

Trends in obesity and type 2 diabetes; ethnic aspects and links to adipokines

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*To Astrid, Sixten and Olof
representing the future*

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Abstract

Objective

The prevalence of obesity and related diseases such as type 2 diabetes mellitus (T2DM) is increasing worldwide, and the Asian Indian population seems to be particularly susceptible to developing T2DM, even at a low body mass index (BMI). In Sweden, the age-adjusted prevalence of diabetes has not increased despite increasing self-reported obesity. However, modern data on the prevalence of obesity and T2DM in Scandinavia are absent.

The biochemical links between obesity and subsequent T2DM are unknown, but the adipocyte-derived hormones leptin and adiponectin (adipokines) have been suggested as potential links because they both are related to insulin and glucose physiology. Some studies have found leptin to be an independent predictor of T2DM in men but not in women, although these results are inconsistent. In contrast, adiponectin has more consistently been linked to development of T2DM in both men and women. Furthermore, the leptin–adiponectin ratio may predict incident T2DM better than either of the two hormones separately.

The aims of this thesis were to describe time trends in obesity and T2DM in northern Sweden, to evaluate leptin and adiponectin as predictors of deterioration in glucose metabolism including T2DM, and to evaluate leptin as a risk marker regarding ethnic differences, circ-annual variation, and intra-individual stability.

Materials and methods

Three large population surveys were used, the Northern Sweden MONICA (MONitoring of Trends and Determinants in Cardiovascular Disease) study, the Västerbotten Intervention Programme (VIP), and the Mauritius Non-Communicable Disease Study. Within the MONICA study, six cross-sectional surveys were performed in Sweden's two northernmost counties, Norrbotten and Västerbotten, between 1986 and 2009. A total of 1000 men and 1000 women ages 25–64 years, also including from 1994 250 men and 250 women ages 65–74 years, were independently chosen for each survey. The overall participation rate was 75%. In 1999, a reinvestigation was performed in 74% of all participants from the three first surveys. Data from the MONICA surveys were used in papers I and IV and data from the reinvestigation survey in paper II.

VIP is an ongoing population intervention program that started in the mid-eighties targeting cardiovascular risk factors and has covered the whole county of Västerbotten since 1991. Inhabitants are invited the years they turn 40, 50, and 60 years old, and the annual participation rate has varied between 48% and 67%. A subset (n=1780) from VIP was used in paper II for the circ-annual leptin analysis, and VIP data linked to the diabetes register in Västerbotten (DiabNorr) were used in a case referent study (640 patients with T2DM) in paper III.

The Mauritius Non-Communicable Disease Study was performed in 1987 in 10 randomly selected (with probability proportional to size) population clusters. All eligible adults ages 25–74 years were invited, and the participation rate was 86% (n=5083). In 1992, a follow-up survey was performed in 49% of the initial participants. The Mauritius survey data were used in paper II.

Results

I. BMI increased in men ages 25–74 years and in women ages 25–44 years in northern Sweden between 1986 and 2004. The prevalence of obesity (BMI ≥ 30) increased in men ages 25–44 and 55–74 years and in women ages 25–44 years. The prevalence of obesity increased from 10.4% to 19.1% in men and from 12.9% to 17.9% in women ages 25–64 years. Waist circumference (WC) decreased in women of all ages and in men ages 55–64 years between 1986 and 1990. After 1990, WC increased again, and the prevalence of abdominal obesity rose markedly in women ages 25–64 years.

II. Differences in circulating levels of leptin, leptin per BMI unit (leptin/BMI), and leptin per cm in WC (leptin/waist) were tested in men and women of Asian Indian, Creole (African), and Caucasian ethnicity. Asian Indian men and women had the highest leptin concentrations and Caucasian men and women the lowest while Creole men and women had intermediate values for leptin, leptin/BMI, and leptin/waist. No circ-annual variation in leptin concentrations was seen in Caucasians. The intra-individual test–retest stability for leptin was equal in men and women of different ethnicities, over 5–13 years, with an intra-class correlation of 0.65–0.82.

III. High adiponectin concentrations predicted decreased risk of T2DM in both insulin-sensitive and insulin-resistant men and women, whereas high leptin levels predicted increased risk for T2DM only in insulin-sensitive men. A high leptin–adiponectin ratio predicted T2DM in both men and women, and men with a high ratio had a shorter time to diagnosis than those with a low ratio.

IV. In northern Sweden, fasting and post-load glucose increased in women ages 24–65 years with 0.2 mmol/l and 0.7 mmol/l, respectively, between 1990 and 2009. Consequently, the prevalence of impaired fasting glucose and impaired glucose tolerance (IGT) rose from 4.5% to 7.7%, and from 7.8% to 14.5%, respectively. In men, post-load glucose increased at 0.5 mmol/l, and the prevalence of IGT rose from 3.5% to 10.1%. The prevalence of diabetes did not increase. An independent relationship between leptin and changes in fasting and post-load glucose was seen in men but not in women.

Conclusion

An increasing obesity and concomitant deterioration in glucose metabolism was seen in northern Sweden in the period studied. High adiponectin concentrations predicted a decreased risk of T2DM in both men and women, whereas high leptin concentrations predicted an increase in fasting and post-load glucose as well as an increased risk of T2DM in men

but not in women. Individual insulin resistance status modified the association between leptin and T2DM, and the leptin–adiponectin ratio may add further predictive information beyond the measures of the separate hormones. In relation to traditional anthropometric measures of obesity, Asian Indian men and women had the highest and Caucasians the lowest concentrations of leptin while Creole (African) men and women had intermediate levels. As a risk marker, leptin has a high intra-individual stability, equal in men and women and among different ethnicities over 5–13 years with no circ-annual variation.

Key words

Type 2 diabetes, leptin, adiponectin, obesity, abdominal obesity, epidemiology, ethnicity, fasting glucose, post-load glucose

Original papers

This thesis is based on the following papers, which in the text will be referred to by their Roman numerals. Articles reprinted with kind permission.

- I. Lilja M, Eliasson M, Stegmayr B, Olsson T, Söderberg S. Trends in Obesity and Its Distribution: Data From the Northern Sweden MONICA Survey, 1986–2004. *Obesity* 2008; 16; 1120-1128.
- II. Lilja M, Rolandsson O, Shaw JE, Pauvaday V, Cameron AJ, Tuomilehto J, Alberti KGMM, Zimmet PZ, Söderberg S. Higher leptin levels in Asian Indians than Creoles and Europids: a potential explanation for increased metabolic risk. *Int J Obes (Lond)*. 2010; 34: 878–885.
- III. Lilja M, Rolandsson O, Norberg M, Söderberg S. The impact of leptin and adiponectin on incident type 2 diabetes is modified by sex and insulin resistance. *Submitted*.
- IV. Lilja M, Eliasson M, Eriksson M, Söderberg S. A rightwards shift of the distribution of fasting and post-load glucose in northern Sweden between 1990 and 2009, and its predictors. *In manuscript*.

Abbreviations

ACE	angiotensin converting enzyme
ADA	American Diabetes Association
AdipoR1	adiponectin receptor 1
AdipoR2	adiponectin receptor 2
AgRP	Agouti-related peptide
α-MSH	α -melanocortin stimulating hormone
AMP	adenosine monophosphate
AMPK	activated protein kinase
ARC	arcuate nucleus
ATP	adenosine triphosphate
BBB	blood-brain barrier
BMI	body mass index
BP	blood pressure
CCK	cholecystokinin
CEPT	cholesterol ester transfer protein
CRP	C-reactive protein
CV	coefficient of variation
CVD	cardio vascular disease
db	diabetes
DiabNorr	the diabetes register in Västerbotten
DXA	dual-energy X-ray absorptiometry
EASD	European Association for the study of Diabetes
eNOS	endothelial nitric oxide synthase
EQUALIS	Swedish national accreditation body
ER	endoplasmic reticulum
fa	fatty
FPG	fasting plasma glucose
GIP	gastric inhibitory polypeptide
GGT	gamma-glutamyltransferase
GLP	glucagon-like peptide
HbA1c	glycosylated haemoglobin / haemoglobin A1c
HDL	high density lipoprotein
HMW	high molecular weight
HOMA	homeostasis model assessment
hs	high sensitivity
ICC	intra class correlation
IDF	International Diabetes Federation
IFG	impaired fasting glucose
IGF	insulin-like growth factor
IGT	impaired glucose tolerance
IL	interleukin
IR	insulin resistance
IRS	insulin receptor substrate
JAK	Janus kinas

h	hour
HOMA	homeostasis model assessment
leptin/BMI	leptin per BMI unit
leptin/waist	leptin per cm in waist circumference
LPL	lipoprotein lipase
MAPK	mitogen-activated protein kinase
MetS	metabolic syndrome
MHO	metabolically healthy but obese
MONICA	Monitoring of Trends and Determinants in Cardiovascular Disease
mRNA	messenger ribonucleic acid
NCEP III	National Cholesterol Education Program's Adult Treatment Panel III
NEFA	non-esterified fatty acid
NPY	neuropeptide Y
NTS	nucleus of the solitary tract
ob	obesity
OGTT	oral glucose tolerance test
PAI-1	plasminogen activator inhibitor-1
PI3K	phosphatidylinositol-3-OH kinase
POMC	pro-opiomelanocortin
PPAR	peroxisome proliferator-activated receptor
RA	receptor agonist
RIA	radioimmunoassay
SI	synergy index
SLIP	serum leptin-interacting protein
SNP	single nucleotide polymorphism
SOCS	suppressor of cytokine signalling
STAT	signal transducer and activator of transcription
Th	T-helper
TNF- α	tumor necrosis factor- α
T2DM	type 2 diabetes mellitus
VIP	Västerbotten Intervention Programme
VMH	ventromedial nucleus of the hypothalamus
WC	waist circumference
WHR	waist to hip ratio
2h	two hours

Sammanfattning på svenska

Syfte

Över hela världen beskrivs en ökad förekomst av fetma och sjukdom med koppling till fetma, som typ 2 diabetes mellitus (T2DM). Människor med sin etniska bakgrund på den indiska halvön förefaller ha en ökad risk att utveckla T2DM, även vid ett lågt body mass index (BMI). I Sverige har enligt flera undersökningar, den åldersjusterade förekomsten av diabetes inte ökat, trots att den självrapporterade förekomsten av fetma stigit. Moderna data på förekomsten av fetma eller T2DM saknas i stor utsträckning från Skandinavien.

Kunskap om den biokemiska kopplingen mellan fetma och utveckling av T2DM saknas. De fettvävsproducerade hormonerna leptin och adiponectin har föreslagits kunna vara en möjlig länk, eftersom de båda påverkar sockeromsättning och insulinnivåer. En del studier har visat att leptin, oberoende av andra riskfaktorer, är kopplat till insjuknande i T2DM hos män men inte hos kvinnor. Andra studier motsäger dessa fynd. För adiponectins del så är studier mera entydiga. Hos både män och kvinnor är höga nivåer av adiponectin kopplat till en lägre risk att insjukna i T2DM. Kvoten mellan leptin och adiponectin (leptin/adiponectin) har föreslagits vara bättre på att förutsäga T2DM än hormonerna var för sig.

Syftet med denna avhandling var att beskriva tidstrenden i utvecklingen av fetma och T2DM i norra Sverige, att bedöma leptin och adiponectins koppling till försämrad sockeromsättning inklusive T2DM, att bedöma i vad mån koncentrationen i blodet av leptin skiljer sig mellan olika etniska grupper samt om nivåerna av leptin varierar över året, eller hos samma individ varierar över tid.

Metod

Tre stora befolkningsundersökningar har använts, Norra Sveriges MONICA (MONitoring of Trends and Determinants in Cardiovascular Disease) studie, Västerbottens Interventions Program (VIP) och the Mauritius Non-Communicable Disease Study. Inom MONICA har sex tvärsnittsstudier genomförts i Norr- och Västerbotten mellan 1986 och 2009. 1000 män och 1000 kvinnor i åldrarna 25-64 år, från 1994 även 250 män och 250 kvinnor i åldrarna 65-74 år, har slumpmässigt inbjudits till varje studie. Den genomsnittliga deltagandefrekvensen var 75%. 1999 inbjöds alla deltagare från de tidigare undersökningarna till en återundersökning, vilken genomfördes på 74% av de initiala deltagarna. Data från MONICA har använts i artikel I och IV och data från återundersökningen i artikel II.

VIP är ett befolkningsinriktat program med syftet att påverka riskfaktorer för hjärt- och kärlsjukdom inklusive T2DM. VIP startade i mitten av 1980-talet och sedan 1991 omfattas hela Västerbotten. Alla innevanare i länet inbjuds det år de fyller 40, 50 eller 60 år till en undersökning. Deltagandefrekvensen har varierat mellan 48% och 67%. Data

från en mindre grupp deltagare i VIP (1780 personer) har använts för att studera årstidsvariationen av leptin i artikel II. Genom att koppla data från VIP till Västerbottens diabetesregister (DiabNorr) identifierades 640 patienter vilka undersöktes i VIP och en tid därefter (minst 1 år) insjuknat i T2DM, samt kontroller utan känd T2DM. Data från dessa individer användes i artikel III.

Studien på Mauritius genomfördes 1987 inom 10 slumpmässigt (med sannolikheten att bli vald beroende på befolkningsstorlek) utvalda befolkningsområden. Alla innevanare mellan 25 och 74 år inbjöds och deltagandefrekvensen var 86%. 1992 gjordes en återundersökning vid vilken 49% av de initialt undersökta deltog. Data från Mauritius har använts i artikel II.

Resultat

I. I norra Sverige ökade BMI bland män i åldrarna 25-74 år samt bland kvinnor 25-44 år gamla mellan 1986 och 2004. Förekomsten av fetma (BMI ≥ 30) ökade bland män i åldrarna 25-44 år och 55-74 år samt bland kvinnor 25-44 år. Bland män i åldrarna 25-64 år ökade förekomsten av fetma från 10,4% till 19,1% och bland kvinnor i samma åldrar från 12,9% till 17,9%. Midjeomfånget minskade bland kvinnor i alla åldrar samt bland män 55-64 år gamla mellan 1986 och 1990. Efter 1990 ökade midjeomfånget igen och förekomsten av bukfetma ökade markant bland kvinnor i åldrarna 25-64 år.

II. Nivåerna i blodet av leptin, leptin per BMI-enhet (leptin/BMI) och leptin per cm i midjeomfång (leptin/midja) jämfördes mellan män och kvinnor från en europeisk befolkning från norra Sverige samt en indisk och en (afrikansk-) kreolsk befolkning på Mauritius. Indiska män och kvinnor hade de högsta nivåerna och svenska män och kvinnor de lägsta nivåerna och kreolska män och kvinnor hade nivåer däremellan av leptin, leptin/BMI och leptin/midja. Ingen årstidsvariation av leptin sågs bland de svenska deltagarna. Leptin hade en hög stabilitet (intra-class correlation, ICC mellan 0,65 och 0,82) mellan första undersökning och återundersökning, lika för män och kvinnor, olika etniska grupper och en uppföljningstid mellan 5 och 13 år.

III. Höga nivåer i blodet av adiponectin var hos både insulinkänsliga och insulinresistenta män och kvinnor kopplat till lägre risk för T2DM, medan det enbart var hos insulinkänsliga män som höga nivåer av leptin var kopplat till ökad risk för T2DM. Ett högt leptin/adiponectin var kopplat till ökad risk för T2DM hos både män och kvinnor, och hos män sågs en kortare tid till insjuknande i T2DM hos dem med en hög kvot jämfört med dem med en låg kvot.

IV. I åldrarna 24-65 år ökade mellan 1990 och 2009 faste- och belastningsglukos bland kvinnor med 0,2 mmol/l respektive 0,7 mmol/l och förekomsten av förhöjt fasteglukos ökade från 4,5% till 7,7% och av nedsatt glukostolerans (IGT) från 7,8% till 14,5%. Bland män i samma åldrar ökade belastningsglukos med 0,5 mmol/l och förekomsten av IGT från 3,5% till 10,1%. Förekomsten av diabetes ökade däremot inte. Hos män, men inte hos kvinnor, sågs en oberoende relation mellan leptin och förändringar i faste- och belastningsglukos.

Konklusion

En ökande förekomst av fetma och en samtidig försämring av sockeromsättningen sågs i norra Sverige under den studerade perioden. Höga nivåer av adiponectin var kopplat till en minskad risk för T2DM bland både män och kvinnor, medan höga nivåer av leptin hos män var kopplat till ökande nivåer av faste- och belastningsglukos liksom till ökad risk för T2DM. Förekomsten av insulinresistens påverkade kopplingen mellan leptinnivåer och insjuknande i T2DM och kvoten mellan leptin och adiponectin kan möjligen ge mer information om risken att insjukna i T2DM än vad leptin och adiponectin kan vara för sig. Även justerat för BMI och midjeomfång så har indiska män och kvinnor högsta nivåer och svenska män och kvinnor lägsta nivåerna av leptin, medan kreolska män och kvinnor från Mauritius har nivåer däremellan. Beträktat som en riskmarkör så har leptin en hög stabilitet över tid hos den enskilda individen och denna stabilitet är lika hos män och kvinnor av olika etnicitet och lika över 5-13 års uppföljning, däremot sågs ingen årstidsvariation av leptin.

Introduction

Ethnicity

The term ethnicity is widely used in the literature but practically never defined. The term is not limited to the country of residence or birth but also refers to migrants of both first and subsequent generations. For people of European background, the terms used in the literature include Europids, Whites, and Caucasians, the two latter also associated with the usually unstated possibility of originating from a non-European country. In this thesis, the term Caucasian is used instead of White or Europid.

The designation South Asians, or Asian Indians, refers to people from the Indian subcontinent (Bangladesh, Bhutan, India, Maldives, Nepal, Pakistan, and Sri Lanka), although the exact definition is almost never given in the literature.

Creole (African) refers in this thesis to the ethnic group in Mauritius that comprises people of mixed African and Malagasy ancestry, including some European and Asian Indian admixture [1].

Obesity

The epidemic of obesity

An ongoing epidemic of obesity has been described worldwide in all continents and is replacing undernutrition and infectious diseases as the most significant global contributor to health problems [2]. In 2005, 23.2% of the adult global population was estimated to be overweight (body mass index, BMI 25.0–29.9 kg/m²) and 9.8% to be obese (BMI ≥30 kg/m²), corresponding to 937 million and 396 million people, respectively. In 2030, with unchanged secular trends, the projected numbers for overweight and obese persons will be 2.16 billion and 1.12 billion, respectively. Estimates from 2008 mention even higher numbers, with 1.46 billion adults being overweight and 502 million being obese worldwide [3]. In addition, the prevalences of childhood obesity and overweight (with use of International Obesity Taskforce cut-offs) [4], have more than doubled or tripled in many countries since the mid-1970s, now often exceeding 25%. Some hopeful reports exist, however, from Australia, France, Sweden, and Switzerland that the prevalence of childhood overweight and obesity might be plateauing or even decreasing [5]. In low- and middle-income countries, the increase in

obesity is mostly seen in towns among the more wealthy, and in particular among women. In high-income countries, obesity is more equally distributed among men and women but more prevalent in rural areas [6] and in socially and economically disadvantaged groups [5] and neighbourhoods [7]. Obesity has a large impact on individual health as well as on health services through a large spectrum of co-morbidities such as type 2 diabetes mellitus (T2DM), hypertension, dyslipidaemia, coronary heart disease, stroke, certain types of cancer, osteoarthritis, and depression [2, 5]. Obesity causes a higher risk of spending many years with disability than both smoking and drinking [8] and is linked to a shortened life expectancy [9].

