	
P for change within group	0.0001	0.02	
12 weeks	100.2 ± 7.7	105.5 ± 7.9	0.11
Change 6-12 weeks	-2.6 ± 2.4	-1.5 ± 1.8	0.2
95% confidence interval	-3.9 to -1.2	-2.7 to -0.7	
P for change within group	0.001	0.003	
Change 0-12 weeks	-5.6 ± 2.8	-2.9 ± 3.1	0.03
95% confidence interval	-7.2 to -3.9	-4.8 to -1.1	
P for change within group	0.0001	0.004	
AUC ^a Glucose ₀₋₁₂₀ (mmol/lxmin)			
Baseline	1104 ± 118	1145 ± 298	0.6
6 weeks	877 ± 161	1024 ± 339	0.15
Change 0-6 weeks	-220 ± 206	-120 ± 255	0.3
95% confidence interval	-339 to -101	-262 to +21	
P for change within group	0.002	0.09	
12 weeks	807 ± 107	1065 ± 250	0.001
Change 6-12 weeks	-70 ± 156	+41 ± 179	0.09
95% confidence interval	-160 to +20	-59 to +140	
P for change within group	0.12	0.4	
Change 0-12 weeks	-290 ± 143	-80 ± 168	0.001
95% confidence interval	-373 to -208	-173 to +13	
P for change within group	0.0001	0.09	
AUC ^a Insulin ₀₋₁₂₀ (nmol/lxmin)			
Baseline	80.5 ± 41.1	69.7 ± 44.7	0.5
6 weeks	63.1 ± 30.0	54.1 ± 37.2	0.5
Change 0-6 weeks	-17.4 ± 27.7	-15.5 ± 16.9	0.8
95% confidence interval	-33.4 to -1.3	-24.9 to -6.2	
P for change within group	0.04	0.003	
12 weeks	56.1 ± 30.1	60.4 ± 46.4	0.8
Change 6-12 weeks	-7.0 ± 16.9	+6.2 ± 25.8	0.12
95% confidence interval	-16.7 to +2.8	-8.1 to +20.5	
P for change within group	0.15	0.4	
Change 0-12 weeks	-24.3 ± 28.4	-9.3 ± 23.3	0.13
95% confidence interval	-40.7 to -8.0	-22.2 to +3.6	
P for change within group	0.007	0.14	

^aAUC, Area under curve for glucose and insulin response to 75 g oral glucose tolerance test. The base of the AUC was set at 0 mmol/l for glucose and 0 nmol/l for insulin.

Paper III

This short-term human study showed beneficial effects of a Paleolithic diet on risk factors for cardiovascular disease when compared to a Mediterranean-like Consensus diet partly based on cereals. A Paleolithic diet thus conferred greater improvement in glucose tolerance (AUC Glucose₀₋₁₂₀), a trend for greater decrease in insulin response to ingested glucose (AUC Insulin₀₋₁₂₀), and a greater decrease in waist circumference (Table 4). Weight loss was on average 4.4 kg with no significant group difference (Table 4). Change of fat mass analyzed in a subset of patients (n=15) did not differ between the groups and explained 50% of the weight change (p=0.002).

Reported food composition differed between the two groups such that subjects in the Paleolithic group had a much lower intake of dairy products, cereals and oil/margarine, and a higher intake of fruits and nuts (Table 5). Reported energy intake was 25% lower in the Paleolithic group (p=0.004, Table 6) despite similar quantities of consumed food by weight (Table 5). After adjustment for energy intake, the improvement of glucose tolerance was still larger in the Paleolithic group (p=0.02), while the larger waist loss and the tendency for larger decrease in insulin response to ingested glucose disappeared. The Paleolithic group also had a 47% lower glycemic load, and in the whole study population glycemic load was positively associated with changes in both waist (r=0.52, p=0.008) and glucose tolerance (r=0.50, p=0.01) but not with change in insulin response to ingested glucose (r=0.30, p=0.15).

In *post hoc* analysis, a positive association between intake of cereals and change in waist circumference explained 42% of waist loss among the whole study population (p=0.0003), and 40% in the Consensus group alone (p=0.016). In contrast, there was a negative correlation between fruit intake and change in waist circumference, which explained 21% of waist loss (p=0.01). Each of these associations remained significant after adjustment for dietary assignment, energy intake, carbohydrate intake or glycemic load.

Table 5: Diet composition (g/day) in the two groups, as estimated from 4-day weighed food records (mean \pm standard deviation)

	Paleolithic group (n=14)	Consensus group (n=15)	P
Fruit	493 \pm 335	252 \pm 179	0.03
Vegetables ^a	327 \pm 233	202 \pm 88	0.07
Potatoes	51 \pm 42	77 \pm 78	0.3
Nuts	11 \pm 12	2 \pm 6	0.02
Meat, fresh	143 \pm 95	97 \pm 67	0.16
Meat products	65 \pm 59	58 \pm 49	0.8
Fish	119 \pm 92	77 \pm 56	0.16
Eggs	29 \pm 23	19 \pm 18	0.21
Beans, peas	8 \pm 21	15 \pm 26	0.5
Cereals	18 \pm 52	268 \pm 96	0.0001
Milk and dairy products	45 \pm 119	287 \pm 193	0.0006
Oil, margarine ^b	1 \pm 3	16 \pm 11	0.0001
Sauce	2 \pm 6	25 \pm 31	0.02
Pastry	1 \pm 3	13 \pm 25	0.12
Jam	1 \pm 3	6 \pm 10	0.12
Total amount of food	1311 \pm 598	1382 \pm 222	0.7
Wine	59 \pm 63	37 \pm 51	0.3
Beer, light ^c	11 \pm 27	27 \pm 47	0.3
Sweet beverages (excluding juice)	18 \pm 46	53 \pm 90	0.2
Juice	38 \pm 75	88 \pm 141	0.3

^aIncluding root vegetables (but excluding potatoes) and beans with pods.

^bButter was not reported to be consumed by anyone.

^cStronger beer or liquor were not consumed, as reported.

Thus, waist loss increased with increasing intake of fruit and decreasing intake of cereals, associations which explained most of the group difference in waist loss.

Notably, after 12 weeks all 14 subjects in the Paleolithic group had normal plasma glucose values at OGTT, compared with 7 of 15 subjects in the Consensus group where five subjects still had diabetic values (Table 7). There was no significant difference between groups in insulin resistance (HOMA-IR), insulin sensitivity (QUICKI, data not shown) or HbA1C (Table 7).

Table 6: Daily intake of macronutrients, dietary fiber, cholesterol, sodium, potassium, magnesium and calcium in the two groups, as estimated from 4-day weighed food records (mean \pm standard deviation)

	Paleolithic group (n=14)	Consensus group (n=15)	P
Energy			
MJ	5.6 \pm 2.2	7.5 \pm 1.3	0.01
Kcal	1344 \pm 521	1795 \pm 306	
Protein			
g	90 \pm 41	89 \pm 20	0.9
g/kg body weight	0.98 \pm 0.4	0.95 \pm 0.2	0.8
percentage of total energy intake	27.9 \pm 6.8	20.5 \pm 3.6	0.002
Total fat			
g	42 \pm 20	50 \pm 13	0.2
g/kg body weight	0.44 \pm 0.2	0.55 \pm 0.2	0.12
percentage of total energy intake	26.9 \pm 6.4	24.7 \pm 4.3	0.3
Fatty acids			
saturated (g)	11.5 \pm 4.8	16.8 \pm 4.2	0.005
percentage of total energy intake	7.7 \pm 2.4	8.3 \pm 1.7	0.4
monounsaturated (g)	16.3 \pm 7.4	19.0 \pm 5.0	0.3
percentage of total energy intake	10.7 \pm 2.6	9.4 \pm 1.9	0.2
polyunsaturated (g)	9.6 \pm 7.5	9.0 \pm 3.0	0.8
percentage of total energy intake	5.8 \pm 2.5	4.4 \pm 1.1	0.06
Carbohydrate			
g	134 \pm 56	231 \pm 48	0.0001
g/kg body weight	1.4 \pm 0.6	2.5 \pm 0.6	0.0001
percentage of total energy intake	40.2 \pm 8.3	51.7 \pm 5.3	0.0002
Glycaemic load ^a	65.1 \pm 30.2	121.6 \pm 27.5	0.0001
Alcohol, percentage of total energy intake	3.9 \pm 4.4	2.3 \pm 3.0	0.3
Fiber (g)	21.4 \pm 13.2	26.8 \pm 7.4	0.2
Cholesterol (mg)	397 \pm 192	295 \pm 122	0.11
Salt (g)			
Sodium	1.9 \pm 0.6	2.9 \pm 0.7	0.0006
Sodium chloride	4.7 \pm 1.6	7.2 \pm 1.7	

^athe product of glycaemic index (with glucose as the reference food) and the amount of carbohydrate, obtained from Foster-Powell et al [110].