There are considerable differences in the prevalence of overweight and obesity in different populations. Based on self-reported data covering the period 1997–2000, the age-adjusted prevalence of obesity in Europe for the population aged 15 years or older ranged from 7–8% in Norway and Switzerland to approximately 20% in Great Britain and Germany. In Sweden, the prevalence was at an intermediate level of 10% [10]. Self-reported data, however, overreport height and underreport weight, leading to an underestimation of the obesity prevalence, for example by 5% points in Sweden and 9.5% points in the USA [11, 12]. With objectively obtained measurements, the obesity prevalence in the USA among people ages 20 years or more has risen from 10.4% in men and 15.0% in women in 1960–1961 [13] to 32.2% in men and 35.5% in women in 2007–2008 [14].

Africa

Few large prevalence studies on obesity in Africa exist. A meta-analysis from West Africa showed in the adult population an obesity prevalence that had more than doubled over 15 years to 10.0%. The increase was largely taking place in women, and the prevalence was highest in urban areas. In South Africa (1999), the department of health reported that the obesity prevalence was 9.1% in men and 29.3% in women [15], and a recent study of civil servants in urban Nigeria showed a prevalence of obesity among males of 8.8% and among females of 17.3% [16], while men and women in rural Uganda had a prevalence of less than 1% and 4% respectively [17]. In Mauritius, using BMI ≥ 25 kg/m² as a cut-off for obesity, obesity prevalence (1992) was 35.7% in men and 47.7% in women. Five years earlier, corresponding figures were 26.1% and 37.9%, respectively [15].

Asia

An increasing prevalence of obesity has also been reported from Asia. Using BMI 30 kg/m² as a cut-off, the prevalence in urban areas in western India was 4.8% in men and 7.8% in women in 1992. In urban areas in northern India in 2004, the percentages were 20.8% and 32.3%, respectively. Using BMI 25 kg/m² as a cut-off, obesity prevalence in urban northern India (2007) was 37.8% in men and 50.3% in women. From China (2008), an obesity (BMI ≥ 25) prevalence of 15–18% in men and 22–27% in women was reported from rural areas, and from the city of Shanghai, for men and women combined, the prevalence was 43.3% [15].

The BMI cut-offs of 25 kg/m² for overweight and 30 kg/m² for obesity were originally based on data from Caucasian populations, but a

recent Indian consensus statement for diagnosis of obesity recommended cut-offs of 23 kg/m² for overweight and >25 kg/m² for obesity in the Asian Indian population [18].

Sweden

Self-reported Swedish data from the years 2000–2001 show a distribution in the prevalence of obesity from 8.0% in the three largest cities to 11.1% in towns in northern Sweden and sparsely populated areas [19]. A study following long-term trends in obesity is a survey of 50-year-old men in Gothenburg, in which the prevalence of obesity more than doubled between 1963 and 2003 with an increase from 6.0% to 13.8%. The prevalence of overweight increased from 37.6% to 45.7% during the same period [20]. In 50-year-old women in Gothenburg (initial survey of women ages 45–54 years), the prevalence of obesity rose from 10.4% to 15.1% between 1980 and 2003. The prevalence of overweight was unchanged though, around 30% [21].

Northern Sweden

Rural areas in western societies tend to have a higher prevalence of obesity than urban areas, and rural areas in northern Sweden in 1999 had a BMI distribution similar to that of rural New York in 1989. This finding could indicate the possibility that rural northern Sweden, with a delay of 10 years, may follow US trends [6]. Two recent studies from northern Sweden indicate, however, that the obesity epidemic is slowing down. Within the Northern Sweden Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) study, there was no increase in BMI between 2004 and 2009 [22]. Within the Västerbotten Intervention Programme (VIP), a trend towards a slower increase in obesity prevalence was seen in middle-aged men and women, and a trend shift was seen in urban areas and among those with a higher education [23]. This pattern could indicate a more positive outlook for obesity trends in Sweden, not following US trends, as has been assumed [6, 24]. A possibility of a slow down in the increase of obesity has recently been reported also from other European countries such as Switzerland [25], and England [26], and in French men [27] and women living in the USA [28], and most importantly in childhood obesity [5].

Abdominal obesity

NCEP and IDF definitions

Abdominal obesity carries a particularly increased risk of cardiovascular disease (CVD) [29, 30, 31] and T2DM [32, 33], and the risk seems to be associated with intra-abdominal (visceral) and not subcutaneous fat accumulation [34]. Two generally used clinical definitions of abdominal obesity exist, the National Cholesterol Education Program (NCEP) Adult Treatment Panel III definition from 2001 and the 2005 definition from the International Diabetes Federation (IDF). Both use waist circumference (WC), and NCEP cut-offs are >102 cm in men and >88 cm in women,

although for men at an increased metabolic risk with multiple metabolic risk factors, WC >94 cm can be used. IDF adds an aspect of ethnic specificity to the sex-specific cut-offs. In Caucasians, WC \geq 94 cm for men and \geq 80 cm for women is used as the cut-off for abdominal obesity, and until further research is performed, the same data are recommended for Sub-Saharan Africans. In South Asian and Chinese men, WC \geq 90 cm and women WC \geq 80 cm are recommended cut-offs [35, 36], which is what the Indian recommendations suggest [18]. Four body sites from immediately above the iliac crest to immediately below the lowest rib are commonly used in different studies to measure WC. All four sites have a high reproducibility (intra-class correlation [ICC] >0.99) and are equally good at predicting total and intra-abdominal adiposity. The spread in mean WC measured at different body sites was 1.6 cm in men and 4.6 cm in women [37].

Waist-to-hip ratio

The waist-to-hip ratio (WHR) is also used to define abdominal obesity, mostly with a cut-off >0.9 in men and >0.85 in women, but other cut-offs have also been used [2]. Hip circumference has in some studies been inversely related to CVD, T2DM, hypertension, dyslipidaemia, and death. The reason is supposed to be an association with increased subcutaneous fat mass but also with increased gluteal and leg muscle mass, where the latter may reflect the amount of physical activity [38]. The subcutaneous gluteofemoral fat acts metabolically differently from the abdominal fat and is associated with a more beneficial lipid profile and negatively associated with inflammatory cytokines [39]. Because WHR also includes measurements of hip circumference, the ratio has been suggested to be more useful than WC. WHR, however, is less reproducible, and the ICC, reflecting the test–retest stability, is lower than for WC [38]. WC explains more of the variance in visceral adipose volume and correlates better than WHR with both visceral adipose volume and total body fat mass. WC and the abdominal sagittal diameter are in women more closely related than WHR to metabolic aberrations, indicating increased risk of CVD and T2DM, but for abdominal sagittal diameter, no international definition of abdominal obesity exists [40].

Time trends

Actual time trends show an increasing prevalence of abdominal obesity in many populations. With 15 years of consecutive surveys, the prevalence of abdominal obesity (NCEP) almost doubled in the adult population in England, and in 2008, the prevalence was 35.7% in men and 43.9% in women. However, the rate of increase has tended to slow over the years [41]. Dutch data for the period 1993–1997 show an increase in abdominal obesity over the period studied. The prevalence in the mid-1990s was similar to that of England at the same time: 14.8% in men and 21.0% in women [42]. Studies from the USA showed a long-standing and gradual increase in WC over the period 1960–2008. In 2007–2008, more than half of the adult (\geq 20 years) population had abdominal obesity (NCEP), at 43.7% men and 61.8% in women. Caucasian men had a higher prevalence (46.8%) than African-American men (38.8%) and Mexican-American men (41.3%).

Among women, the prevalence was highest among African-Americans (72.3%) and Mexican-Americans (71.6%) compared to Caucasian women (59.9%). In 1960–1962, the prevalence of abdominal obesity was 12.7% among men and 19.4% among women, with no differences in WC between the Caucasian and African-American populations [43, 44].

Africa and Asia

The prevalence of abdominal obesity differs markedly also in Asia. In 2007, Korean men had a prevalence (NCEP) of less than 2% while women had a prevalence of 18.3%, and an increasing trend was seen in men between 1998–2003 [45]. Northern urban India had a reported prevalence (NCEP) in 2005 of 34.5% in men and 55.6% in women; prevalences (IDF, South Asian cut-offs) in southern urban India in 2007 were 56.2% in men and 35.1% in women [15]. In Africa, rural Uganda reported a male prevalence (IDF) of 1.0% and female of 31.2% [17], and corresponding figures in rural Benin were 3.5% in men and 52.9% in women. With increasing urbanisation, the corresponding prevalences in Benin rose to 22.0% and 83.0% in men and women, respectively [46].

Sweden

The Swedish study of 50-year-old men in Gothenburg showed an increase in mean WC from 91.7 cm in 1973 to 94.5 cm in 2003. WC was not measured in the 1963 survey, but four years later at age 54, the 1963 survey group had a mean WC of 87.0 cm [20]. In 50-year-old women, mean WC increased from 80.1 cm to 83.1 cm between 1985 and 2003 [21].

Specific cut-offs for general and abdominal obesity in Asian Indians

The metabolic syndrome (MetS) is an often-used term with somewhat different definitions and cut-offs for an obesity-related cluster of cardiovascular risk factors. All definitions include elevated blood glucose, raised blood pressure (BP), and dyslipidaemia together with obesity and/or abdominal obesity. Of note, according to NCEP, is the presence of general or abdominal obesity not mandatory for the diagnosis of MetS. The rationale for different cut-offs for obesity and abdominal obesity in Asians compared to Caucasians is based on the fact that MetS, or parts of the syndrome that are definable as T2DM, leads to an increased CVD risk at a lower WC or BMI in Asians [18, 47].

Diabetes

The epidemic of diabetes

Concomitant with the previously described obesity epidemic is an alarming and global increase in T2DM. As with obesity, the prevalence of T2DM differs largely among but also within countries. IDF has presented diabetes prevalence estimates for 2010 for different countries and regions for people ages 20–79 years adjusted to the world population. Numbers include all types of diabetes, but T2DM contributes a vast majority of the prevalence.

One problem is the lack of prevalence data in many countries where data exist only for the prevalence of known diabetes, i.e., data on previously undetected diabetes are not included. In Europe, representative nationwide data are available only from Turkey, and in most other countries, the total prevalence of diabetes is estimated by multiplying the prevalence of known diabetes by 1.5 [48]. All prevalence and incidence data subsequently presented include all types of diabetes if not otherwise stated.

According to IDF, European prevalence figures range from 1.6% in Iceland to 9.7% in Portugal, with Sweden at 5.2%, and the mean prevalence for Europe at 6.9%. Out of seven global regions, Africa has the lowest prevalence (3.8%) and North America the highest prevalence (10.2%). Prevalence differs largely within region, however; as an example, the Western Pacific region has a range from 1.6% in Mongolia to 30.9% in Nauru. Within the same ethnic groups, a more than six-fold difference in prevalence is seen, mostly with the lowest prevalence in rural areas in low-income countries and the highest prevalence in urban areas in high-income countries [48]. Within the same country, large differences in the prevalence of diabetes also exist, as in India where recent studies showed a range from 5% to 20% in different populations [49].

Globally, the number of patients with diabetes in 1994 was estimated to be 100 million. In 2010, the number was estimated to be 285 million, and the prediction for 2030 is 439 million patients. In addition, 344 million were estimated to have an impaired glucose tolerance (IGT) in 2010, and the number is predicted to reach 472 million in 2030. The largest increase in diabetes and IGT in 2030 will be in South Asians [48]. A recent large review estimated the age-adjusted number of patients with diabetes globally to be even higher, at 347 million in 2008, an increase from 153 million in 1980. This increase was seen in all regions, in particular in the Oceania region, which had the highest prevalence. In addition, increasing fasting plasma glucose (FPG) levels were seen in most regions. Aging and growing populations could explain 70% of the increased diabetes prevalence, but 30% was attributed to other factors [50].

Asia

Among four Asian countries, India had the highest prevalence but also, compared to China and Japan, a peak in the prevalence at an age 10 years younger [51]. Asian Indians have a younger onset of diabetes also in comparison to Caucasians [52], and a gradually younger age at onset has been seen over time [53]. The number of adult patients with diabetes in India is expected to increase from 51 million in 2010 to 87 million in 2030 [48]. A high prevalence of diabetes [54, 55] as well as CVD [56] in Asian Indian migrants compared to indigenous populations is also described from different parts of the world.

Africa

There are few studies on diabetes prevalence using the same methodology repeatedly over many surveys in the same area. In the Mauritius non-communicable Disease study, repeated population surveys were performed between 1987 and 2009 using the same methodology. In

2009, in the population ages 20–74 years, the age-standardised prevalence of diabetes was 22.3% in men and 20.2% in women, an increase of over 60% in both men and women of Asian Indian and Creole ethnicity since 1987. Of note was a marked increase in the prevalence of impaired fasting glucose (IFG) seen between 1987 and 2009 in Asian Indians compared to Creoles, 224% and 39%, respectively. During the same period in the Chinese population, diabetes prevalence increased by 17% and IFG prevalence decreased by 2% [57].

Sweden

In contrast to what would have been expected as a consequence of increasing obesity [22, 23], many Swedish studies have shown no increase in the age-adjusted incidence or prevalence of diabetes. Three register studies in southern Sweden covering a whole community (Laxå) or whole counties (Skaraborg and Kronoberg) have all demonstrated a stable age-adjusted prevalence of T2DM. The Laxå study, covering 1972–2001, showed no increase in the age-adjusted incidence during the whole period and a stable prevalence after 1988. In 2001, the prevalence of T2DM in the total population was 3.9% in men and 4.1% in women, and the incidence was 3.6/1000 in men and 3.0/1000 in women [58]. The incidence of T2DM (1998–2001) in the adult (>20 years) population in Kronoberg county in Sweden was similar—3.92/1000 in men and 3.64/1000 in women—and 93.1% of all cases with diabetes were T2DM [59]. The Skaraborg study (1991–1995) showed an incidence of T2DM of 2.66/1000 inhabitants. The incidence was stable over the years, and an increasing prevalence of 6%/year was attributed to an increased survival of patients with T2DM [60].

A study that has followed a longer time trend is the Gothenburg study in 50-year-old men, with repeatedly performed surveys every 10 years between 1963 and 2003. The study showed no increase in the prevalence of self-reported diabetes but found an increase in the total (self-reported and screening detected) prevalence of diabetes, from 3.6% in 1963 to 6.6% in 2003 [20]. In 50-year-old women in Gothenburg, five surveys have been performed between 1980 and 2003, with the latest survey finding a prevalence of 2%. The prevalence did not change over the years [21].

The incidence and prevalence of T2DM increases with age [50]. Data from Kronoberg county showed a peak in the incidence for ages 65–85 years and a mean age at diagnosis of 67.0 years [59], similar to the mean age at diagnosis of 66.6 years found in Skaraborg county [60]. Two surveys from Stockholm county (1997–1999) [61] and from the city of Gothenburg (2001–2004) [62] in 60-year-old men and women and in 64-year-old women, respectively, have studied the prevalence of diabetes. The prevalences were 9.7% and 5.1% in 60-year-old men and women, respectively. The Gothenburg study in 64-year-old women showed a prevalence of previously known diabetes and screening-detected diabetes of 4.7% and 4.8%, respectively.

Northern Sweden

A stable prevalence of diabetes has been reported from two studies from the Northern Sweden MONICA, where the prevalence of diabetes in people ages 25–64 years was unchanged between 1986–1999. In 1999, the prevalence of previously known (self-reported) diabetes was 2.7% in men and 2.2% in women, and the prevalence of screening-detected diabetes was 3.3% in men and 3.1% in women [63]. An updated report from 2009 found the prevalence of self-reported diabetes was 2.7% in men and 2.4% in women ages 25–64 years, which had remain unchanged since 1986. The prevalence of self-reported diabetes in the 65–74-year group was higher at 15.3% in men and 7.8% in women [22]. In contrast, VIP, a large intervention programme in Västerbotten county with over 100,000 participants, showed an increasing prevalence of diabetes between 1990 and 2007. In the most recent surveys (2002–2007), the prevalence in people ages 40–60 years was 6.8% in men and 4.3% in women, a significant increase of 44% and 17%, respectively, compared to the initial surveys (1990–1995) [64].

Asian Indians at specific risk for T2DM

The particular susceptibility among Asian Indians to T2DM, MetS, and CVD, with onset at young age and increased risk even at low BMI, is of specific interest for both research and health management [47, 48, 51, 52, 54, 55, 65]. Possible mechanisms are multifactorial and not restricted to Asian Indians and more probably reflect a quantitative and not a qualitative difference between ethnicities. Of note, the increased prevalence of obesity-related diseases such as T2DM and CVD in Asian Indians is concomitant with an ongoing increase in the prevalence of obesity [66].

Asian Indian babies have about 1 kg lower birth weight than Caucasian babies, but they have more adiposity and less muscle mass and higher insulin and leptin concentrations than Caucasian babies. This difference may indicate a higher prevalence of disadvantageous foetal programming resulting from intrauterine undernutrition, which may increase the risk for T2DM [66].

Traditionally, a high intake of carbohydrates is seen in South Asians. Compared to Caucasians, there is also a low intake of fibre, fruit, and fresh vegetables. A lower level of physical activity in South Asians compared to Caucasians is reported in both children and adults [66].

The change in lifestyle related to “westernisation” or “Cocacolonization” [67] seen in many fast-developing nations is an important factor for the increased prevalence of T2DM beyond what could be expected from an aging and growing population. The changes include migration from rural areas to urban areas, less physical activity and mechanisation, and an altered diet [50, 66].

Both BMI and WC underestimate the total amount of fat (percent body fat) in South Asians and other non-Caucasian populations, and BMI significantly underestimates the amount of visceral adipose fat mass. As an example, South Asian migrants in the USA with the same percent body fat as Caucasians had a WC 10 cm lower [66]. Also compared to African-Americans, Asian Indians have a larger amount of visceral fat mass

as measured by dual-energy X-ray absorptiometry (DXA) [68]. One reason for the underestimation of fat mass by BMI is that South Asians, in particular women, have lower skeletal muscle mass than Caucasians and also a lower bone mineral content [66, 69]. African-Americans, on the other hand, have a higher muscle mass and bone mineral content than Caucasians [70]. The low muscle mass in Asian Indians is associated with reduced insulin sensitivity, which could thus be part of the pathogenesis for T2DM [66].

Both the total fat percent and the abdominal fat mass are higher in Asian Indians than in Caucasians. In addition, the amount of triglyceride depots in the liver is doubled in Asian Indians compared to Caucasians, both with a BMI of 21–23 kg/m². Linked to the increased fat accumulation in the liver, disturbances such as higher insulin resistance, higher concentrations of insulin, interleukin (IL)-6, and leptin, and lower adiponectin concentrations were seen, and the prevalence of MetS was higher. The intramyocellular triglyceride depots, a marker of insulin resistance, were increased in Asian Indians compared to Caucasians. A large subcutaneous adipocyte size independently predicts insulin resistance and T2DM, and South Asians have larger adipocyte volume than Caucasians, a finding correlated with lower glucose disposal rate and plasma adiponectin levels [66, 71], higher insulin resistance [72], and compared to adipocyte hyperplasia, higher leptin concentrations [73].

An excessive lipolysis with increased release of non-esterified fatty acids (NEFAs) is supposed to stimulate gluconeogenesis and triglyceride production and to increase lipid overload in various non-adipocyte tissues including β -cells, thus inducing β -cell dysfunction and apoptosis. Both baseline and fasting NEFAs are higher in Asian Indians compared to Caucasians. In addition, the insulin-mediated suppression of NEFAs, measured after 2 hours (2h) of an oral glucose tolerance test (OGTT) is reduced in Asian Indians [66, 74].

Only a few small studies have compared circulating levels of the adipocyte-derived hormones leptin and adiponectin (adipokines) in Asian Indians with that of other ethnicities. Even after adjustment for body fat, leptin levels are higher in Asian Indians compared to Caucasians and Chinese, and adiponectin levels are lower in Asian Indians compared to Caucasians [71, 74, 75].

Finally, an increased susceptibility to diabetes and MetS is seen in many ethnicities, e.g., Asian, Hispanic, Indian, and African populations, compared to Caucasians [48,76, 77, 78]. It could thus be relevant to describe Caucasians as having a decreased susceptibility to T2DM and MetS.

The metabolically healthy obese

Epidemiologically and clinically, it is evident that not all obese individuals develop metabolic changes, and the concept of the metabolically healthy but obese (MHO) individual has emerged. Most often through the hyperinsulinaemic–euglycaemic clamp or other indices, such as the homeostasis model assessment (HOMA), MHO individuals have been identified, often using the NCEP definition of MetS. Approximately one third

of obese individuals have been defined as MHO, with the value depending on the definition used [79]. The prevalence of MHO is slightly higher in women, decreases with age, and is influenced by lifestyle factors such as physical activity and smoking [80]. Ethnicity influences the prevalence of MHO, and non-Hispanic Caucasians have a lower prevalence than African-Americans. MHO individuals have less visceral fat but the same amount of subcutaneous fat and less ectopic fat in liver and muscle. Furthermore, people with MHO have higher adiponectin levels, both of total and high molecular weight (HMW) adiponectin compared to other obese individuals. The subclinical inflammation often seen in obesity is lower in MHO, with lower concentrations of IL-6 and C-reactive protein (CRP) as an example. The response to weight loss differs between MHO and other obese people, with beneficial metabolic effects seen only in the non-MHO population [79, 80].

Adipose tissue

In humans, the adipose tissue, predominantly white as compared to brown, is mostly localised subcutaneously, and surrounds the viscera. The adipocyte is mainly involved in the post-alimentary storage of triglycerides in response to a meal-induced rise in glucose and lipids and a concurrent rise in insulin. In fasting, insulin concentrations drop, and lipolysis of stored triglycerides starts, a process that also is influenced by glucocorticoids, glucagon, epinephrine, and the sympathetic nervous system [81, 82].

The adipose tissue is built up by adipocytes in a vascular–stromal surrounding with macrophages, fibroblasts, endothelial cells, and pre-adipocytes. The latter can generate new adipocytes throughout life [81, 82], an ability that is decreased in patients with T2DM, potentially increasing the risk of ectopic fat accumulation. In T2DM patients, genes involved in insulin signalling and lipid metabolism in pre-adipocytes are down-regulated while genes involved in inflammation and apoptosis are up-regulated [83]. As a consequence of overeating, both fat cell number and size can increase, but the regulatory mechanisms behind these mechanisms are unclear. Hypertrophy of the adipocytes is linked to disadvantageous metabolic changes such as increased inflammation, increased release of NEFAs, decreased adiponectin, and increased leptin production [81, 82].

A small amount, around 50 g, of the fat cells are brown fat, mostly located in the supraclavicular region or thorax. Although previously considered rare in adults, recent studies demonstrate the apparent existence of brown fat in most adults. Brown fat cells are mitochondria rich and can produce heat, a “non-shivering thermogenesis”, through uncoupling of adenosine triphosphate (ATP) production. In most people, brown fat is active and thus detectable only in cold surroundings. The volume and activity of brown fat is higher in women and decreases with increasing overweight and age, and the activity is higher in wintertime and inversely correlated with BMI and total and visceral fat volumes [84, 85, 86]. The development and activity of brown fat is controlled by the sympathetic nervous system by neurons from the hypothalamus and brain stem, which induce thermogenesis. The major pathway controlling thermogenesis is the

leptin–melanocortin pathway, which indicates a role for the white adipose tissue in inducing activity in the brown fat [87].

Furthermore, the white adipose tissue plays a central role in energy homeostasis. In response to hormonal and neural signalling and through secretion of adipokines, the adipose tissue influences feeding, immunity, thermogenesis, and a wide range of neuroendocrine functions [81]. Compared to the visceral adipose tissue, the subcutaneous adipose tissue is more sparsely innervated and more readily stores excess calorie intake [88]. The autonomic nervous system innervates the white adipose tissue with separate neurons for the subcutaneous and the visceral fat depots. Sympathetic nervous stimulation initiates catabolism, reduces adipogenesis, and stimulates lipolysis while the parasympathetic nervous stimulation leads to decreased lipolysis and an anabolic state [82].

The amount of visceral fat increases with age, in men more continuously, but in women a more pronounced increase is not seen until after menopause. Excess energy intake increases the accumulation of visceral fat, but this is not a prerequisite because age and male sex are strong determinants together with a genetic effect. Heritability explains around 50% of the variance in the amount of visceral fat compared to only 5% of the variance in subcutaneous fat and in BMI (all adjusted for fat mass). Also, low birth weight and low weight at the age of one year are associated with increased visceral fat [40]. Early overfeeding of lactating rats increases the amount of visceral fat and cell size, surprisingly expressing lower levels of leptin. As the opposite would have been expected, a potential inference is a dysfunctional adipocyte [89]. Visceral fat is more sensitive to weight reduction than subcutaneous fat in both men and women, which has been attributed to a higher metabolic activity and a higher lipolytic rate in the visceral depot. The abdominal subcutaneous adipose tissue decreases in men after the age of 50 while in women, the decrease starts one to two decades later in life [40], changes that influence the interpretation of WC.

Adipocytes from different regions of the body demonstrate different responses to catecholamine-induced lipolysis with a gradient from femoral and gluteal fat cells over subcutaneous abdominal fat cells to the highest responsiveness in visceral adipocytes. Insulin induces an inhibition of this catecholamine-stimulated lipolysis, to a lesser extent in visceral adipocytes than in subcutaneous adipocytes, although the glucose uptake is similar in subcutaneous and visceral fat [40, 90]. The adipocytes from obese individuals, irrespective of region of the body, have a high lipolytic activity, especially in the visceral fat, and the increased release of NEFAs to the portal vein could potentially interfere with liver glucose production and insulin clearance, leading to hyperinsulinaemia and accelerated gluconeogenesis [40].

Other hormones are also related to adipocyte metabolism. Growth hormone, testosterone, and oestrogens all regulate the distribution of fat storage towards visceral or subcutaneous depots and are thus important for the sex difference in fat distribution, and thyroid hormones have a catabolic effect on the adipocyte [40, 88].

Obesity per se is associated with a state of low-grade inflammation. Non-adipose cells— pre-adipocyte endothelial cells,

fibroblasts, leukocytes, and macrophages—are responsible for most of the inflammatory response seen. Changes have been demonstrated both in histology, with an increase in activated macrophages, and in biochemistry, with an increase in various inflammatory cytokines, e.g., tumour necrosis factor- α (TNF- α) and IL-6, both mostly produced by non-adipocytes such as macrophages. IL-6 is the major cytokine initiating hepatic CRP production, a marker of ongoing low-grade inflammation. CRP has been associated with increased cardiometabolic risk and has a predictive value beyond the Framingham risk score for CVD [91, 92], and traditional risk markers for T2DM. Both TNF- α and IL-6 have been linked to interference with insulin signalling and to insulin resistance [82, 93].

The hormone resistin also effects inflammation. In rodents, it is produced by adipocytes but in humans by macrophages. The hormone has structural similarities with adiponectin, increases with feeding and obesity, and induces hyperglycaemia and insulin resistance in animal models. The role of resistin in humans is unclear [94].

With weight loss comes a reduction in the number of activated macrophages and the concentration of inflammatory adipokines [81, 94]. Similar changes accompany increased physical activity, with a reduction of inflammatory adipokines including leptin and an increase in the anti-inflammatory adipokines adiponectin and IL-10 [82, 95]. The concomitantly reduced visceral adiposity is mainly the result of a reduced adipocyte size; before physical activity induces a weight reduction, it can yield an improved adipocyte function, measured as improved insulin sensitivity and a more beneficial adipokine profile [82].

In obese mice, endoplasmic reticulum (ER) stress in the liver and in the adipose tissue has been demonstrated. This ER stress interferes with the processing and synthesis of proteins, leading to an accumulation of misfolded proteins [96]. In obese mice, local hypoxia in the adipose tissue has been demonstrated, and hypoxia is a known inducer of ER stress. The reason for the hypoxia has not been elucidated but may be caused by the enlarged adipocytes having a diameter greater than the diffusion capacity of oxygen. Hypoxia also dysregulates the expression of adipokines, leading to lower levels of adiponectin, higher levels of leptin, and more inflammation. This dysregulation of adipokine expression has also been demonstrated in human adipocytes in vitro [82, 97, 98].

The adipocyte and other cells in the adipose tissue release a large number of hormones and cytokines with a wide range of functions and targeting organs. Most adipokines increase in obesity except adiponectin, which has an inverse relationship with the amount of adipose tissue. Adipokines affect food intake and fat mass (e.g., leptin), insulin resistance (e.g., adiponectin, resistin, visfatin, omentin, retinol-binding protein 4, and vaspin), and inflammation (e.g., adiponectin, resistin, IL-6, TNF- α , adipisin). Various adipokines also affect vasodilatation (e.g., apelin), BP (e.g., angiotensin II, angiotensin-converting enzyme [ACE], angiotensinogen), fibrinolysis (e.g., plasminogen activator inhibitor-1 [PAI-1]), lipid metabolism (e.g., lipoprotein lipase, hormone-sensitive lipase, cholesterol ester transfer protein [CEPT], retinol-binding protein 4), and macrophage attraction and activation. A spectrum of essential body functions such as

fertility, growth, metabolism, and feeding behaviour is regulated through leptin. Finally, both the adipocyte-derived and macrophage-derived adipokines exert autocrine and paracrine regulation of their functions, which can, for example, aggravate local inflammation [82, 94].

Leptin

The history of leptin

In 1950, a severely obese mouse trait with an autosomal recessive syndrome also including hyperphagia, infertility, and a variety of hormonal and metabolic disturbances was described. Early onset of diabetes and insulin resistance and a reduced locomotor activity were observed in the mice. The mutation, located at chromosome 6, was designated “obese” and the mouse trait as *ob/ob*. In 1966, a mouse trait with a very similar phenotype was found. This mutation, “diabetes”, was found on chromosome 4, and the trait was called *db/db* [99]. In 1994, research showed that the *ob* gene encodes a peptide chain 167 amino acids in length [100], soon designated as leptin, after the Greek word *leptos*, for thin. Treatment with recombinant leptin totally reverses the symptoms of the *ob/ob* mice, which completely lack leptin. In the *db/db* mouse, treatment with recombinant leptin has no effect because the trait involves high levels of circulating leptin, and the dysfunction was soon shown to be attributable to a receptor dysfunction [99].

Total leptin deficiency is extremely rare in humans, having been described only in a few children. Treatment with recombinant leptin, however, dramatically reverses obesity, hyperphagia, and other symptoms [101]. Dysfunction of the leptin receptor is also a very rare cause of obesity in humans. In almost all cases of human obesity, the obesity is associated with hyperleptinaemia, but without any known receptor dysfunction [102, 103].

Circulating levels of leptin reflect the amount of stored fat and thus act as a key communicator informing a wide range of organs, in particular the brain, about metabolic and nutritional status. Feeding, energy balance, and body weight are regulated by the hormone, which also interacts with other hormonal systems, acts as a growth factor for various cell types, and acts as a permissive factor for fertility [102, 104, 105].

The hormone

In humans, the *ob* gene encoding leptin is localised at chromosome 7q31.3, producing a protein that is 167 amino acids long. After translocation to the microsomes, a signal peptide is removed, leaving a protein with a length of 146 amino acids and a molecular weight of 16 kDa that is the circulating leptin molecule. No significant storage of leptin exists in the adipocyte, and the basal secretion occurs at a stable rate, although pulsatile [102, 106]. The secretion is highest in the middle of the night, the opposite of what is seen for cortisol [104].

Leptin is mainly produced by the adipocytes in the white adipose tissue [107], although the hormone is also expressed in many other tissues. Reports exist regarding expression in humans in placenta, mammary

epithelial cells, and infant bone marrow and in various animals in brown adipose tissue, myocytes, testes and ovaries, the gastric mucosa, and hair follicles. In rats, a production of leptin from trans-differentiated stellate cells in the liver after an injury has been demonstrated. As leptin concentrations rise in humans with liver cirrhosis, it is a possibility that the same phenomenon observed in the rats could take place in people [102, 104, 105]. In humans, glucose, insulin, glucocorticoids, TNF- α , oestrogens, and alcohol increase the concentration of leptin whereas androgens, growth hormone, somatostatin, β 3-adrenergic agonists including isoprenaline, thiazolidinediones, NEFAs, and cigarette smoking reduce leptin levels [105, 108, 109, 110]. Leptin down-regulates the production of corticosteroids in the adrenal glands [111], and interestingly, significantly higher leptin levels have been described in socially isolated and depressed men, but not in women, even after taking BMI, lifestyle, and metabolic variables into account [112].

Leptin is partially bound to proteins in the plasma. Reports indicate a higher proportion of leptin bound to plasma proteins in lean individuals compared to obese people, approximately 45% and 20%, respectively. A proportion of the hormone is also tissue bound, presumably being of importance as a contributor to stable plasma concentrations [102, 113]. Leptin is cleared from the circulation by the kidneys, and as a consequence, higher levels are seen in humans with renal failure. Renal epithelial cells degrade leptin after filtration [102], possibly after binding to the leptin receptor [114]. The clearance rate does not differ between lean and obese individuals [104, 106]. The half-life for the free as well as for the protein-bound leptin is similar as for other peptide hormones, approximately 3.4 minutes and 71 minutes, respectively [105].

The effect of leptin on energy balance and appetite is mainly mediated by neurons in the hypothalamus where the longer version of the receptor is also highly expressed. In rodents, leptin is transported across the blood-brain barrier (BBB) by a saturable process, but the presence of this mechanism in humans is not clear. Through capillaries close to the median eminence, a diffusion of molecules of the size of leptin can take place; thus, leptin reaches the ventrobasal hypothalamus. It has been suggested that leptin could pass the BBB by receptor-mediated transcytosis by the short form of the receptor that is highly expressed in micro vessels. These receptors are up-regulated in hyperleptinaemia, but it is also possible that the receptors are involved in leptin clearance and not in transcytosis [113]. Leptin can pass the BBB in mice lacking a functional receptor, indicating that other transport mechanisms, at least partly, can explain the BBB transport. In the cerebrospinal fluid, the concentration of leptin is approximately 1% of that in plasma [113]. While leptin levels in obese individuals are more than three fold that found in lean individuals, the leptin levels in the cerebrospinal fluid are elevated by only 30%, indicating that leptin passes the BBB by a saturable pathway [115].

The production of leptin mirrors the total amount of ob mRNA in the adipose tissue. Obese persons have a higher amount of ob mRNA than lean persons, and larger adipocytes, which are the major determinant of high amounts of ob mRNA. In humans, leptin expression is

higher in subcutaneous than in visceral adipocytes, which may be the opposite of what is found in rodents. Overfeeding in rats, even without significant weight gain, is followed by an increase in ob mRNA [105, 106]. A short-term fast of 22 hours without weight loss in humans, on the other hand, is accompanied by decreased leptin production. The relative fall in leptin levels, as a response to fasting is greater in lean than in abdominally obese individuals [106, 116].

Receptors and signalling

The leptin receptor exists in six known isoforms, five short and one long. All receptors share the same extracellular binding domain, but the intracellular domains differ in length. One of the short isoforms lacks both the transmembrane and intracellular domains and exists as a soluble receptor in the circulation. Only the long receptor can fully activate the Janus kinase (JAK)–signal transducer and activator of transcription (STAT) pathway, and the short isoforms can only weakly activate JAK and do not activate STAT [102, 113]. The long receptor is primarily localised in the hypothalamus but is also identified in adipose tissue, in the gonads, and in the adrenal glands, while the short forms are present in a wide variety of tissues e.g., in hypothalamus, gonads, prostate, adipose tissue, choroid plexus, vascular endothelium, kidney, liver, lung, gonads, skeletal muscle, pancreas, bone marrow, spleen, and hematopoietic cells, in particular macrophages [102, 106, 111, 117, 118].

In hypothalamus, most of the details on leptin signalling have been gathered through studies on knock-out mice. In the JAK/STAT signalling pathway, activation of STAT3, STAT5 and JAK2 explain most of the energy homeostasis and of other physiological processes, but notably only partially the control of glucose homeostasis. In particular STAT3 signalling seems to be crucial for energy homeostasis. Other cytokines, prolactin and growth hormone also act on STAT5, and on the JAK/STAT pathway. Leptin induced signalling is suppressed by a negative feedback by suppressor of cytokine signalling (SOCS)3 induced by STAT3. Notably, cellular inflammation also induces SOCS3 signalling [110].

By JAK2 activation of the insulin receptor substrate-phosphatidylinositol-3-OH kinase (IRS-PI3K) pathway, central leptin signalling significantly improve peripheral insulin sensitivity independent of food intake and body weight. The mechanisms for the improvement, also seen in severely insulin deficient mice, seems to be mediated via increased hepatic insulin sensitivity, suppressed gluconeogenesis in the liver, but also via increased peripheral tissue glucose uptake. PI3K signalling, together with the sympathetic nervous system also regulates lipid metabolism in white adipose tissue. Insulin also activates PI3K signalling and thus contributing to increased peripheral insulin sensitivity and inhibition of food intake [110].