Table 7: Other glucometabolic variables (mean \pm standard deviation)

	Paleolithic group (n=14)	Consensus group (n=15)	P for difference between groups
HbA _{1c} (%)			
Baseline	4.76 \pm 0.26	4.89 \pm 0.79	0.6
6 weeks	4.61 \pm 0.25*	4.84 \pm 0.72	0.3
12 weeks	4.64 \pm 0.22	4.85 \pm 0.69	0.3
Change 0-12 weeks	-0.13 \pm 0.26	-0.03 \pm 0.39	0.4
95% confidence interval	-0.28 to +0.02	-0.24 to +0.17	
P for change within group	0.09	0.7	
Normal glucose levels ^a (n)			
Baseline	2	2	0.8
6 weeks	10	10	0.7
12 weeks	14	7	0.0007
Diabetic glucose levels ^b (n)			
Baseline	10	9	0.4
6 weeks	1	3	0.2
12 weeks	0	5	0.01
lnHOMA-IR			
Baseline	1.37 \pm 0.45	1.52 \pm 0.67	0.5
6 weeks	1.00 \pm 0.39*	1.16 \pm 0.52*	0.3
12 weeks	0.90 \pm 0.38	1.22 \pm 0.52	0.07
Change 0-12 weeks	-0.46 \pm 0.44	-0.30 \pm 0.47	0.3
95% confidence interval	-0.71 to -0.21	-0.56 to -0.04	
P for change within group	0.002	0.03	
Insulin/Glucose ₀₋₃₀			
Baseline	172 \pm 125	145 \pm 110	
6 weeks	135 \pm 61	133 \pm 144	0.5
12 weeks	139 \pm 72	112 \pm 126	1.0
Change 0-12 weeks	-33 \pm 94	-33 \pm 71	0.5
95% confidence interval	-87 to +21	-73 to +8	1.0
P for change within group	0.2	0.11	
Incremental Glucose AUC ₀₋₃₀ ^c			
Baseline	48 \pm 20	54 \pm 20	0.4
6 weeks	48 \pm 19	60 \pm 40	0.3
12 weeks	44 \pm 20	62 \pm 26	0.06
Change 0-12 weeks	-4 \pm 24	+7 \pm 21	0.19
95% confidence interval	-18 to +10	-4 to +19	
P for change within group	0.6	0.2	
Incremental Insulin AUC ₀₋₃₀ ^c			
Baseline	7.1 \pm 4.0	7.5 \pm 5.9	0.8
6 weeks	6.2 \pm 3.0	6.2 \pm 5.6	1.0
12 weeks	5.5 \pm 2.9	6.1 \pm 4.8	0.7
Change 0-12 weeks	-1.6 \pm 3.1	-1.5 \pm 2.7	0.9
95% confidence interval	-3.4 to +0.2	-3.0 to +0.07	
P for change within group	0.08	0.06	

*P<0.05 by paired t-test for change within group (6 week level is compared with baseline and 12 week level is compared with 6 week level); ^aFasting venous plasma glucose \leq 6.0 mmol/l and 2-hour venous plasma glucose <7.8 mmol/l at OGTT (despite increased capillary blood glucose at screening); ^bFasting venous plasma glucose \geq 7.0 mmol/l or 2 hour venous plasma glucose \geq 11.1 mmol/l at OGTT; ^cincremental area under curve during the first 30 min of oral glucose tolerance test, using levels at 0 min as the base of the area.

Paper IV

This short-term study showed a Paleolithic diet to be more satiating per calorie when compared to a Mediterranean-like Consensus diet partly based on cereals. A Paleolithic diet thus conferred a significantly higher Satiety Quotient for energy and a lower consumption of energy per meal (Table 8). There were no significant differences between groups in the Satiety Quotient for weight or in weight consumed per meal (Table 8). The greater satiating capacity per calorie may have been caused by the significantly lower energy density of the Paleolithic diet (Table 8), and would be consistent with previous reports of a negative correlation between energy density and the satiating capacity of common foods [48, 124]. Other possible explanations of the Paleolithic diet's greater satiating capacity per calorie is the significantly higher relative intake of protein [125, 126] and the lower intake of salt [127], saturated fatty acids [128] and carbohydrates in the Paleolithic group (Table 6), although other studies has indicated no effect of varying intake of macronutrients such as carbohydrates on satiety [129].

Table 8: Measures of subjective satiety, meal frequency, energy and weight of food and drink, and Satiety Quotients, i.e. satiety adjusted for energy intake and weight of food and drink (mean ± standard deviation)

	Paleolithic group (n=13)	Consensus group (n=14)	P
Subjective satiety at meal initiation (0 minutes)	-1.0 ± 0.9	-1.0 ± 0.5	0.9
Subjective satiety 30 minutes after meal initiation	1.6 ± 0.7	1.7 ± 0.3	0.4
Change in subjective satiety 0-30 minutes	2.6 ± 1.0	2.8 ± 0.6	0.6
Time between meals including snacks ^a (hours)	4.6 ± 1.4	4.9 ± 1.7	0.6
Meals per day including snacks	4.9 ± 1.1	4.4 ± 0.8	0.2
Energy from food and drink per meal ^b (kJ)	1226 ± 516	1755 ± 380	0.005
Weight of food and drink per meal ^b (g)	311 ± 131	377 ± 85	0.13
Energy density of food and drink ^b , daily average (kJ/g)	4.0 ± 0.6	4.8 ± 1.1	0.04
Satiety Quotient for energy per meal (MJ ⁻¹)	2.5 ± 1.4	1.6 ± 0.4	0.03
Satiety Quotient for weight per meal (kg ⁻¹)	10.4 ± 6.5	7.6 ± 1.7	0.13

^aTime between meals was calculated by dividing total number of meals (including snacks) with total time between meals including night hours. ^bDrink containing energy, e.g. excluding water, coffee and tea.

Group differences in type of carbohydrate consumed could also explain group differences in satiating capacity per calorie, since the major source of carbohydrate in the Consensus group were cereals, which, according to Holt et al [48], are less satiating than fruit, the major source of carbohydrate in the Paleolithic group (Table 5).

There was otherwise no significant difference between groups in any of the following measures: subjective satiety at meal initiation or 30 minutes after meal initiation; change in subjective satiety between these points of time; length of time between meals or number of meals or snacks per day (Table 8). We also found that our above mentioned marked improvement of glucose tolerance after advice to eat a Paleolithic diet was independent of all other outcome measures.

Leptin levels and free leptin index decreased, and leptin receptor levels increased significantly in both groups, with no significant difference between groups, although a trend for greater relative decrease in leptin levels in the Paleolithic group was noted (Table 9). The dietary variable that was most strongly and significantly associated with change in serum leptin was reported absolute cereal intake (Figure 2). Lower intake of cereals thus correlated with greater decrease in leptin, and this correlation remained significant when changes in weight, but not waist, were controlled for, indicating that cereals may be linked with leptin through effects on visceral fat (data not shown).

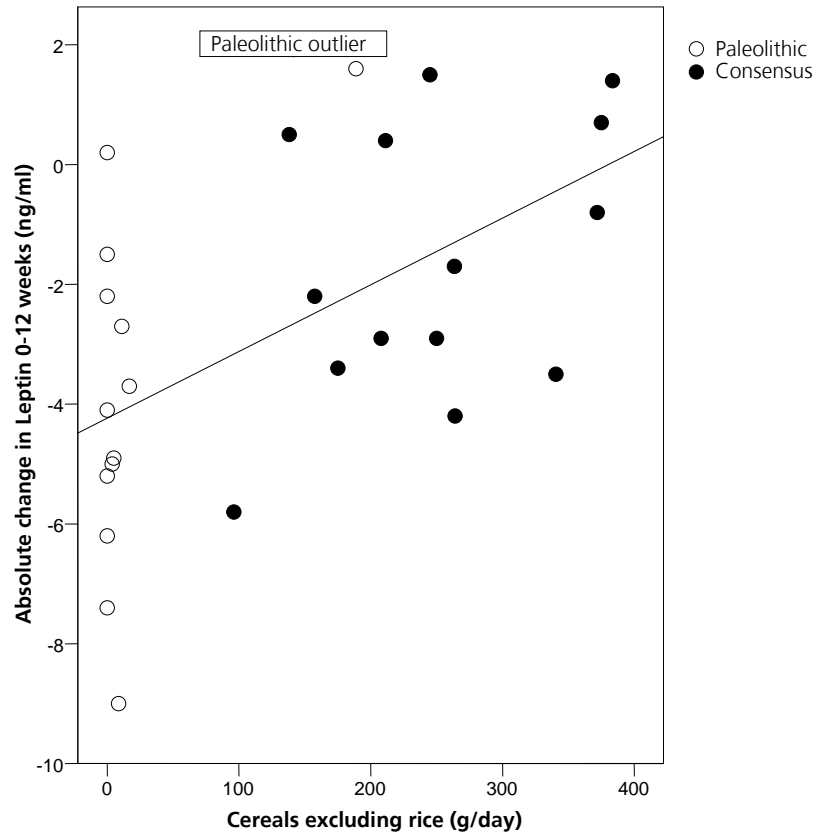
Notably, one subject in the Paleolithic group was an extreme outlier in terms of cereal intake, with intakes corresponding to those of the Consensus group despite the interventional advice to avoid cereals (Figure 2). When this outlier was excluded, the difference between groups in relative leptin change between baseline and 12 weeks became significant (Table 9). The greater relative decrease in the Paleolithic group could indicate greater increase in leptin sensitivity [62]. This would hypothetically induce effects equivalent to those reported from rats injected with leptin, where satiation increased and energy intake per meal decreased [130], an effect which closely resembles the other results from this study.