The soluble receptor provides the primary binding of leptin in the circulation and is supposed to delay clearance. The amount of soluble receptors is highly correlated to the total amount of cell surface-bound receptors. In analogy to what is described for IL-6, it has been suggested that the soluble receptor can potentiate the effect of leptin. Studies have indicated that the soluble receptor increases insulin sensitivity by acting on the IRS-

PI3K pathway. This interaction could at least in part explain the findings of an inverse relationship between the soluble receptor, and insulin resistance, incident diabetes [119], and MetS [120] respectively.

Sex differences

In women, leptin concentrations are approximately three fold that in men, independent of the amount of fat [106]. The difference could at least in part be attributed to a difference in body composition with a larger percent of body fat [104], a larger amount of subcutaneous fat [102], and larger adipocytes in women under age 50 years [121]. Leptin decreases with increasing age independent of BMI changes, and in a more pronounced way in women [104]. The sex difference in leptin levels is apparent at birth [122], and older children have similar levels of leptin as adults [106]. Androgens suppress the synthesis of leptin while the role of oestrogens is somewhat inconsistent [102, 104, 105], although most studies show that oestrogens increase leptin. An increased release of leptin *in vitro* by oestrogens has been described [123], but oestrogen substitution in post-menopausal women does not change leptin levels [104].

Leptin levels vary in women through the menstrual cycle more than 1.5 fold and throughout pregnancy more than 2.7 fold. Leptin per BMI unit varies almost two fold in individual women through the menstrual cycle as well as through pregnancy. Concentrations of leptin peak during the luteal phase, concomitant with the progesterone peak. The increase in leptin levels during pregnancy more closely correlates with an increase in BMI. Leptin levels plateau during the third trimester despite a continuous increase in oestrogens throughout the pregnancy. A concomitant increase in insulin and cortisol and contribution of leptin from the placenta have been suggested as an explanation for the observed increase. Within one month after delivery, leptin levels are cut by half, which indicates that the increase cannot solely be attributed to an increase in obesity during pregnancy [123].

Leptin signalling in the ventral premammillary nucleus differs between male and female mice. In the male mice, these neurons are important for the metabolic regulation of food intake, body weight, and glucose homeostasis. The same effect, but to a much lesser extent, is seen in the female mice, where neurons predominantly exert the effect of regulating fertility [124]. Whether the same difference is found in humans is not known. The response to leptin injected into the third cerebral ventricle differs between male and female rats. The food intake is reduced in both sexes, measured as a 4-hour intake, but only in female rats when measured as 24 hours of food intake. The opposite response has been seen after injection of insulin, resulting in a 24-hour reduction of food intake in male rats [125]. Ovariectomised rats demonstrate an increased food intake, weight gain, and increasing leptin levels. Substitution with oestrogen restores the symptoms, possibly by increasing the responsiveness to leptin through an increased expression of the long form of the leptin receptor in the hypothalamus [126].

Ethnicity

Only small studies have examined potential differences in circulating leptin levels among ethnicities, controlling for various anthropometric measures of obesity. One study found that insulin-resistant Asian Indian men had higher levels than insulin-resistant Caucasian men [74], and in lean men, the same ethnic difference has been seen, with Chinese men at intermediate leptin levels [127]. African-Americans have higher leptin concentrations than Caucasians [128, 129], and in South Africa, women of African ethnicity have higher leptin levels than Caucasian women [130]. South-American Indians have lower levels than Caucasians [131, 132], but many studies have shown no difference in leptin concentrations among ethnic groups [133, 134, 135]. A recent study reported that South Asians have the least favourable adipokine profile of high leptin and low adiponectin among all studied populations (Caucasians, Chinese, South Asians, and Canadian Aboriginals) [136].

Circ-annual and intra-individual stability

In many seasonally breeding animals, leptin acts as a permissive factor for fertility. Driven by the photoperiodic length, a marked circ-annual variation in appetite, fat accumulation, and leptin levels is seen. Concomitant with the variation in leptin levels, there is a varying responsiveness to exogenous leptin [137, 138, 139]. Melatonin alone does not influence leptin levels [138, 139], but in the presence of insulin, leptin expression is enhanced by melatonin in rat adipocytes [140].

In humans, only three small studies have addressed the topic. Two longitudinal studies with a combined total of 43 participants showed no seasonal variation in leptin levels [141, 142], while a cross-sectional study of 65 individuals showed a slight variation of leptin by season [143].

The intra-individual stability of leptin over time has been studied only in 17 young women, with an ICC of 0.58 [144].

Epigenetics

In mice, undernutrition during the gestational period and during lactation leads to a higher food intake and adiposity in offspring. Altered methylation of the leptin gene thus leads to a permanently decreased expression of the gene in the pups but no global methylation in the DNA [145]. Increased glucose levels during pregnancy change the foetal programming, and IGT in the mother leads to DNA methylation and decreased expression of the leptin gene [146].

Physical activity

Only more strenuous (>800 kcal) physical activity, lasting more than 1 hour, acutely reduces plasma leptin levels [147, 148], and moderate physical activity without weight reduction is not sufficient. Strenuous physical activity over two weeks reduced circulating leptin levels independent of changes in WC [95]. In 10 obese women, a brisk 20-minute walk resulted in a reduced appetite and increased perception of fullness and satiety, changes of the same magnitude as those resulting from intake of

snacks. An association between leptin concentrations and the rating of appetite and satiety was found only after the brisk walk, not after snack intake or physical inactivity [149]. A potential explanation could be a reduced transport of leptin over the BBB in obesity. This reduction has been described in obese mice and can be reversed by fasting [150], possibly also in humans [115]. The transport of leptin over the BBB is enhanced by catecholamines [151], which are elevated by moderate physical activity [152].

Hunger and feeding

Recent studies indicate that leptin influences feeding and reward systems at different levels in the brain, not only in the hypothalamus. Neuroimaging in obese patients shows that leptin supplementation reduces the response to pictures of food in the insula as well as in the frontal areas. A similar response has been seen in the insula in patients with leptin deficiency after supplementation [153]. These findings could indicate that leptin decreases the response in some important taste and reward areas in the brain.

A major target for leptin is the hypothalamus, influencing both behaviour and metabolism, and leptin inhibits orexigenic neurons and neuropeptides, such as neuropeptide Y (NPY) and Agouti-related peptide (AgRP), which stimulate food intake. Both leptin and insulin reduce signalling via NPY and AgRP [110, 154]. Leptin also decreases energy expenditure as well as stimulating anorexigenic signalling such as α -melanocortin-stimulating hormone (α -MSH), a cleaved product of pro-opiomelanocortin (POMC). In brief, the activity and gene expression of NPY, AgRP, and POMC are modulated in the arcuate nucleus (ARC) by leptin. In the ARC, signalling from other parts of the brain, such as the “satiety centre” in the ventromedial nucleus of the hypothalamus (VMH), is integrated, leading to downstream signalling that affects metabolism and feeding [154].

In the hypothalamus, leptin is an important regulator of body weight and energy homeostasis, but it is far from being the only regulator. A wide range of circulating hormones contribute, including insulin together with several gastrointestinal hormones, e.g., ghrelin and cholecystokinin (CCK), and metabolites such as glucose and lipids together with serotonergic and dopaminergic neurons [155].

Leptin also interacts with the gut and its hormones, of which the most widely studied interaction is that with the satiety peptide CCK. Leptin potentiates the effect of CCK, which acts on vagal fibres in the nucleus of the solitary tract (NTS) in the brain stem, [110, 156] and possibly also modulates its release. Leptin through vagal neurons, also modulates feeding by suppressing gastric emptying [156].

A low general locomotor activity is seen in *ob/ob* mice as well as in leptin-deficient humans, but food seeking is markedly increased. After administration of exogenous leptin, the general locomotor activity is increased and food-seeking activity normalised, an effect that presumably is mediated via hypothalamic neurons. In starving wild-type mice, an increased food seeking is seen, a behaviour that is partially reversed by exogenous leptin. In wild-type mice fed ad-lib, exogenous leptin has little effect on

feeding, which could indicate that the physiological role of leptin is more as a signal in starvation rather than in obesity [157, 158].

Leptin resistance

Three animal models have widely been used in studies on leptin, the *ob/ob* and *db/db* mice traits and the use of injected leptin into the intracerebral fluid. The two former models, although resulting in marked obesity, differ in some aspects from normal human obesity. The *ob/ob* mouse totally lacks leptin and is, in contrast to ordinary human obesity, notably sensitive to exogenous leptin. The *db/db* mouse has an abundance of leptin but exhibits no signalling downstream of the malfunctioning leptin receptor. An injection of leptin into the cerebral ventricle appears to yield a leptin concentration that by far exceeds the physiological range [99, 100, 102, 113], so results from studies with these models may not be fully applicable to human obesity.

The concept of leptin resistance has emerged from the fact that obesity normally is accompanied by increased leptin levels despite the physiological role of leptin to reduce food intake and body weight. Because leptin signalling seems to function in some aspects such as fertility, energy expenditure, and sympathetic regulation, the concept of selective leptin resistance, i.e., restricted to the control of food intake, has been suggested [159].

There are several potential mechanisms for the development of leptin resistance. Dysfunctional receptors arising from genetic mechanisms are found in the *db/db* mice, and in Zucker fatty (*fa/fa*) rats, a status very rarely found in humans [160]. Leptin, like other hormones, seems to self-regulate its signalling by reducing receptor expression. In various mouse models of obesity, reduced expression of the hypothalamic leptin receptors is found. In transgenic mice overexpressing leptin, a down-regulation of the receptor and consequently of its signalling is seen [160]. Potentially, a resistance could develop through reduced transportation of leptin across the BBB. The transportation is thought to be through a saturable transport system in the choroid plexus and vascular endothelium. Obese humans and mice have a lower cerebrospinal-to-serum ratio of leptin, supportive of the idea of a saturable transport, and in obese mice, administration of leptin to the cerebrospinal fluid induces a short-term weight reduction [160]. In diet induced obesity in rodents, the ability of leptin to activate STAT3 is reduced, and at the cellular level, a negative feedback by STAT3 promoted accumulation of SOCS3, inhibits leptin JAK/STAT signalling. SOCS3 deficient mice have increased leptin sensitivity and are protected against diet-induced obesity and increased SOCS3 signalling in POMC causes obesity and decreased leptin responsiveness. Other members of the SOCS family also regulate signalling from cytokines, including leptin [110, 159, 160]. The expression of SOCS3 is up-regulated by hypothalamic inflammation, potentially causing obesity. Interestingly, rats fed on a high fat diet show a decreased responsiveness to leptin concomitant with hypothalamic inflammation, measured by elevated mRNA and protein levels of TNF- α , IL-6 and other pro-inflammatory cytokines [110]. Leptin is a major regulator of total and feed seeking locomotor activity. Notably, STAT3

signalling in mice promote increased locomotor activity [161, 162], indicating that leptin resistance may influence physical activity.

A number of circulating serum leptin–interacting proteins (SLIPs) have been described, and one of them is CRP. In vitro studies show that CRP prevents leptin from binding to the receptor, and in *ob/ob* mice, infused human CRP attenuates the effect of leptin on food and weight regulation as well as on glucose and lipid metabolism. Studies suggest that while leptin enhances CRP production in the liver, CRP regulates leptin signalling. Of interest, leptin increases CRP levels in non-obese individuals but not in the obese, which supports the existence of leptin resistance [159, 160]. Finally, ER stress has been described as a potential explanation for leptin resistance. High fat diet can induce ER stress in rodents, which as a consequence increase weight and demonstrate a reduced responsiveness to the anorexigenic effects of leptin and insulin. Of interest, a centrally administered ER stress inhibitor could restore food intake and weight [110, 159].

Other researchers have argued that leptin resistance does not exist and that the abundance of food and mechanisation in modern society has made it possible for humans to achieve the BMI set by their individual regulatory systems [163]. In animals, obesity would in many ways be disadvantageous because it increases the risk of predation. Since human ancestors 2 million years ago formed social groups and could protect themselves with weapons, predation has no longer acted as a strong evolutionary force in people. In the absence of natural selection and through random mutations, a drift has occurred in the set point of the individual BMI, but in modern society, a majority of the population has the potential to achieve that BMI [163]. If true, this “drifting set point” model may have large implications for the effect of dietary intervention for obesity as well as the future development of the obesity epidemic.

Some examples of peripheral leptin resistance have been described [159, 160, 164], which could allow for an alternative interpretation of the potential pathophysiology in hyperleptinaemia. Instead of increased leptin levels causing inflammation, endothelial dysfunction, platelet aggregation, oxidative stress, and other potentially harmful changes, the opposite could be true: namely, that as a consequence of peripheral leptin resistance, leptin cannot exert its beneficial effects [164,165].

Inflammation

The structure and function of leptin resembles that of other pro-inflammatory cytokines, and their concentrations often covary [160]. Leptin activates monocytes, thus modulating the production of TNF- α and IL-6, and activates T lymphocytes and macrophages. Leptin not only stimulates T cell production but also induces a shift towards an increase in pro-inflammatory T1 helper (Th1) cells over anti-inflammatory T helper2 (Th2) cells. The production of CRP in the liver may be regulated through an effect on IL-6 but also may be regulated directly [160, 166]. Of note, a potential role for leptin in various non-autoimmune inflammatory situations, as well as in autoimmune diseases such as type 1 diabetes, has been suggested [160, 167].

Sympathetic and parasympathetic nervous system

Leptin increases sympathetic signalling in brown adipose tissue, skeletal muscle, kidneys, and adrenal glands [102], with effects on lipolysis, lipid, and glucose metabolism. In white adipose tissue, sympathetic nervous signalling directly decreases leptin production, but leptin production will also subsequently decrease through an increased lipolysis [109, 168]. A feedback system between the sympathetic nervous system and leptin is thus indicated. The vagal nerve serves as a neural communicator between hypothalamus, visceral fat (reducing inflammation and increasing leptin and resistin), the β -cell (stimulating insulin secretion), liver and other visceral organs. Vagal afferent neurons are activated by leptin, and presumably influence meal termination and satiety [156, 169, 170, 171, 172].

Cardiovascular effects

Leptin has been linked to CVD risk factors and events [164], possibly through inflammatory cells and cytokines linked to the formation of atherosclerosis [160]. Hypertension is more frequent in hyperleptinaemia and could possibly be caused by increased sympathetic signalling in the kidneys. Leptin increases platelet aggregation, might be pro-thrombotic, and affects endothelial function and smooth muscle cell proliferation [160, 164]. In people, increased leptin levels have been linked to subsequent acute cardiovascular events, restenosis after coronary angioplasty, and stroke [164, 173, 174]. Of interest, a sex difference has been described for the risk of stroke, with leptin being an independent risk marker in men but not in women [175].

Lipids and lipid metabolism

In cultured human and murine macrophages, leptin stimulates the secretion of lipoprotein lipase (LPL), which is pro-atherogenic because it promotes sub-endothelial lipoprotein accumulation. Especially in hyperglycaemia, leptin stimulates accumulation of cholesterol esters in foam cells. An inverse relationship is found between leptin and high density lipoprotein (HDL) cholesterol and with apolipoprotein A1, and leptin increases the hepatic clearance of HDL in mice, thus leading to an unfavourable cholesterol profile [164].

The lipolysis in adipocytes is stimulated by leptin [168], as is the muscular clearance of NEFAs and triglycerides [160]. In brown adipose tissue, thermogenesis is stimulated by leptin [87]. Leptin decreases the content of triglycerides in liver, pancreas, and skeletal muscle. But unlike what is seen in starvation, the increased lipolysis is not accompanied by increasing NEFAs or ketones in plasma [168], and reduced plasma triglycerides have been demonstrated in patients with lipodystrophy (with loss of subcutaneous adipose tissue and thus reduced leptin concentrations) on treatment with leptin [176]. It has been proposed that the role of hyperleptinaemia is to increase survival in famines by permitting surplus storage in the adipocytes without concomitant risk of ectopic, harmful lipid deposition [177].

Leptin and glucose metabolism

Leptin-deficient mice and humans develop diabetes early, a status that is rapidly normalised on treatment with leptin. A similar effect is seen in patients with lipodystrophy and diabetes. Worth noting, this effect is seen prior to a decrease in weight, indicating an interaction between leptin and glucose metabolism and possibly with insulin [160].

In humans, plasma leptin and insulin concentrations mirror each other, and the pulsatility of the secretion is highly synchronised [178]. Further, increasing leptin levels are accompanied by an increased insulin resistance independent of BMI [160]. Leptin secretion from adipocytes is stimulated by glucose and insulin, and patients with an insulinoma have increased leptin levels. In the potential bi-directional regulation between the adipocyte and the pancreatic islets, leptin decreases insulin secretion by directly acting on the β -cells. A reduction in the transcription and synthesis as well as the secretion of insulin has been shown in human and rodent pancreatic cells [160, 178]. Most studies on isolated β -cells have been performed with supra-physiological concentrations of leptin, but even with physiological doses of leptin in rats, a decreased insulin secretion has been demonstrated [178]. In addition, enhanced glucose uptake and oxidation in skeletal muscle and reduced gluconeogenesis from the liver have been demonstrated both *in vitro* and *in vivo* after both peripheral and intracerebral administration of leptin [110, 160, 178]. The positive effect of centrally administered leptin on glucose metabolism, is mediated via vagal nerve fibres from NTS and notably, is also seen in severely insulin deficient animals [110]. The expression of leptin does not differ in subcutaneous and epicardial adipose tissue between diabetic patients and non-diabetic individuals of the same BMI, potentially because of counter-regulatory mechanisms [179]. The potential effect of leptin on glucagon is not fully elucidated, but in rats with induced diabetes, glucagon levels were normalised by leptin, and the glucagon effect in liver cells was reduced *in vitro* [105, 110].

As a response to obesity and the increasing demands for insulin, studies have identified a compensatory β -cell proliferation, induced by insulin and insulin-like growth factor (IGF) and mediated by the insulin receptor substrate (IRS)-2 pathway. Adiponectin blocks the degradation of IRS-2, while glucagon-like peptide (GLP)-1 and gastric inhibitory polypeptide (GIP) stimulate IRS-2. Leptin seems to exert a stimulatory effect on IRS-2 through the same pathway as GLP-1 and GIP but also has an inhibitory effect through other paths [180, 181]. Potentially in obesity, reduced adiponectin concentrations can no longer protect the β -cell mass, while the increased leptin levels exert a negative effect [180]. A concomitant decrease in GLP-1 and GIP, often but not consistently described in diabetes [182], could contribute to a further decline in β -cell mass.