Table 9: Leptin, leptin receptor and free leptin index (mean ± standard deviation)

	Paleolithic group (n=14)	Consensus group (n=15)	P for difference between groups
Fasting plasma leptin (ng/ml)			
Baseline	10.7 ± 3.9	13.5 ± 11.0	0.4
6 weeks	6.6 ± 3.0	10.9 ± 8.5	0.09
12 weeks	7.1 ± 3.2	11.0 ± 8.4	0.11
Change 0-6 weeks	-4.0 ± 3.1	-2.6 ± 3.3	0.2
P for change within groups 0-6 weeks	0.0003	0.01	
Relative change 0-6 weeks (%)	-34 ± 25	-19 ± 20	0.08
Change 0-12 weeks	-3.6 ± 3.0	-2.5 ± 4.0	0.4
P for change within groups 0-12 weeks	0.0006	0.03	
Relative change 0-12 weeks (%)	-31 ± 26	-18 ± 22	0.15
Relative change 0-12 weeks (%)*	-35 ± 21	-18 ± 22	0.04
Fasting plasma leptin receptor (ng/ml)			
Baseline	19.0 ± 8.9	14.9 ± 5.7	0.14
6 weeks	20.5 ± 12.3	19.6 ± 8.7	0.8
12 weeks	20.6 ± 10.8	18.1 ± 5.7	0.4
Change 0-6 weeks	1.5 ± 7.2	4.7 ± 8.2	0.3
P for change within groups 0-6 weeks	0.5	0.04	
Relative change 0-6 weeks (%)	13 ± 45	37 ± 50	0.2
Change 0-12 weeks	1.5 ± 7.0	3.2 ± 7.2	0.5
P for change within groups 0-12 weeks	0.4	0.10	
Relative change 0-12 weeks (%)	17 ± 51	33 ± 49	0.4
Free leptin index			
Baseline	0.7 ± 0.5	1.1 ± 1.3	0.3
6 weeks	0.5 ± 0.5	0.7 ± 0.7	0.3
12 weeks	0.5 ± 0.4	0.6 ± 0.4	0.3
Change 0-6 weeks	-0.3 ± 0.6	-0.4 ± 0.7	0.5
P for change within groups 0-6 weeks	0.09	0.04	
Relative change 0-6 weeks (%)	-29 ± 47	-33 ± 29	0.8
Change 0-12 weeks	-0.2 ± 0.5	-0.5 ± 1.0	0.4
P for change within groups 0-12 weeks	0.08	0.08	
Relative change 0-12 weeks (%)	-28 ± 43	-30 ± 33	0.9

*Paleolithic outlier in terms of cereal intake excluded.

Figure 2: Relation between reported intake of cereals excluding rice and change in leptin between baseline (0 weeks) and end of study (12 weeks). Paleolithic outlier in terms of cereal intake is annotated. Fitted line for all subjects.



Conclusions

These first randomized controlled trials on the effects of a Paleolithic diet show beneficial effects on risk factors for cardiovascular disease and satiety.