In female spontaneously diabetic mice, leptin is found to accelerate autoimmune diabetes [183], indicating a potential β -cell harming effect of leptin mediated by the immune system. Leptin could possibly both increase and decrease insulin sensitivity. By way of increased muscular glucose oxidation, reduced gluconeogenesis in the liver, and reduced

triglyceride accumulation in liver and muscle, increased insulin sensitivity could occur, but induction of SOCS3 could have the opposite effect [160].

Leptin supplementation improves glucose metabolism in patients with leptin deficiency or severe lipodystrophy or patients with Rabson–Mendenhall syndrome, a rare condition with extreme insulin resistance thought to result from insulin receptor deficiency. On the other hand, leptin treatment in humans with normal obesity yields no positive effect on plasma insulin levels or glucose tolerance as measured by OGTT. These studies were small, however, and individuals with glucose intolerance were excluded; further, OGTT may not be a sufficiently sensitive measurement [178, 184].

Leptin and diabetes

Studies are inconsistent as to whether leptin is associated with subsequent diabetes or not. An independent relationship has been described in men but not in women based on sex-stratified analyses [185, 186, 187, 188], but other studies have found no relationships [189, 190], and still others have identified even a protective role after multiple adjustments [191]. Snijder et al. have argued that the latter result could be attributable to an overadjustment in the statistical modelling, or impreciseness in the diagnosis of diabetes [190].

Treatment with leptin

Leptin supplementation for children without endogenous leptin production has been very successful [101]. Furthermore, supplementation for patients with severe lipodystrophy and diabetes and adult patients with leptin deficiency and diabetes markedly ameliorates hyperglycaemia [176, 178]. Patients with Rabson–Mendenhall syndrome benefit from leptin injections [178]. In normal obesity, treatment effects are less impressive, which has been attributed to leptin resistance. Exogenous leptin, indeed, reduces weight at a greater rate in people with milder obesity and in those with relatively lower levels of leptin, which could support the concept of leptin resistance [159]. On the other hand, trials using leptin to achieve weight reduction in obesity demonstrate a dose-response effect on weight reduction by the given dose of leptin. To achieve a 10-fold increase in mean weight reduction (7.1 kg vs. 0.7 kg), however, the dose of leptin had to be increased 30 fold (from 0.01 mg/kg/day to 0.3 mg/kg/day). Treatment with leptin has also been attempted in small studies involving patients after voluntary weight loss with the aim of preventing a rebound weight gain. After weight reduction, the participants' total energy expenditure was decreased out of proportion to the change in body composition, by approximately 300–500 kcal/day. The changed energy expenditure was attributed to a change in skeletal muscular work efficiency, decreased sympathetic nervous signalling, and decreased circulating concentrations of thyroxine and triiodothyronine. After supplementation with leptin, the metabolic changes were restored and the weight reduction maintained [163, 192].

Adiponectin

The hormone

The hormone, a protein consisting of 244 amino acids, is mainly produced by adipocytes, but in humans also by bone marrow, osteoblasts, foetal tissue, myocytes, cardiomyocytes, and salivary gland epithelial cells. In chickens, production has been described from the pituitary gland, liver, diencephalon, skeletal muscle, ovary, spleen, and kidney [193]. Adiponectin circulates in different multimeric forms but also as a proteolytic fragment; the globular form that has structural similarities with TNF- α . The basic version is a trimer that can exist as hexamers and multimers (HMW adiponectin) [193, 194, 195]. The hexamer and HMW forms are the major configurations in plasma, partly because of a longer half-life. The different versions of circulating adiponectin have different main target organs; e.g., HMW adiponectin is particularly active in the liver and in endothelial cells [193]. The HMW form has been shown to be the most biologically active and the most potent at insulin sensitisation [196, 197].

In contrast to most other adipokines, circulating levels of adiponectin are inversely correlated with anthropometric measures of obesity such as BMI, the amount of visceral fat, and particularly WHR [193]. Circulating levels of adiponectin are reduced with increasing age independent of obesity [198]. Glucocorticoids, TNF- α , growth hormone, β -adrenoreceptor agonists, and prolactin down-regulate the production of the hormone while insulin, at least in the acute setting, IGF-1 and peroxisome proliferator-activated receptor (PPAR) agonists increase circulating levels [193, 197, 199]. Transgenic mice overexpressing adiponectin down-regulate the expression of messenger ribonucleic acid (mRNA), indicating a self-regulatory feedback loop [193]. The expression of adiponectin in diabetic patients and non-diabetic individuals of the same BMI is equal in subcutaneous and epicardial adipose tissue, despite marked differences in circulating levels, which may be the result of counter-regulatory mechanisms [179]. The adiponectin levels in the cerebrospinal fluid are low in mice, only about 1/1000 of that in serum, and one small study in humans showed no detectable adiponectin in the cerebrospinal fluid. In mice, most of the adiponectin is in the globular and low-molecular-weight forms, and with venous injection, globular adiponectin does not pass the BBB [200, 201].

The insulin-sensitizing effects of adiponectin are mediated by activation of adenosine monophosphate (AMP)-activated protein kinase (AMPK), p38 mitogen-activated protein kinase (MAPK), and PPAR- γ . The hormone also has anti-atherosclerotic and anti-inflammatory properties and possibly also anti-cancer and cardioprotective functions [193].

Receptor and signalling

Three receptors have been identified—the adiponectin receptors 1 and 2 (AdipoR1, AdipoR2) and the T-cadherin receptor. AdipoR1 is most abundantly expressed in skeletal muscle and AdipoR2 in skeletal muscle and the liver. Both AdipoR1 and AdipoR2 are expressed in pancreatic

β -cells, where interestingly, the receptor expression is increased by exposure to NEFAs [193, 199]. The AdipoR1 and AdipoR2 receptors are widely distributed in hypothalamus and the brain stem and also in the pituitary gland. This distribution suggests that adiponectin is important in the regulation of feeding and energy expenditure, although results of studies conflict [193]. In part, the discrepancies can be the result of intra-cerebrospinal administration of adiponectin in some studies, which results in concentrations considerably in excess of physiological levels. Adiponectin receptors are also found in various other organs such as the ovaries, and in endothelial cells and adipocytes. In subcutaneous adipose tissue, the expression of AdipoR1 and AdipoR2 is down-regulated in obesity and mostly restored after weight reduction. In animal models, expression of AdipoR1 and AdipoR2 receptors responds both to fasting and to insulin, differently in different organs, and possibly also with a different response to fasting in different species. In skeletal muscle, the expression of the receptor is inversely regulated by insulin [193].

The T-cadherin receptor is most abundantly expressed in the heart and in larger arteries. The receptor lacks the intracellular domain needed for signal transduction and binds only the hexameric and HMW forms of adiponectin. With this competing receptor, the signalling via AdipoR1 and AdipoR2 receptors is modified. In various test models, the expression of the T-cadherin receptor is up-regulated by oxidative stress, progesterone, epidermal growth factor, and arterial injury and down-regulated by fasting [193].

Sex differences

Women have higher circulating levels of adiponectin and in particular of HMW adiponectin. Exogenous testosterone in humans decreases adiponectin levels while oestrogens have no effect [193].

Ethnicity

Adjusted for age and WC, South Asians and Chinese men and women have lower adiponectin concentrations than Caucasians and Canadian Aboriginals. In all ethnicities except for South Asians, an inverse relationship has been seen between BMI and adiponectin. In addition, the most pronounced increase in HOMA-insulin resistance (IR) per unit decrease in adiponectin was seen in South Asians and Canadian Aboriginals [136].

Physical activity

An increase in adiponectin is seen after intense short-term physical activity, with a delay until 30 minutes after exercise, but studies in obese and in non-athletes are limited. Training that improves fitness seems to increase adiponectin levels [95, 148].

Inflammation

Adiponectin demonstrates a wide range of anti-inflammatory properties, from influencing differentiation of bone marrow cells to an effect

on the expression of inflammatory and anti-inflammatory cytokines from macrophages and other monocyte-derived cells. The anti-inflammatory cytokines IL-10 and IL-1 receptor agonist (RA) are increased, and pro-inflammatory cytokines such as IL-6 and TNF- α are suppressed as a general response to adiponectin, although with a slightly different response depending on the adiponectin isoform. However, TNF- α can regulate and decrease the synthesis of adiponectin. TNF- α is produced mainly by the adipose tissue, which could in part explain the reduced production of adiponectin in obesity [193]. Finally, in humans, circulating levels of adiponectin have been linked to the severity of inflammatory joint diseases, including rheumatoid arthritis [193].

Sympathetic and parasympathetic nervous system

The expression of adiponectin mRNA is markedly down-regulated in adipocytes exposed to β -adrenergic stimulation, indicating a potential regulatory control by the sympathetic nervous system [202]. Favouring such a mechanism is the finding that a blockade of β -adrenergic signalling in patients with hypertension increases adiponectin levels [203] and that cold exposure acutely increases norepinephrine and decreases adiponectin levels [204]. On the other hand, adiponectin decreases renal sympathetic signalling in rats in a dose-dependent way, which leads to decreased BP [193]. Parasympathetic activation does not influence adiponectin mRNA [172].

Cardiovascular effects

Experimentally, adiponectin prevents cerebral ischaemia in mice through an endothelial nitric oxide synthase (eNOS) dependent mechanism [205], and reduces the size of an experimental cardiac infarction in mice [193]. Through different mechanisms, adiponectin can influence atherosclerotic progress in the arteries by reducing inflammation, formation of foam cells, and subendothelial lipid accumulation; furthermore, adiponectin increases endothelial nitric oxide production, thus inducing vasodilatation [193].

Most cases of cardiovascular disease are associated with low adiponectin levels, but opposing findings exist. Several reports have shown an increased mortality risk in patients with cardiac heart failure or coronary artery disease with high levels of adiponectin. It is possible that these results reflect a counter-balancing increase in adiponectin concentrations, which is proposed to be initiated as a response to an excessive pro-atherosclerotic endothelial process [179, 194, 198]. Another potential mechanism could involve a concomitant rise in brain natriuretic hormone, which highly covaries with adiponectin in patients with cardiac heart failure. The former hormone reflects the severity of the cardiovascular disease, and the latter reflects a compensatory mechanism initiated by vascular stress. Concomitant renal failure or unintended weight reduction as an explanation is considered less likely, but a potentially harmful effect by adiponectin is possible [198].

Adiponectin can potentially modify three major paths that could contribute to the development of hypertension. In addition to

endothelial dysfunction and sympathetic signalling, the renin–angiotensin system also interacts with adiponectin. Angiotensin II receptor blockers increase adiponectin levels, and injection of angiotensin II induces a decrease in adiponectin levels [193]. Adiponectin, independent of traditional risk factors, predicts hypertension [193, 194]. Most anti-hypertensive medication seems to modify adiponectin levels in a way opposite to their effect on insulin sensitivity. Thiazide diuretics and β -blockers decrease adiponectin levels while spironolactone, angiotensin-converting enzyme inhibitors, and angiotensin II blockers and also calcium channel blockers in some studies increase circulating levels [194].

Lipids and lipid metabolism

In rodents, adiponectin regulates lipid metabolism in skeletal muscle through activation of AMPK, PPAR α , and p38 MAPK, thus increasing fatty acid oxidation and glucose use. In the liver, activation of AMPK and PPAR α increases oxidation and suppresses the synthesis of fatty acids [193]. In humans, adiponectin associates inversely with dyslipidaemia and with HDL cholesterol levels, the latter association being more evident with increasing obesity. Adiponectin may also regulate the catabolism of HDL. Lipid-lowering treatment with statins does not influence serum levels [193, 194].

Adiponectin and glucose metabolism

Adiponectin exerts effects on glucose metabolism but also seems to protect β -cells from cell death [180]. Both human and rodent β -cells express AdipoR1 and AdipoR2, and the expression of AdipoR1 mRNA is decreased in obese mice compared to lean mice. Adiponectin-deficient knockout mice and lipotrophic mice are insulin resistant and respond with less insulin secretion to a glucose load. These mice respond with a significantly improved glucose metabolism after adiponectin supplementation, and similarly, the secretion of insulin increases in rat β -cells by exogenous adiponectin. Adiponectin counteracts the free fatty acid–induced apoptosis of β -cells and attenuates an acute pancreatitis in obese mice [193].

Although studies are few, chronic insulin treatment has shown no effect on circulating levels of adiponectin, and acute insulin treatment studies are small and inconsistent [194]. The reason for the discrepancies may be the varying insulin resistance status, as indicated in a recent study [197]. This study by Hajri et al. also demonstrated that insulin regulates circulating levels and expression of adiponectin, indicating a bi-directional regulation between insulin and adiponectin. Of interest, that study also linked the low-grade inflammation seen in obesity [93] to the dysregulation of adiponectin and potentially also of insulin. Also worth noting, TNF- α induces insulin resistance and also promotes lipolysis and reduces adipogenesis [93, 197].

Hajri et al. used both in vitro experiments and the hyperinsulinaemic clamp technique in obese, insulin-resistant, and lean insulin-sensitive persons [197]. Prior to the clamp, the obese insulin-

resistant individuals had reduced concentrations of both total and HMW adiponectin. In lean individuals, insulin significantly increased circulating adiponectin, while in the obese insulin-resistant participants, adiponectin levels did not change. Furthermore, the expression and release from adipose tissue of TNF- α and IL-6 increased in the obese individuals as a response to insulin. In adipose tissue, TNF- α but not IL-6 strongly counteracted the stimulatory effect of insulin, and in particular the secretion of HMW adiponectin was blocked by TNF- α . On exposure to insulin, the expression and release from adipose tissue of adiponectin increased three fold [197], a finding supported by other studies [206].

As described previously, adiponectin increases glucose use in skeletal muscle in rodents. The hormone also modifies insulin sensitivity by an increase in fatty acid oxidation in skeletal muscle and in the liver, and by a decrease in hepatic glucose production [193]. In *ob/ob* mice, adiponectin promotes a subcutaneous adipose tissue hyperplasia, which also results in improved insulin sensitivity [79].

In patients with T2DM, treatment may change adiponectin levels. Metformin and most sulfonylureas, except glimepiride, do not change circulating levels. The reason that glimepiride acts differently from other sulfonylureas, increasing adiponectin, is thought to be its insulin-mimetic or insulin-sensitizing properties. Thiazolidinediones induce increased adiponectin expression through an insulin-sensitizing effect, mediated by PPAR γ 2 activation in adipose tissue, and treatment increases the HMW form. Acarbose also increases adiponectin, although the mechanism is unknown [194]. In adipocytes, the GLP-1 agonist exenatide induces secretion of adiponectin, presumably through the protein kinase A pathway [207], and GIP, another incretin, potentially down-regulates the expression of adiponectin in adipocytes [208].

Adiponectin and diabetes

In many studies, high adiponectin levels predict, independently of other risk factors, a decreased risk of subsequent T2DM. The decreased risk is found in men and women of many ethnicities, and the relationship is dose dependent [209], and may be more pronounced in obese individuals [198]. In a meta-analysis, the risk of incident T2DM was 0.72 (0.67–9.78) per 1 Log $\mu\text{g/ml}$ increase in adiponectin [209]. Patients with T2DM and insulin resistance also have lower levels of adiponectin [194]. A recent study demonstrated that low adiponectin levels predicted incident T2DM only in insulin-resistant individuals and not in those who were insulin sensitive [210].

In a predictive model evaluating different biological paths leading to T2DM, adiponectin contributed 32.1% (16.8–49.1%) of the calculated risk after adjustments for age, sex, anthropometry, and lifestyle. In the model used, high-sensitivity CRP, gamma-glutamyltransferase, and HDL cholesterol, representing inflammation, fat accumulation in the liver, and MetS, respectively, contributed less (15.5–23.5%) [211]. This study thus supports the importance of adiponectin as a contributor to incident T2DM.

In line with HMW adiponectin being the most potent insulin sensitiser [196, 197], some epidemiological studies have argued that the

HMW-to-total-adiponectin ratio is a better predictor of metabolic disturbances [194], and better correlates with HOMA [193]. Others have found HMW adiponectin to be a better predictor than total adiponectin of incident T2DM [212]. However, the correlation between total and HMW adiponectin was very high ($r=0.89$), and an equal capability for total and HMW adiponectin to predict metabolic disturbances has also been demonstrated in a recent study [213].

The leptin–adiponectin ratio

The leptin–adiponectin ratio may better classify MetS than either of the two hormones alone, with a high sensitivity and specificity [214, 215]. The ratio also correlates better than HOMA with insulin resistance–based measurement in both diabetic and non-diabetic individuals [216, 217], and correlates better with a clamp-derived measurement than HOMA or other indexes [216]. Potentially, the non-fasting leptin–adiponectin ratio could be used in clinical practice, as the correlation with a fasting ratio is high ($r=0.95$) and widely exceeds that of non-fasting and fasting HOMA ($r=0.45$) [218]. The results are conflicting as to whether the ratio is a better predictor of incident diabetes than either of the two hormones [189, 219].

Diagnosis of diabetes

Diagnostic criteria

According to the World Health Organization (WHO) criteria from 1999, two major diagnostic options for the diagnosis of diabetes exist based on the use of plasma or whole blood glucose levels [220, 221]. The diagnosis could be based on either a value for FPG above threshold or a value for 2h glucose measurement above the threshold. Both venous and capillary whole blood as well as venous and capillary plasma could be used, all with different cut-offs. Table 1, adapted from Alberti and Zimmet [221], also shows cut-offs for IGT and IFG, both associated with an increased risk of progress to T2DM and macrovascular diseases [221, 222]. In the clinical setting, a confirmatory test is mandatory for the diagnosis of diabetes and should be undertaken on a separate day. In patients with typical symptoms of hyperglycaemia, however, no confirmatory test is needed. If an OGTT is performed for epidemiological or population screening reasons, one fasting or one 2h glucose value above the threshold is sufficient for the diagnosis of diabetes.

Table 1. Cut-off values for the diagnosis of diabetes mellitus, impaired fasting glucose (IFG), and impaired glucose tolerance (IGT).

	Whole blood		Plasma	
	Venous	Capillary	Venous	Capillary
Diabetes mellitus				
fasting glucose (mmol/L)	≥ 6.1	≥ 6.1	≥ 7.0	≥ 7.0
and/or				
2h post load glucose (mmol/L)	≥ 10.0	≥ 11.1	≥ 11.1	≥ 12.2
Impaired glucose tolerance (IGT)				
2h post load glucose (mmol/L)	≥ 6.7	≥ 7.8	≥ 7.8	≥ 8.9
Impaired fasting glucose (IFG)				
fasting glucose (mmol/L)	≥ 5.6	≥ 5.6	≥ 6.1	≥ 6.1

For the diagnosis of IGT or IFG, a glucose level below the threshold for diabetes is mandatory.