The major conclusions for each paper were:

- I. Leptin resistance may be a metabolic sign of human maladaptation to dietary cereals, which could partly explain the observed global association between cardiovascular disease and agrarian-based dietary habits. Lectins could be a cereal constituent with sufficient properties to cause leptin resistance.
- II. A Paleolithic diet confers higher insulin sensitivity, lower C-reactive protein and lower blood pressure than a cereal-based diet in domestic pigs.
- III. A Paleolithic diet improves glucose tolerance more than a Mediterranean-like diet.
- IV. A Paleolithic diet is more satiating per calorie than a Mediterranean-like diet.

Future aspects and recommendations

The results of this work open up the possibility of altering recommendations for dietary treatment of cardiovascular diseases as well as dietary recommendations for the general population. However, current dietary recommendations should not be changed only because of the results from these few and small trials. Instead, the beneficial effects of a Paleolithic diet in these first randomized controlled trials underline the need for further research.

Firstly, more clinical trials should be made on the effects of a Paleolithic diet on cardiovascular diseases and associated risk factors. We are currently in the end-stages of a trial on patients with diabetes type 2 in primary health care. This cross-over trial is comparing the effects of a Paleolithic diet with the generally recommended cereal based diet on risk factors for cardiovascular diseases. The patients in this primary health care trial are also reporting their subjective impressions of each diet. This will give us more qualitative knowledge on the differences between these diets.

Secondly, the effects seen in these trials of a Paleolithic diet compared to a cereal based diet on satiety and leptin support a possible link between leptin resistance and a diet based on cereals. We are currently investigating this possibility further with *in vitro* experiments on interactions between cereal lectins and leptin. Hopefully, we will either be able to refute the possibility of such a link, or we will make some serious headway in our understanding and treatment of cardiovascular diseases.

Acknowledgements

This thesis is a collaboration of many, and I would like to thank everyone who in any way has contributed to its final conclusion.

The most important people of all, **the patients**, for giving generously of their time and blood. Thanks to volunteers like them we can gather new knowledge to benefit all of us.

My supervisor, **Staffan Lindeberg**, for unending support, humor, curiosity and patience in teaching me the ways of scientific research. Our chance encounter during a brief working period at Sjöbo Health station several years ago turned out to have a great and lasting impact on my life.

Co-supervisor, **Bo Ahrén**, for supporting and believing in me, and for always giving swift answers to all questions. **Lilian Bengtsson**, **Lena Kvist** and **Maria Sörhede Winzell** at professor Ahrén's lab for excellence at work, humor and patience with a family doctor unskilled in laboratory work.

Co-author and research collaborator **Stefan Olsson**, for always sharing your immense knowledge in biology, and for effortlessly providing a constant flow of innovative ideas and suggestions.

Professor **Birgitta Hovélius**, for your support, encouragement, and for being a true inspiration.

Co-authors **Thorkild C Bøg-Hansen**, **Anita Dole**, **Giovanni Pacini**, **Frank Sundler**, **Nils Wierup**, **Stig Steen**, **Trygve Sjöberg**, **Martin Ugander**, **Johan Frostegård**, **Leif Göransson**, **Yvonne Granfeldt**, **Emma Borgstrand**, **Jerry Soffman**, **Kit Sjöström** and **Charlotte Erlanson-Albertsson**, for fruitful collaboration.

Docent **Lennart Truedsson**, for giving me a playful first encounter with medical research during summer work at the laboratory of clinical immunology, and for always taking time to listen.

My boss at Arlöv Health Station, **Ingrid Henrikz**, for unconditional support and encouragement, and for your devotion to primary health care.

Diabetes nurse **Ulla-Carin Branell** at Arlöv Health Station, for having an open mind and for fantastic commitment in our current study on Paleolithic diet to diabetics in primary health care.

Psychologist **Ann Christin Berggren** at Arlöv Health Station, for a good sense of humor, and for a smashing cover photograph.

All **colleagues and fellow-workers** at Arlöv Health Station, for your

encouraging interest in my research and for making Arlöv Health Station a fun place to work at.

My sister-in-law **Karen**, for always taking time to give linguistic support.

My parents **Siv** and **Gunnar**, for a loving childhood, and for always being there with excellent support and advice.

My brother **Rickard**, for linguistic support and for being my best friend.

My wonderful children **Linnéa** and **Louise**, for your love and laughter, and for your patience with daddy's "boring research on pigs".

My beloved wife **Rose-Marie**, for your love and for giving me a wonderful life. This thesis would not have been possible without you.

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