The use of glycosylated haemoglobin (HbA1c) as an alternative or additive option for the diagnosis of diabetes has been proposed. With the recent international standardisation of the HbA1c method, the arguments for the use of HbA1c as a diagnostic tool are stronger, and the American Diabetes Association (ADA) now recommends HbA1c as an additive diagnostic tool [223, 224]. The recommended cut-off for the diagnosis of diabetes is HbA1c 6.5% (corresponding to 48 mmol/mol), and HbA1c values in the range 5.7–6.4% (corresponding to 39–47 mmol/mol) should be considered as indicating an impaired glucose metabolism.

Validity of diagnosis

Not many studies on the validity of a T2DM diagnosis exist. A study in five European countries with 2556 patients with newly diagnosed diabetes in primary health care showed that the diagnosis was in accordance with WHO criteria in 82% of cases. The number that met the diagnostic criteria of WHO ranged from 90% in Spain to 69% in England, and 13% (range 6.8%–27%) of the diabetes diagnoses were false positive, with patients mostly having IGT. The numbers diagnosed by FPG or by 2h glucose were not described. A total of 25% of the newly diagnosed patients had symptoms at diagnosis [225], presumably reducing the numbers who were misclassified.

Self-reported diabetes shows a very high specificity and a concordance (kappa value around 0.8) with medical records and testing, in contrast to self-reported hypertension or hypercholesterolaemia, with kappa values around 0.55 and 0.50, respectively [226].

Glucose measurements – pre analytic considerations

Food intake and physical activity can both influence glucose measurements, an effect that is not restricted to a direct influence after a meal or a physical activity. A low carbohydrate intake in the days before an OGTT markedly increases post-prandial glucose levels [227], and the WHO recommendations state that the intake of carbohydrates should be at least 150 g per day at least 3 days before an OGTT. Physical activity should be as usual at least 3 days before an OGTT, and actual medication, inactivity, and infections should be taken into account because they all can influence the test results markedly. A continuous fall in glucose levels in an OGTT around 120 minutes after the glucose load intake argues for a collection of the 2h sample within a time span of ± 5 minutes [220].

In a whole blood sample, consumption of glucose by blood cells can markedly and rapidly reduce glucose levels, arguing for a rapid separation of plasma or rapid cooling on ice [228], and a fall in glucose levels of 0.5 mmol/l or more was observed after 2 hours at room temperature. In contrast, plasma glucose measurements are stable over at least 2 days at a temperature from +4 °C to + 37 °C. Because the haematocrit substantially influences glucose levels in a whole blood analysis, WHO recommended in 2006 the use of venous plasma glucose as a standard method for all measuring and reports [220].

Glucose measurements –analytic considerations

The glucose measuring instrument imprecision (coefficient of variation, CV) is important for the test–retest stability of analysis. A reference method for glucose analysis is proposed to have a CV not exceeding 2%, which is a precision that a modern benchtop analyser can achieve [229]. Other studies reveal, on the other hand, that 41% of clinical laboratory instruments used for glucose measurements had, from the reference methods, a significant bias leading to a potential misclassification of more than 12% of the patients [223].

HbA1c

HbA1c reflects the average glucose levels during the last three months. It has a very low day-to-day variability and a test precision at least as good as the best glucose measuring instruments and assays, and values are stable after sampling although a confirmatory test for the diagnosis of diabetes is recommended [223, 224]. Anaemia, haemoglobin abnormalities, uraemia, and pregnancy influence the result, and in pregnancy, HbA1c is not recommended as a diagnostic tool, which together with the economic cost has led WHO to not accept HbA1c as a diagnostic tool for diabetes [220].

Test-retest stability of OGTT and diagnosis of diabetes

Many studies have described the test–retest stability of glucose measurements and of the diagnosis of diabetes. Especially, the 2h glucose levels show high variability. In a Danish study of a screening population ages 40–69 years, a repeated OGTT was undertaken after 14 days. The intra-individual CVs were 7.9% and 13.8% for FPG and 2h glucose,

respectively. Repeated capillary blood glucose varied around ± 1 mmol/l and ± 3 mmol/l for FPG and 2h glucose, respectively, and a diagnosis of diabetes was confirmed in 76% of the cases [230]. Other studies have shown a day-to-day variability of FPG of around 12–15% [223].

The rationale for a diagnosis of diabetes is that it identifies a patient at risk for macro- and microvascular complications. Of interest, one study found that only 28% of a population with newly diagnosed diabetes met both the FPG and the 2h glucose criteria for diagnosis [231], and a concordance for HbA1c and FPG has been identified at 47% [232]. Accordingly, different populations will be identified with different diagnostic tools as having diabetes. The populations detected will differ by size and may also differ in their risk for complications [220, 223] and potentially in their risk factor profile, possibilities that might influence the interpretation and comparison of different studies.

Epidemiology and risk factors

Epidemiology

The word epidemiology is of Greek origin (*epi*=among, *demos*=people, *logos*=science), giving the meaning of the word: the science of diseases among people. The Greek physician Hippocrates (460–377 B.C.) was the first to describe how the environment influences the occurrence of diseases [233]. The first modern epidemiologist was Dr. John Snow, who studied the spread of cholera in London in 1848–1854 [234]. *The Dictionary of Epidemiology* defines epidemiology as: "the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to the prevention and control of health problems" [235].

Various study designs can be used in epidemiological research. In the papers presented in this thesis, we have used cross-sectional, cohort, and nested case–control methodologies. Repeated cross-sectional surveys using the same methodology in an independently chosen random sample can yield not only the prevalences of risk factors and diseases but also the time trends in the population being described. Cross-sectional studies can also be used as a relatively cheap method of searching for associations and generating hypotheses. The nested case–control study is a method of making a cohort study less expensive. Both cases and controls are chosen from an already existing defined cohort, for which there is some existing information on exposure and risk factors. The cohort and nested case–control methodologies are superior to the cross-sectional case–control study in the evaluation of causality [236].

Risk factor

The definition of risk factor varies. In 2006, WHO gave the following description: "A risk factor refers to an aspect of personal habits or an environmental exposure, that is associated with an increased probability of occurrence of a disease" [237]. *The Dictionary of Epidemiology* defines a risk factor as "an aspect of personal behaviour or life style, an environmental

exposure, or an inborn or inherited characteristic, that, on the basis of epidemiological evidence, is known to be associated with health-related condition(s) considered important to prevent" [238]. Nothing in the definitions mentions a direct causality or that a risk factor, on its own, is sufficient to cause a disease. Others argue that the term "risk factor" should be replaced by three narrower and distinct terms—risk marker, risk factor, and cause—to avoid premature claims of intervention or treatment. The use of the term "risk factor" should be restricted to the situation in which modifying the risk factor in an experiment demonstrates an influence on the prevalence and/or incidence of a disease. Without such knowledge, the term "risk marker" should be used. Only a factor essential for the occurrence of a disease should be called a "cause" [239].

Hill, in a 1965 paper [234] presented criteria for the probability of a true clinical causality of an identified risk factor. The criteria were as follows:

- 1) *Strength*: The stronger the association is, the more plausible is a causality.
- 2) *Consistency*: With more studies, in different settings and with a different design giving the same results, the possibility of causality will be strengthened.
- 3) *Specificity*: The more specifically individuals with a risk factor are affected, and those without the risk factor are not, the stronger is the possibility of causality. Hill here warns, however, that this criterion has to be interpreted cautiously in common diseases.
- 4) *Temporality*: Exposure to a risk factor must precede the disease.
- 5) *Biological gradient*: A dose-response gradient strengthens the probability of causality.
- 6) *Plausibility*: Important, though at the same time to quote Hill: "What is biologically plausible depends upon the biological knowledge of the day".
- 7) *Coherence*: Are our findings in coherence with what is taking place in society and is seen in other fields of science?
- 8) *Experiments*: Can removal of a risk factor reduce or even prevent disease? Hill considered this to be most supportive of causality of all presented criteria.
- 9) *Analogy*: Can sometimes be used. If we know that a chemical compound causes a disease, then if another similar compound is suspected to cause the same disease, the analogy criterion could be used.

Aims of this dissertation

The general aims of this dissertation are as follows:

- To describe prevalences and time trends in general obesity and abdominal obesity in northern Sweden using objective measurements and not self reports.
- To describe prevalences and time trends in fasting and 2h post-load glucose, and in disturbances in glucose metabolism, including diabetes in northern Sweden.
- To investigate leptin, adiponectin and their ratio as predictors of future type 2 diabetes (T2DM), and to evaluate potential sex differences in the associations.
- To test the hypothesis that trends in fasting and 2h glucose in the population relate to circulating levels of leptin and to evaluate potential sex differences in the associations.
- To evaluate potential ethnic differences in circulating levels of leptin, after adjustment for anthropometric measures of obesity.
- To evaluate the intra-individual and circ-annual stability of leptin in humans.

Materials and methods

Västerbotten and Norrbotten counties in Sweden

The two counties Västerbotten and Norrbotten in northern Sweden are similar in size, each with a population of 250,000 inhabitants and, in ages below 65 years, with slightly fewer women than men. A total of 4.4% of all inhabitants in Västerbotten and 3.1% in Norrbotten in 2009 were born outside Europe [240]. The total population has been fairly stable over the survey years 1985–2009 [241]. Life expectancy (2006–2010) was in Västerbotten equal to that for Sweden as a whole at 79.1 years in men and 83.2 years in women. In Norrbotten, corresponding figures were 78.2 years for men and 82.7 years for women. The median income in the two counties in 2009 was the same as that for the country [240].

Mauritius

Mauritius is a multi-ethnic subtropical island in the Indian Ocean with approximately 1.3 million inhabitants in 2011, and 7.5% of the population is 65 years or older. Life expectancy in 2011 was 71.0 years in men and 78.1 years in women, and the majority (68%) of the population is of Asian Indian origin. Another 27% are Creole (African background), 3% are Chinese, and 2% are Caucasians. Gross domestic product per capita was estimated in 2010 at \$14,000 compared to \$39,100 in Sweden [242].

Study populations

The Northern Sweden MONICA

The MONICA surveys were started by WHO in the early 1980s to follow trends in cardiovascular risk factors globally. Altogether, 41 study centres in 26 countries in four continents joined the MONICA project. Two of these centres were Swedish—in Gothenburg and in northern Sweden—with the two northernmost counties of Norrbotten and Västerbotten participating [243]. The worldwide MONICA study ended after 10 years [244, 245], but the survey is ongoing in northern Sweden and is planned to continue at the time of this writing.

The survey procedure in the Northern Sweden MONICA (designated here as MONICA) has been published previously [246]. In brief, cross-sectional population-based surveys were performed in 1986, 1990, 1994, 1999, 2004, and 2009. For each survey, an independently chosen sample of 250 men and 250 women of different age groups (25–34, 35–44, 45–54, and 55–64 years) was invited. From 1994 onwards, the age group 65–74 years was also included. The participation rate has declined somewhat over the survey years, from 81.2% in 1986 to 69.2% in 2009. Non-participants are younger, report a lower education level, and are less often married/cohabitating and more frequently smoke and have diabetes [22, 247].

In 1999, a reinvestigation was performed, to which all men and women chosen for the previous surveys were invited for a follow-up using the same methodology. The mean participation rate of the three first

surveys was 79%, and 3772 of the 5129 initial participants were reinvestigated.

All MONICA surveys have been performed from January to April. At each survey, participants were asked to complete a questionnaire covering medical and social history, lifestyle, psychosocial situation, and food consumption frequency. Blood samples were obtained and BP and anthropometric measurements taken. Blood samples were stored at the Medical Biobank at Umeå University Hospital for future research [248]. A standard OGGT was performed after an overnight fast in a random subset of 65% of the non-diabetic participants, except in 1999 because of logistical reasons [246], as all participants invited to previous surveys were reinvestigated that year. Country of birth was registered, but not ethnicity.

Västerbotten Intervention Programme (VIP)

Since 1985, VIP has been an ongoing community intervention program, targeting cardiovascular and diabetes prevention in Västerbotten county. The methodology has been presented previously [248, 249]. In short, all inhabitants in the county are invited to a health survey at their nearest health centre the year they turn 40, 50, or 60 years old. Initially, inhabitants turning 30 years old were also invited. The survey design is similar to that of the Northern Sweden MONICA with blood sampling, stored blood samples in the Medical Biobank, an OGTT in all non-diabetic participants, and anthropometry and BP measurements [249]. On average, 86% of the participants have donated blood to the Medical Biobank [250]. Since 1992, the whole county has been covered by the VIP surveys, and the participation rate has varied between 48–67%, with higher figures in recent years. By 2007, 110,000 health surveys had been performed in 85,000 unique individuals [249], and the surveys run throughout the year except for July. An analysis in 1998 showed only a small social selection bias between attendees and non-attendees, although a tendency towards younger age, lower income, and more prevalent unemployment in non-participants was seen [251]. Country of birth was registered, but not ethnicity.

Mauritius Non-communicable Disease Study

With increasing industrialisation in the post-World War II era, an increasing prevalence of non-contagious diseases as a cause of mortality was seen in Mauritius, which led the government to initiate an intervention program in the mid-1980s. A baseline population survey was performed in 1987, and details of this study have been described earlier [1]. In 1987, ten population clusters were randomly chosen in the country, with a probability proportional to size. An area in China Town in the capital, Port Louis, was also purposely selected. This area was excluded from analysis in paper II because of the small numbers of participants. All eligible adults, ages 25–74 years, were invited, and 5083 persons participated, giving a response rate of 86%. In 1992, a total of 2480 of the initial participants entered a follow-up survey that used the same methodology.

The surveys were performed in April and May with a similar methodology as in MONICA and VIP, with questionnaires, anthropometry,

and OGTT and blood sampling after an overnight fast. Ethnicity was registered, however, based on self reports [1, 57].

The diabetes register in Västerbotten (DiabNorr)

Computerised medical records have enabled registration of all patients in Västerbotten county with a diagnosis of diabetes. Through linking inpatient and outpatient registers to the VIP register in 2002, a total of 1923 diabetes patients were identified who had previously participated in VIP, and 68% of them consented to participate in DiabNorr, which together with 5% deceased patients resulted in 1413 eligible diabetes patients. Research nurses visited all health institutions in the county to collect data from the medical records. Each case was reviewed by a specialist in internal medicine and classified according to the 1999 WHO recommendations [220]. The study population and the validation processes for the diagnosis up to 2002 have been described previously [252]. In 2007, the same procedure was repeated, but now the VIP register was also linked to the pharmaceutical registry at the National Board of Health and Welfare to identify patients on anti-diabetic treatment. Altogether, 1501 eligible diabetes patients were identified for the study in paper III.

Study design

Paper I

The aim was to describe prevalence's of obesity and abdominal obesity based on both NCEP III and IDF definitions, as well as the time trends.

In this study, we used anthropometric measures from the first five cross-sectional surveys performed between 1986 and 2004 within the MONICA study. Altogether 8857 participants were included, and the overall participation rate was 77.0%.

Paper II

The aim was to explore if circulating levels of leptin, both crude and adjusted, differed between ethnicities, and to analyse “test–retest stability” and circ-annual variation of leptin.

Participants from the first three cross-sectional surveys in MONICA and the first cross sectional survey in Mauritius were re-examined after 5-13 years and 5 years respectively, giving cohorts of 5993 individuals of different ethnicity and follow-up time. All pregnant women were excluded from analysis. A subset of participants from the Lycksele area, who were examined between 1988 and 1992 in the VIP survey, was used to test the potential circ-annual stability of leptin. The number of participants was 1780 men and women, and the participation rate was 74%. Lycksele is a small Swedish town in the Västerbotten county, located 200 km south of the polar circle, and the daylight length varies between 4 and 21 hours over the year.

In Mauritius in 1987, WC was measured at the minimum circumference between the xiphisternum and umbilicus, and in 1992, the same measuring site as in MONICA was used, at the mid-point between the

iliac crest and the lower margin of the ribs. As a consequence, mean WC measurements from 1987 were 1.5 cm lower in men and 2.7 cm lower in women [37]. This situation was just recently recognised [57], and a new analysis with adjusted WC was performed on data published in paper II (see results).

Paper III

The aim of this study was to evaluate leptin, adiponectin, and their ratio as predictors of incident T2DM, and whether participant sex, and insulin-resistance status influenced these associations.

For the establishment of a nested case-control study, participants with T2DM from the diabetes register were matched with other participants within the VIP survey without any known diabetes. For each patient, controls without known diabetes were chosen, and they were group matched for age group, sex, examination date and area of residence. For the latter, there were three groups, one for each hospital area (Umeå, Lycksele and Skellefteå) with surrounding communities. Altogether 1501 patients with diabetes and 1564 controls were identified.

Year of diagnosis of T2DM was known, and June 30 was set as the day of diagnosis. Patients diagnosed <1 year after their survey in VIP were not included in the analysis. A total of 371 men and 269 women with T2DM, mean duration 6.4 years (range 1.0–15.5 years) remained for the analysis

Paper IV

The aim of this study was to describe time trends in FPG and 2h glucose levels, and frequencies of IFG, IGT, previously known and screening detected diabetes in the population in northern Sweden between 1986 and 2009. A second aim was to evaluate if changes in fasting and 2h glucose were uniformly distributed along the entire glucose distribution or not, and a third aim was to evaluate leptin and traditional risk factors as predictors of trends in FPG and 2h glucose.

This study was entirely based on the MONICA survey, and we used OGTT data from four surveys between 1990 and 2009, and information about previously known diabetes from all six surveys between 1986 and 2009. For the OGTT studies, a random subsample of 65% of all participants was invited. Only four surveys were included as no OGTTs were performed in 1999 for logistic reasons, and information about the sampling frame in 1986 for the OGTT was missing; thus, some individuals not primarily randomised to the OGTT group may have been included in the OGTT group. As a consequence, OGTT data from 1986 were omitted. In total 10 586 individuals (participation rate 75.6%) participated in surveys 1986–2009. In the surveys 1990, 1994, 2004 and 2009 altogether 7138 individuals participated (participation rate 75.1%), and an OGTT was performed in 3582 individuals.

Anthropometry

In the MONICA, VIP, and Mauritius surveys, weight was measured in light clothes without shoes, in MONICA to the nearest 0.2 kg and in VIP to the nearest 0.5 kg. Height was measured to the nearest centimetre, and BMI (kg/m^2) calculated [1, 246, 253]. Hip circumference was measured at the maximum circumference. WC was measured midway between the iliac crest and the rib margin in MONICA and in VIP, although in VIP, WC was introduced into the survey protocol in 2003 [246, 249]. In Mauritius in 1987, WC was measured at the minimum circumference between the xiphisternum and umbilicus and in 1992 at the mid-point between the iliac crest and the lower margin of the ribs, and the measurements from 1987 were adjusted by adding 1.5 cm in men and 2.7 cm in women to the original measurement [37].

Blood pressure

BP was measured twice after 5 minutes in a sitting position with a Hawksley random zero sphygmomanometer (and from 2009 with the OMRON M7 monitor in half the sample), and mean BP was registered in MONICA [22, 246]. In VIP, BP was measured once with a mercury sphygmomanometer after 5 minutes in a sitting position [249], although some initial participants had their BP measured in a recumbent position. For these latter, BP was adjusted to values reflecting a sitting position in accordance with double BP measurements in 1850 participants [254].

Questionnaires and blood sampling

Questionnaires were administered covering various lifestyle questions including physical activity and food intake, medication, medical and social history, level of education, and heredity. Smoking habits, and for MONICA and VIP the use of the moist snuff "snus", were self reported. For further analysis, participants were dichotomised into daily smokers vs. non-daily smokers, and daily snus users vs. non-daily snus users.

In VIP and MONICA, prevalence of previously known diabetes was based on self-reports. Most non-diabetic participants in VIP and a random subset of 65% in MONICA were invited to a standard 75 g OGTT (performed in 50.8%), and the diagnosis of survey-detected diabetes was set in accordance with WHO definitions [1, 22, 220, 246, 249].

Blood samples were drawn after an overnight fast (100% for Mauritius and 80% for MONICA and VIP), or a minimum of 4-hours fast (20% in MONICA and VIP) and analysed within 24 hours without freezing. Most participants donated blood to the biobank for future research [1, 22,248], and samples for leptin and adiponectin were analysed in plasma and serum stored at $-70\text{ }^{\circ}\text{C}$.

Laboratory procedures

Leptin and adiponectin

In both Umeå and Melbourne, the same method was used for the analysis of leptin in stored samples from MONICA and VIP and from

Mauritius, respectively. A double-antibody radioimmunoassay (RIA) (Millipore, Billerica, MA, USA) was used for leptin and for adiponectin. Total CV for leptin was 4.7% at both low (2–4 ng/ml) and high (10–15 ng/ml) levels. For adiponectin, the total CV was 15.2% at low (2–4 µg/ml) and 8.8% at high (26–54 µg/ml) levels.

Glucose, insulin, and C-peptide

In MONICA, venous plasma was used for glucose analysis, and until 1999, the hexokinase method (Boehringer Mannheim Automated Analysis for BM/Hitachi system 717, Mannheim Germany) was used in a single laboratory. For the surveys of 2004 and 2009 a Hemocue benchtop analyser (Quest Diagnostics, Madison, NJ, USA) was used. In 2004 a parallel analysis was undertaken using both methods, and based on 195 OGTT samples, a linear regression model was developed and the measurements from 2004 and 2009 were adjusted accordingly. From 1985–2004 in VIP, a Reflotron benchtop analyser (Roche Diagnostics, Basel, Switzerland) was used; thereafter, a Hemocue bench top analyser was used (Quest Diagnostics, Madison, NJ, USA).

Fasting serum insulin and C-peptide levels were analysed with Roche Elecsys assays on a Modular E170 analyser (both Roche Diagnostics, Basel, Switzerland). Total CV for insulin was <5% at 20 mIU/l and <4% at 100 mIU/l. Total CV for C-peptide was <6% at both 0.6 nmol/l and 3.2 nmol/l. HOMA2 models were calculated using an online calculator (<http://www.dtu.ox.ac.uk/homacalculator/index.php>).

Lipids

In MONICA, total cholesterol was analysed with an enzymatic method (BM Monotest Cholesterol CHOD-PAP; Boehringer Mannheim GmbH, Mannheim, Germany) between 1986 and 1994. From 1999, a dry chemistry method was used (Vitros 950; Kodak Ektachem, Rochester, NY, USA). The measurements have a CV of 3.6% at 3.9 mmol/l and 3.1% at 6.7 mmol/l and were accredited by the Swedish national accreditation body (EQUALIS). In VIP, total cholesterol and triglycerides were analysed on a Reflotron benchtop analyser (Roche Diagnostics, Basel, Switzerland). In individuals with increased cardiometabolic risk, further analysis on lipid fractions was made at the nearest hospital, but these data were omitted because of small numbers.

Statistics

All calculations were made on SPSS (Chicago, IL, USA) version 11.0.4 to version 18.

Paper I

Univariate analysis of variance and analysis of covariance were used to explore data over different survey years, and data were analysed separately for men and women, for separate age groups (with a span of 10 years), as well as for ages 25–64 years as one group. In the post hoc analysis, a Bonferroni method was chosen to adjust for multiple comparisons.

Prevalence estimates were standardised using the direct method against the population in Norrbotten and Västerbotten in 2004. All pregnant women were excluded.

Paper II

Because of a skewed distribution of leptin, all analyses were performed both using non-transformed leptin and logarithmically transformed (\log_e) leptin. Univariate analysis of variance and analysis of covariance were used to explore data over different survey years and ethnic groups and for the analysis of potential variability of leptin over months and seasons. A conservative method (Bonferroni) was chosen in the post hoc analysis to account for multiple comparisons. To evaluate the independence of ethnicity in relation to known determinants of leptin, a stepwise sex-stratified linear multivariable regression was performed, using dummy variables with Caucasian ethnicity as the reference. An ICC, two-way mixed-type consistency, single measurements, was used for the analysis of the test–retest stability of leptin. All pregnant women were excluded, and the Chinese group in Mauritius was excluded because of small numbers.

Paper III

Student's unpaired *t*-tests were used to test for differences in means between cases and referents. The sex-specific distribution of HOMA2–IR among referents was used to define insulin sensitivity status, dichotomised into two groups with a cut-off between quartiles three and four. Values below cut-off were defined as insulin sensitive and above cut-off as insulin resistant.

The sex-stratified distribution of continuous variables in referents was used to categorise quartiles. The influence of studied variables on incident T2DM, stratified for sex and insulin sensitivity status, was tested by univariate and multivariable conditional logistic regression analysis. The distribution of cases and controls over quartiles was tested with a chi-squared test for linear trend. The association between logarithmically transformed (\log_e) values of leptin, adiponectin, and BMI was explored with a BMI-adjusted, sex-stratified partial correlation in referents, using a two-tailed test.

A synergy index (SI) [255] was used to analyse the potential interaction of adiponectin and leptin on incident T2DM, and $SI > 1.0$ was considered indicative of synergy and < 1.0 as indicative of antagonism. The effect of leptin, adiponectin, and their ratio (dichotomised into high and low values based on cases only) on time to diagnosis was tested using Kaplan–Meier analysis (log-rank test).

Paper IV

Because of its skewed distribution, leptin was logarithmically transformed (\log_e) prior to analysis. Univariate analysis of variance and analysis of covariance were used to explore data over different survey years. For the analysis of linear trends, the Mantel–Henszel χ^2 test was used for proportions, and linear regression was applied for continuous variables. A linear regression model was used to analyse the interaction of survey year on

FPG and 2h glucose and for the analysis of annual change in glucose levels and the prevalences of IFG and IGT. A sex- and survey-specific percentile analysis was performed to form equally sized deciles for FPG and 2h glucose. A Tukey mean-difference plot was constructed to demonstrate changes in FPG and 2h glucose between the survey years 1990 vs. 2009. In the plot, the difference in mean glucose for corresponding deciles was plotted against the total mean of the combined deciles. A linear trend analysis was performed, separately for each decile, over the four surveys 1990–2009.

Ethical considerations

The Ethics Committee at Umeå University approved all studies included in this thesis linked to the MONICA and VIP surveys, and the Mauritius surveys were approved by the Alfred Healthcare Group Ethics Committee (Melbourne, Australia) as well as by the Ministry of Health in Mauritius. An informed consent was obtained from all participants in all surveys.

Results and discussion

Paper I

Results

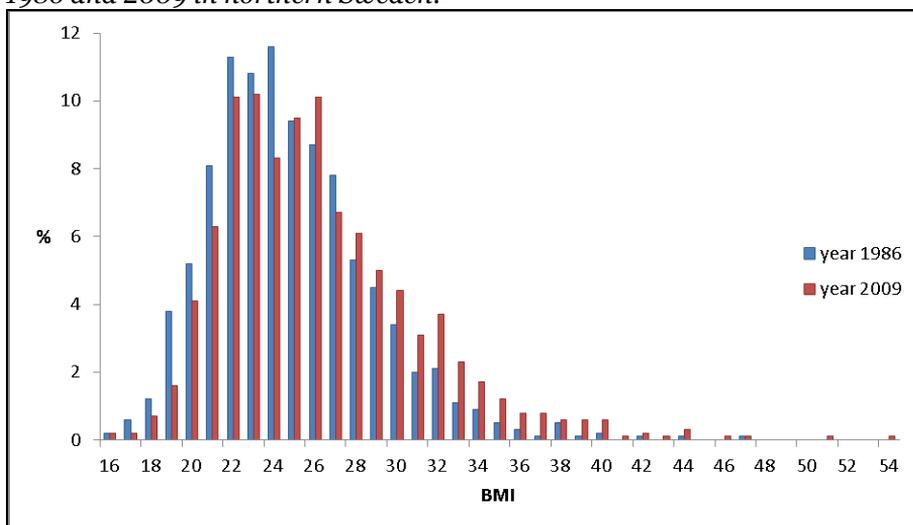
Between 1986 and 2004, increasing weight and BMI were seen in men of all ages, and in women in the younger age groups. For ages <65 years, the annual increase in BMI was 0.1 kg/m², with the most pronounced increase in young men and women. The prevalence of overweight and obesity increased in men in most age groups while an increase in women was seen only for ages <45 years. Again, the most pronounced increase was seen in the youngest age groups. As an example, obesity and overweight were as prevalent in men ages 25–34 years in 2004 as in men 20 years older in 1986. The age-standardised prevalence of obesity for ages 25–64 years rose from 10.4% to 19.1% in men and from 12.9% to 17.9% in women between 1986 and 2004.

WC decreased between 1986 and 1990 in all women, about 6 cm in younger groups. A decrease was also seen in men ages 45–54 years. After 1990, WC increased again, most markedly in women. Between 1986 and 1999, hip circumference increased but thereafter decreased, leading to an initial decrease in WHR followed by an increase. The most pronounced increase in the prevalence of abdominal obesity (NCEP definition) between 1986 and 2009 was seen in men ages 25–34 years, from 3.4% to 15.8%. In women abdominal obesity did not increase over all surveys, but did so between 1990 and 2004, and the prevalence increased (NCEP) with 24.6% points in women 45–64 years old.

Discussion

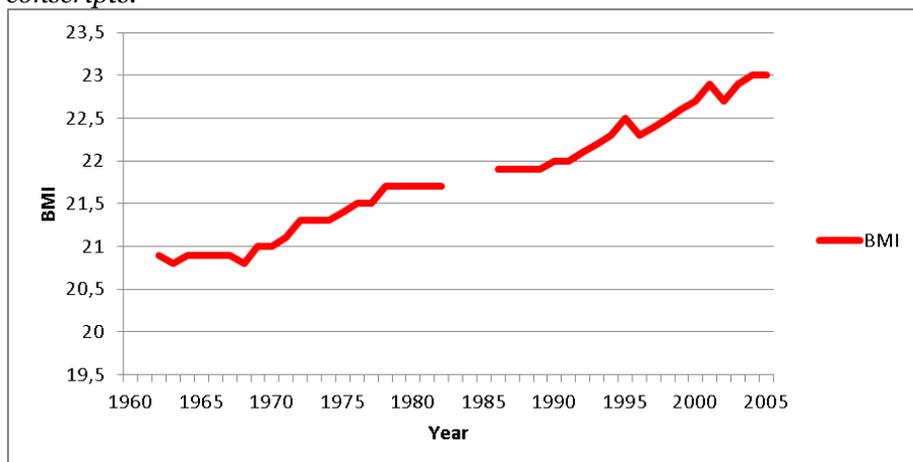
The most striking finding is the increasing general and abdominal obesity, in particular in the youngest ages, a finding that raises concern for future increases in obesity related diseases. The prevalence of obesity we obtained by objective measurement exceeded self-reported obesity prevalence 1999–2000 [19] by approximately 5% points, which is in line with what has been described previously [11, 12]. The increasing obesity was caused by a rightward shift in the distribution curve of BMI, and not by an increased skewness, as indicated in Figure 1, where we present an updated analysis including also the 2009 survey, in men and women ages 25–64 years. Separate analyses for men and women yielded similar results and are not shown.

Figure 1. Distribution of BMI (kg/m²) in men and women 25-64 years old 1986 and 2009 in northern Sweden.



The trend of increasing obesity was not restricted to the period after 1986. Up to 2007, almost all Swedish young men had to be conscripted, and Figure 2 shows the national Swedish trend, between 1962 and 2005 in BMI in 18-year-old male conscripts [240]. Data from 50-year-old men in Gothenburg show a similar long-lasting increase in mean BMI, from 24.8 in 1963 to 26.4 in 2003 [20].

Figure 2. Mean BMI (kg/m²) 1962–2005 in 18-year-old Swedish male conscripts.



The changes in abdominal obesity are of particular interest, partly because of its association with subsequent T2DM and CVD [29, 30, 31, 32], but also because of the marked decrease in WC seen in women between

1986 and 1990. Potentially, the decrease in WC could be attributed to health-promoting activities with a combined individual and community approach in the survey areas in the mid- and late-1980s, such as the initiation of VIP in the Norsjö community [256]. After 1990 up to 2004, WC and the prevalence of abdominal obesity rose, in particular among women. Between 1999 and 2004, the prevalence of abdominal obesity (NCEP) rose more rapidly in Swedish women ages 45–64 years than among women of the same age in the USA [257]. Between 2004 and 2009, BMI [22] and hip circumference had flattened, and WC decreased in both men ($p=0.002$) and women ($p=0.001$) 25–64 years old; see Table 2 (adapted from paper IV). When analysed separately, each age group in men and women showed an insignificant decrease in WC between 2004 and 2009 (data not shown). Between 1986 and 2009, WC was unchanged in women but increased significantly in men ($p=0.001$). These results support findings from VIP, some European countries, and women in the USA that the obesity epidemic may be slowing [23, 25, 26, 27, 28].

Table 2. Mean BMI (kg/m²), waist and hip circumference (cm), and leptin (ng/ml) levels for men and women ages 25–64 years in northern Sweden 1986–2009.

	1986	1990	1994	1999	2004	2009
Men						
N	821	769	730	663	705	650
BMI (kg/m ²)	25.6 (3.5)	25.9 (3.3)	26.2 (3.7)	26.6 (3.5)	27.1 (4.0)	27.0 (4.1)
waist (cm)	93.1 (9.5)	91.5 (9.2)	92.9 (10.2)	95.0 (9.8)	96.3 (11.1)	94.5 (11.6)
hip (cm)	98.0 (6.1)	97.9 (6.0)	99.3 (6.7)	103.2 (6.6)	100.7 (6.9)	100.9 (7.1)
leptin*(ng/ml)	3.6 (1.9)	3.6 (1.9)	4.0 (1.9)	5.3 (2.0)	5.0 (2.2)	4.4 (2.1)
Women						
N	793	799	752	713	761	673
BMI (kg/m ²)	25.0 (4.4)	25.0 (4.4)	25.3 (4.4)	25.9 (4.5)	26.2 (5.0)	26.3 (5.4)
waist (cm)	85.3 (12.3)	79.4 (11.0)	82.9 (12.4)	83.8 (12.0)	85.5 (12.7)	83.4 (13.3)
hip (cm)	98.6 (8.7)	97.8 (9.0)	100.0 (8.8)	103.0 (8.5)	100.7 (9.2)	101.4 (10.3)
leptin*(ng/ml)	10.7 (1.9)	10.2 (1.8)	11.0 (1.8)	15.5 (1.9)	14.6 (2.0)	11.4 (2.1)

Values given are numbers and means, for leptin geometric mean =* and (SD)

waist = waist circumference, hip = hip circumference

Paper II

Results

Asian Indian men and women had higher values for leptin, leptin/waist, and leptin/BMI than both Creole and Caucasian men and women. This finding remained stable after adjustments for WC or BMI. Because WC was measured differently in Mauritius in 1987 and 1992 [57], measurements from 1987 were adjusted according to previous studies [37]. A new analysis with adjusted WC was performed, and the ethnic differences between Asian Indians and other ethnicities in leptin levels, adjusted for obesity measurements, remained unchanged. Creole men, in the new analysis, had higher leptin, leptin/waist, and leptin/BMI values than Caucasian men. Creole women, in comparison with Caucasian women, still had significantly higher leptin/waist values (when adjusted for smoking, age, and WC) than Caucasian women, but leptin/BMI and leptin no longer remained significantly different with the same adjustments. See Table 3 (corresponding to Table 2 in paper II) analysed with adjusted WC measurements.

A new linear regression analysis was also performed on explanatory variables for the variance in logarithmically transformed leptin. With this new analysis, the fourth paragraph in the results for paper II should read as follows: "A model including age, BMI, WC, and smoking status explained 42.0 % and 51.4% of the variance of logarithmically transformed leptin in men and women, respectively. Adding ethnicity could explain an additional 10.3% ($p < 0.0005$) in men and 1.1% ($p < 0.0005$) in women. With a stepwise method, an Asian Indian ($\beta = 0.576$, $p < 0.001$) and a Creole ($\beta = 0.407$, $p < 0.001$) background together with BMI ($\beta = 0.048$, $p < 0.001$), age ($\beta = 0.004$, $p < 0.001$), and WC ($\beta = 0.032$, $p < 0.001$) remained associated with logarithmically transformed leptin in men; and in women, an Asian Indian background ($\beta = 0.187$, $p < 0.001$) and BMI ($\beta = 0.078$, $p < 0.001$), WC ($\beta = 0.012$, $p < 0.001$), and smoking regularly ($\beta = -0.099$, $p < 0.001$) were independently associated with logarithmically transformed leptin levels".

ICC for leptin was high, at 0.82 for logarithmically transformed leptin for all cases, and there was no difference depending on sex, follow-up time (5–13 years), or ethnicity. No circ-annual variation in leptin levels was seen.

Table 3. Leptin levels and ethnicity, corresponding to Table 2 in paper II, but here analysed with adjusted waist circumference measurements.

	<i>Asian Indian</i>	<i>Creole</i>	<i>Europid</i>	P, post hoc
<i>Men</i>				
N	822	251	1712	
Leptin (ngml ⁻¹) ^a	5.79 (0.97)	4.88 (0.17)	3.94 (0.07)	***abc
Leptin (ngml ⁻¹) ^b	6.50 (0.10)	5.92 (0.17)	3.44 (0.07)	***bc**a
Leptin/BMI ^a	0.22 (0.004)	0.19 (0.006)	0.15 (0.003)	***abc
Leptin/BMI ^b	0.25 (0.004)	0.22 (0.006)	0.14 (0.003)	***abc
Leptin/waist ^a	0.066 (0.001)	0.056 (0.002)	0.042 (0.001)	***abc
Leptin/waist ^b	0.072 (0.001)	0.065 (0.002)	0.038 (0.001)	***bc**a
<i>Women</i>				
N	988	348	1787	
Leptin (ngml ⁻¹) ^a	15.79 (0.24)	12.32 (0.38)	12.63 (0.17)	***ab
Leptin (ngml ⁻¹) ^b	16.78 (0.25)	13.00 (0.40)	11.95 (0.18)	**ab
Leptin/BMI ^a	0.60 (0.01)	0.47 (0.01)	0.48 (0.01)	**ab
Leptin/BMI ^b	0.62 (0.01)	0.49 (0.01)	0.47 (0.01)	**ab
Leptin/waist ^a	0.192 (0.003)	0.149 (0.004)	0.148 (0.002)	**ab
Leptin/waist ^b	0.200 (0.003)	0.156 (0.005)	0.143 (0.002)	***ab*c

Numbers and means (s.e.) are shown for leptin (ngml⁻¹), leptin (ngml⁻¹) per BMI unit (kg m⁻²) (leptin/BMI) and leptin (ngml⁻¹) per cm in waist circumference (leptin/waist).

Pregnant women were excluded. * = p<0.05, ** = p<0.005, *** =p<0.0005. Post Hoc:

a, Asian Indians-Creoles; b, Asian Indians-Europids; c, Creoles-Europids.

^aData are adjusted for age, smoking status and BMI.

^bData are adjusted for age, smoking status and waist circumference.

Discussion

The main finding in this study was that Asian Indians have, compared to Caucasians and Creoles, higher values for leptin, leptin/waist, and leptin/BMI. This finding supports what previously has been demonstrated in smaller studies comparing Asian Indians and Caucasians [71, 74, 75, 136], though studies comparing Asian Indians with people of African ethnicity are lacking. Creole men had higher leptin levels, adjusted

for obesity measurements, than Caucasian men, and Creole women had higher leptin/waist values, adjusted for WC than Caucasian women. This is in line with what has been demonstrated comparing African-Americans or South Africans with Caucasians [128, 129, 130]. Because leptin has been linked to both CVD [164] and diabetes [185, 186], diseases to which Asian Indians have shown an increased susceptibility [66], this finding in a large study is of interest as one potential explanatory factor.

Only a few small studies have looked at the intra-individual and circ-annual stability of leptin [141, 142, 143, 144], which is of interest regarding the potential predictive value of leptin. The intra-individual stability of leptin is equal to that of plasma cholesterol (ICC 0.6–0.8) [258], which is considered a stable biomarker. No circ-annual variation in leptin levels was seen. The material was large but not longitudinal, but the results are supported by two small longitudinal studies [141, 142].

Paper III

Results

In both men and women, leptin and the leptin–adiponectin ratio predicted an increased risk of T2DM and adiponectin a decreased risk in the univariate analysis. In all adjustment models tested, the predictive capacity for the leptin–adiponectin ratio and for adiponectin remained in men and women. In men, high leptin remained an independent predictor of T2DM in all tested models, but not in women once BMI was introduced as a covariate.

After stratification for insulin sensitivity and adjustment for BMI, leptin remained associated with incident T2DM only in insulin-sensitive men. Adiponectin and the leptin–adiponectin ratio remained associated with subsequent T2DM in both insulin-sensitive and insulin-resistant individuals.

To test for a significant interaction between leptin and adiponectin, SI was calculated. The unadjusted SI was 1.24 (0.88–1.74) in men and 1.45 (0.98–2.15) in women, but with adjustment for BMI, the SI was attenuated to ≈ 1.0 . The median time from survey to diagnosis of T2DM was shorter in men for participants with a high leptin–adiponectin ratio compared to a low ratio, but this pattern was not seen in women or for the separate hormones.

Discussion

This study is the first to demonstrate a different risk associated with leptin in insulin-sensitive and insulin-resistant men. A recent study has demonstrated that low adiponectin levels predict T2DM only in insulin-resistant individuals [210]. Taken together, these two studies could indicate an incompletely understood interaction of adipokines and insulin resistance status in the progress to T2DM. The divergence regarding leptin as a potential risk factor for T2DM [185, 186, 189, 190], could possibly be explained by differing insulin sensitivity, but also by over adjustment [190].

For adiponectin, the findings in this study are in line with previous research [209], while for the leptin–adiponectin ratio, there was a strong association with incident T2DM, and previous studies are inconsistent [189, 219]. Men with a high leptin–adiponectin ratio had a significantly shorter time to diagnosis; this is a new finding that supports the concept of additional predictive information with the ratio compared to the separate hormones.

Paper IV

Results

Between 1990 and 2009, FPG and 2h glucose increased in women aged 25 to 64 years, and 2h glucose in men aged 25 to 64 years. The increase was seen in most deciles, although it was more pronounced in the upper deciles. The prevalence of IGT almost doubled in women and tripled in men, from 7.8% to 14.5% and from 3.5% to 10.1%, respectively. The prevalence of IFG rose in women, from 4.5% to 7.7%, but was unchanged in men. The prevalences of previously known diabetes and screening-detected diabetes and the total prevalence of diabetes were stable over the period studied. In 2009, the total prevalence of diabetes was 6.4% in men and 6.5% in women.

Leptin in men but not in women could independently explain a minor part of the variance in FPG and 2h glucose, with an explanatory capacity being equal to that of traditional risk factors.

Discussion

The prevalence of diabetes did not increase during the period studied, but the increase in both FPG and 2h glucose together with the pronounced increase in IFG and IGT indicates that northern Sweden can anticipate an increase in T2DM. The VIP study, with a much larger study population, indicated that an increased prevalence already is at hand [64]. The prevalence of IFG was higher in men and that of IGT was higher in women, which is typically seen [1, 259], but the relative increases of IFG and IGT were higher in women and in men, respectively, in this study. Because IFG relates more to a decline in basal insulin secretion and in insulin sensitivity in the liver, and IGT relates more to muscular insulin sensitivity [187], the trends seen could reflect a changing patho-physiological trend in men and women.

The finding that leptin partly could explain the variance of FPG and 2h glucose in men, has to our knowledge, not previously been reported. The findings support studies demonstrating that leptin independently predicts T2DM in men but not in women [185, 186], as it also did in paper III.

Methodological considerations

This thesis is based on three large ongoing population surveys with an overall high participation rate and without signs of a major selection bias [1, 251, 252]. The long-lasting repeated surveys using the same

methodology are a strength but also are linked to methodological problems. New methods and analysing equipment need to be introduced, which might require adjusted measurements, i.e., the glucose measurements in MONICA.

The validated diagnosis of T2DM in DiabNorr is a strength. As most diabetes patients are detected in routine care, this will most likely underreport the true incidence and thus reduced the statistical power in paper III.

A large majority of the population was born in Sweden or Finland and presumably Caucasian, but ethnicity was not registered in MONICA or in VIP, only country of birth. In paper II a misclassified ethnicity would, most likely reduce the ability to detect ethnic differences.

Leptin was analysed in two laboratories (Melbourne and Umeå), but the same assay was used on stored samples at both sites, and we do not think that this would have influenced the analysis, as the gender differences and associations with BMI were identical.

General discussion

This thesis describes an increasing prevalence of obesity in men and women 25-64 years old in northern Sweden between 1986 and 2009. In the survey counties, Norrbotten and Västerbotten, a more pronounced increase in obesity was seen in younger age groups, which raises concern for the future. A decreasing WC, and a BMI that is flattening off between the two most recent surveys, 2004 and 2009, indicates the possibility of a slow down in the epidemic of obesity. Such a slow down has been described in childhood obesity in some countries, including Sweden [5], in adults in Västerbotten county in northern Sweden [23], and also internationally [25, 26, 27, 28]. Although this is a somewhat hopeful message, obesity still is a major cause of disease and death, and the actual prevalence of obesity by far exceed that seen one or two generations back [2].

We could not demonstrate any increase in the prevalence of diabetes between 1990 and 2009, but the trends in FPG and 2h glucose together with the increasing prevalences of IFG and IGT are alarming. While our data indicate that it is a matter of time till increased prevalence of diabetes will be at hand, recent data from the VIP surveys in Västerbotten [64], demonstrating an increasing prevalence of diabetes in Västerbotten county, indicate that it is a matter of statistical power. As both IFG and IGT, and not only T2DM, are linked to an increased CVD risk and subsequent costs in health care and society [259], prevention is a major challenge. The increase in FPG and 2h glucose was seen in most deciles of the glucose distribution, indicating that prevention has to target the whole society, not only individuals at the highest risk of developing T2DM.

The risk of T2DM is not equally distributed in society [7, 49], and not between ethnicities, where Asian Indians have a particularly increased susceptibility for T2DM [48, 66]. We have, in a large material, studied differing circulating leptin concentrations as a potential explanation for the increased T2DM risk in Asian Indians. We show that Asian Indian men and women have increased concentrations of leptin compared with Caucasian and Creole men and women, respectively. This difference remained after adjustment for obesity measures. Furthermore Creole men, and women, had in general higher obesity adjusted levels of leptin compared with Caucasians. We also demonstrate that leptin in men, but not in women, independently can explain some of the variance in FPG and 2h glucose, and that leptin in men is an independent predictor of incident T2DM. After stratification for insulin sensitivity status, leptin remained an independent predictor of T2DM only in the insulin sensitive men, whereas high adiponectin levels predicted decreased risk, and a high leptin–adiponectin ratio predicted an increased risk of T2DM in both insulin sensitive and resistant men and women. The leptin–adiponectin ratio may thus add further predictive information beyond that of the separate hormones.

With new emerging risk factors or potential risk factors for T2DM, such as gut microbiota [260], metabolomics [261], vitamin D deficiency [262] or single nucleotide polymorphisms (SNPs) [263], what is the place for leptin, adiponectin and their ratio? As predictors, in a clinical

setting or in a screening situation, not much is won by adding e.g. adiponectin or SNPs to traditional non-invasive risk tools (questionnaires and anthropometry). With the exception of FPG, blood sampling only to a minor extent increases the predictive ability beyond that of the non-invasive methods [263]. When in search for potential pathophysiological mechanisms through which an increased T2DM risk is mediated, the quest is different. Adjusting for any metabolic risk factor with uncertain patho-physiological role, potentially in the same causal pathway, could in that situation be troublesome [190].

What about the probability of leptin, adiponectin and the leptin–adiponectin ratio truly being causative, according to the criteria set up by Hill [234], and the potential mechanisms for such an increased T2DM risk?

Strength, consistency and temporality

Adiponectin in a predictive model including age, sex, anthropometry and life style, contributed with 1/3 of the calculated risk for incident T2DM, and CRP, GGT and HDL cholesterol all contributed less [211]. In addition adiponectin consistently remained as a predictor of incident T2DM in many studies despite various adjustments [209], as it did in paper III in this thesis.

For leptin, a hypothetical obesity/insulin resistance factor (including BMI, fasting insulin, proinsulin and leptin) and a glycaemia factor (including FPG, 2h glucose and NEFAs) predicted incident diabetes and could explain almost 70% of the variance. In contrast, three other hypothetical factors (an inflammatory factor, a blood pressure factor and a dyslipidaemia factor) did not contribute significantly [264]. In men, leptin is an independent predictor of incident T2DM in an accumulating number of studies [185, 186, 187, 188], as it was in paper III in this thesis, but other studies oppose these results [189, 190, 191]. This discrepancy can be due to the impreciseness in the diagnosis of diabetes [190], and notably, in paper III, all cases of T2DM were strictly validated. Findings in paper III also indicate the possibility that the degree of insulin resistance in participants in different surveys could influence the results. In paper IV, we demonstrate that around 5% of the variance in FPG and 2h glucose could be explained by leptin in men. Presumably, the impreciseness in various measurements reduces the ability to explain the variance. Notably, a similar small amount of the variance could be explained by the traditional risk factors, and leptin remained an independent risk factor in men.

For the leptin–adiponectin ratio previous studies are few and inconsistent [189, 219], but paper III indicates the possibility of stronger predictive information of the ratio, than of the separate hormones.

Specificity

For adiponectin, a recent study showed that low adiponectin levels predicted increased risk of T2DM only in insulin resistant men and women [210], and in paper III we demonstrate that leptin predict incident T2DM in insulin sensitive men, and not in insulin resistant. These findings need to be reproduced by others, but they may indicate an incompletely

understood patho-physiological mechanism. Whether the sex difference, where leptin predicts diabetes [185, 186, 187, 188] and stroke [175] in men but not in women, is caused by difference in leptin signalling [125, 126] or other mechanisms, is unclear.

Biological gradient

A gradually increased risk of incident T2DM with increasing levels of leptin [186], and increasing leptin–adiponectin ratio [219], and a decreasing risk with increasing levels of adiponectin [209] was shown previously, and in paper III in this thesis. Of note, while most studies show a decreased CVD risk linked to high levels of adiponectin, several studies have found the opposite [179, 194, 198]. However, high adiponectin levels have not been associated with an increased risk of diabetes.

Plausibility and potential mechanisms

Both leptin and adiponectin are important for the glucose metabolism [110, 160, 197]. Many potential explanations through which decreased adiponectin levels and increased leptin levels could result in T2DM exist, but present knowledge is mostly based on animal models or in vitro studies, and rarely on studies in humans. Thus studies, and the following model on a potential patho-physiology leading to T2DM has to be interpreted cautiously.

Both leptin and adiponectin act as insulin sensitizers in skeletal muscle and liver, but they also, in a bi-directional way communicate with the pancreatic β -cell [110, 160, 197]. With increasing obesity, circulating levels of leptin increase, mirroring the amount of body fat [102], while the levels of adiponectin decrease. The factors permitting leptin levels to increase without a counter-regulatory answer on feeding and body weight, and the cause of decreased adiponectin levels in obesity are only partly known [159, 197].

Only strenuous physical activity acutely reduces leptin levels [147, 148], but through effect on weight [148] and the perceived hunger [149] leptin levels are potentially influenced indirectly. An increasing number of animal studies have demonstrated mechanisms of a decreased central responsiveness “leptin resistance” leading to hyperleptinaemia in obesity. ER stress in hypothalamus induced by overeating or inflammatory cytokines, e.g. CRP, interfering with the receptor, and a reduced BBB transport of leptin are three potential mechanisms [159, 160]. A negative feedback mechanism induced by increased leptin concentrations is another. An increased SOCS3 activity, that down-regulate the signalling in STAT3, the most potent pathway for the control of energy homeostasis, is induced by increased leptin levels. To that overeating can in rodents induce an inflammation in hypothalamus, were inflammatory cytokines further increase SOCS3 signalling [110]. In adipose tissue, increased leptin levels activate and stimulate an inflammatory answer, and the subsequent increase in TNF- α further up-regulates leptin [160, 166].

TNF- α and adiponectin bi-directionally down-regulate each other, while physical activity increase levels of adiponectin and decrease

TNF- α [95, 148]. Potentially leptin induced increase in TNF- α could be a main initiator of the obesity related decrease in adiponectin, together with a concomitantly reduced physical activity [197].

As a consequence of reduced adiponectin levels [197] and possibly ectopic fat accumulation [93], the body needs more insulin. While insulin in lean persons increase adiponectin release, no such effect is seen in insulin resistant individuals or during chronic insulin treatment [194, 197]. A reduced β -cell mass is seen in patients with T2DM over time. Reduced levels of adiponectin could partly be an explanation, as the hormone reduces the degradation of IRS-2 [180, 181].

Insulin resistance increases over time in patients progressing to T2DM [199]. This model is supported by the findings that increased leptin levels predict incident T2DM in insulin sensitive men [paper III], while low adiponectin levels predict T2DM in insulin resistant men and women [210], which could potentially indicate a pathophysiological temporality. The finding that the leptin–adiponectin ratio possibly is a better predictor of T2DM than the separate hormones [paper III], could indicate some degree of additive effect on incident T2DM, but an alternative interpretation is that the ratio better reflects actual insulin levels.

Coherence and analogy

The coherence and analogy criteria are not relevant.

Experiments

As described above, only few studies exist in humans and the animal studies performed have explored, and eventually intervened, at separate potentially causative steps. Yet no intervention studies exist, that truly could identify leptin or adiponectin as having a causative role in the progress towards T2DM.

Future questions

An important topic for further research is to reveal more of the background to leptin resistance and its clinical implications on diseases, but also in prevention. Could leptin resistance in part explain the difficulties in clinical practice to help patients to change life style and reduce weight? A similar question is whether leptin resistance partly could explain the difficulties for obese individuals to increase physical activity? Indications exist, as locomotor activity, feeding and energy expenditure all are regulated by STAT3 signalling, which potentially could be down-regulated in insulin resistance.

Animal intervention studies using leptin and adiponectin, as well as their antagonists, targeting progress to T2DM are needed to better elucidate the potential causative role of the hormones, and their possible future clinical use.

The most important issue though, is prevention of the epidemic of obesity and of T2DM seen worldwide. The papers in this thesis clearly demonstrate an increasing obesity and deterioration in glucose metabolism also in northern Sweden. Frustratingly, the results of various

preventive methods are often not encouraging. Much effort therefore has to be spent in search for better and validated preventive methods. The prevention has to take place both at an individual level, and in all of the society to succeed. To quote the German doctor, scientist and politician Rudolf Virchow (1821-1902):

"Medicine is a social science, and politics is nothing but medicine on a large scale. Medicine, as a social science, as the science of human beings, has the obligation to point out problems and to attempt their theoretical solution: the politician, the practical anthropologist, must find the means for their actual solution".

Conclusions

- Between 1986 and 2004, the prevalence of obesity increased in men 25-44 years old, and 44-74 years old, and in women 25-44 years old in northern Sweden. During the same period, the prevalence of abdominal obesity (NCEP and/or IDF definitions) increased in men 25-64 years old and in women 35-64 years old.
- Between 1986 and 2009, fasting plasma glucose (FPG) and post load, 2h glucose concentrations increased in women 25 to 64 years old, and 2h glucose increased in men 25 to 64 years old, and the increase was seen in most deciles of the glucose distribution. The prevalence of impaired fasting glucose (IFG) increased in women but was unchanged in men, and the prevalence of impaired glucose tolerance (IGT) increased in both men and women. The prevalence of both known diabetes, and of survey detected diabetes was unchanged.
- In both men and women, the leptin–adiponectin ratio independently predicted an increased risk of incident type 2 diabetes (T2DM), and adiponectin predicted a decreased risk of incident T2DM, while leptin only in men was an independent predictor of incident T2DM.
- Leptin could in men, but not in women, explain a minor part of the variance in FPG and 2h glucose. The explanatory capacity of leptin was equal as that of traditional risk factors.
- Adjusted for obesity measurements, smoking habits and age, Asian Indian men and women had higher levels of leptin, leptin per cm in waist circumference (leptin/waist) and leptin per BMI unit (leptin/BMI) than Caucasian and Creole men and women, respectively. Creole men had higher leptin, leptin/waist and leptin/BM than Caucasian men and Creole women had higher levels of leptin/waist when adjusted for waist circumference (WC) than Caucasian Women.
- Leptin has a high intra-individual stability, equal in Asian Indians, Creoles and Caucasians, in men and women and over 5-13 year of follow-up time.
No circ-annual variation in leptin was seen.

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