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A study of changes in glucose metabolism and inflammatory markers in morbidly obese patients undergoing bariatric surgery

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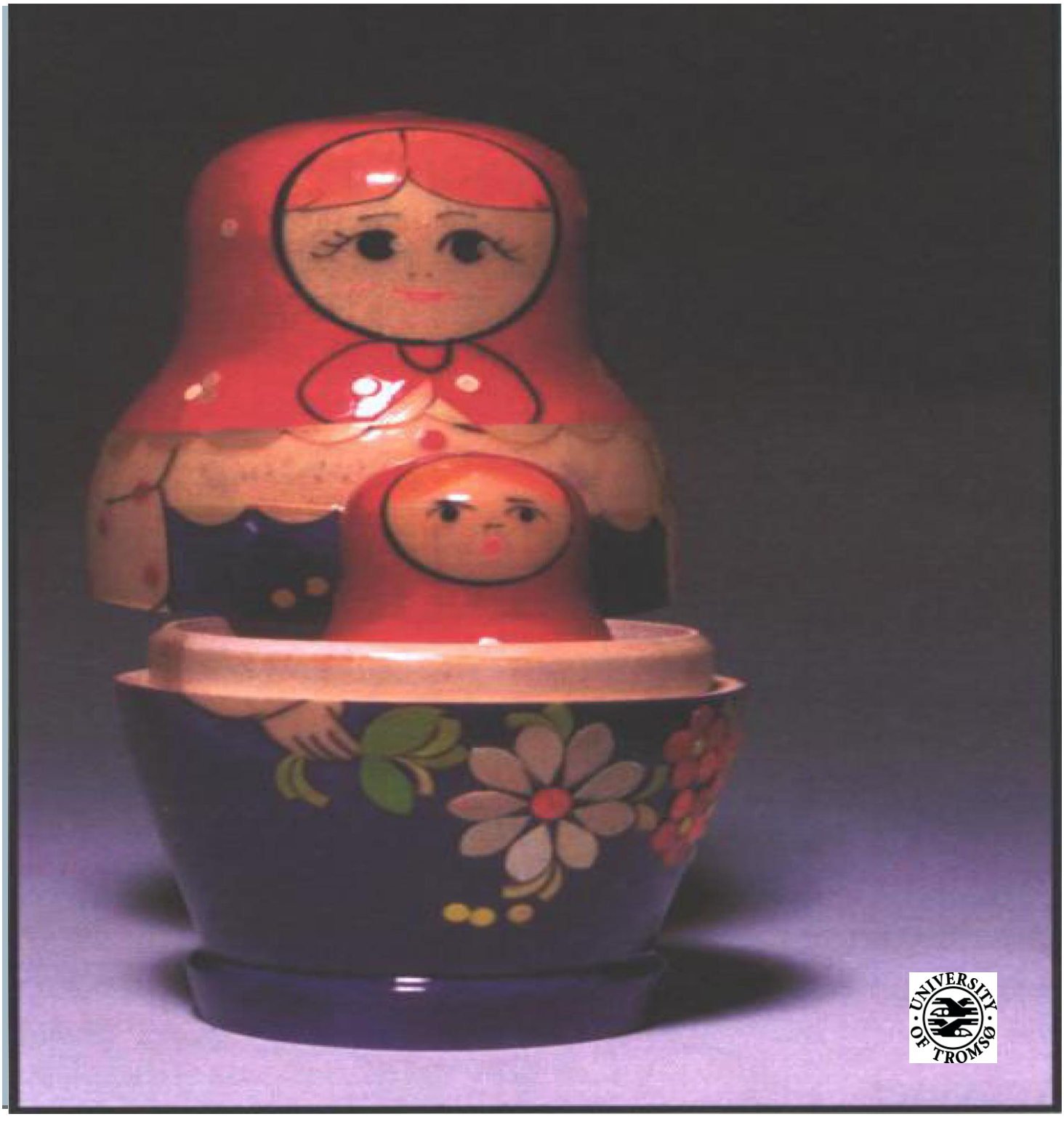
A dissertation for the degree of Philosophiae Doctor – June 2015



A study of changes in glucose metabolism and inflammatory markers in morbidly obese patients undergoing bariatric surgery

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Content

Acknowledgements

In 2004 we started performing bariatric surgery at our hospital. I was a resident at that time and had Jens Fromholt Larsen as my mentor. When we started up treating a "new patient group" – morbidly obese patients – we wanted to implement research. He contacted Knut Tore Lappegård and Erik Waage Nielsen for help drafting a project protocol. I was asked to join the group as a ph-student and I 'am glad I accepted that offer. I would like to thank Jens Fromholt Larsen for giving me this opportunity.

It has been a long and at times struggling journey. Without a never-ending inspiring and patient support from my supervisors Knut Tore Lappegård and Erik Waage Nielsen I would not be standing here today. Your knowledge in immunology, statistics and how to perform science has been priceless. You never lost faith in me. You were always available for discussions no matter where you were and at any time. You thought me a language I was not familiar with, namely the language of proper scientific writing. I am so grateful to the both of you.

Marius Trøseid; my co author in Paper III and IV has given me valuable knowledge in the field of immunology. You have a lot of energy, creativity and work capacity, making us able to analyze LPS and sCD14. The collaboration with your research group at Ullevål hospital has been very important.

It is a long time ago but starting a new research project is time consuming, and doing it part time in addition to clinical activity is difficult. Without the support and understanding from Department of Surgery it would not have been possible to conduct.

These research projects have involved several departments at this hospital; Department of Medicine, Department of Anesthesiology, Department of Radiology, Department of Clinical Chemistry and Somatic research laboratory. I would like to thank everyone participating in these projects.

Special thanks to the staff at the Regional center for treatment of morbid Obesity (RSSO) – especially Lisbet Kristensen and Nina Lillegaard – nurses at RSSO - without your dedication and contribution in this field this has not been possible. To Anita Langås – secretary at RSSO - for keeping track of the patients.

To Hanne Thoresen and Jeanette Andersen for performing and analyzing CT scans at the Department of Radiology. To everyone working in the operating theater where bariatric surgery was performed and fatty tissue samples was obtained.

To my colleagues at the Department of gastrointestinal surgery – who patiently have filled in for me when I was away and remembered taking samples of the patients in my control group. You have always been supportive.

To my Swedish colleagues – from Erstad hospital in Stockholm and Västerås Central hospital – for helping us develop the surgical skills in the field of bariatric surgery we hold at our department today.

To Somatic research laboratory - where I have stored my "biobank" - Hilde, Judith, Grethe, Anne and Dorthe. I have had the pleasure of working with the most leading, dedicated and thorough bioengineers in Norway!

Thanks to my family; my mother, my parents in law, my sisters and brothers and your families. You have had faith in me the whole time! My sister Guri –helping me coping in bad and in good days – even helping me tidying in the freezer at the research lab!

To my best friend and husband Harald! You are always there for me! It has been a hard time coping with clinical work, scientific work in addition to the most important – our family. I have worked a lot; long working days, evenings and weekends and you have managed the rest without complaining. To our wonderful two sons; John and Ole – you are the best!

Torunn Kristin Nestvold

Bodø 2015

Abbreviations

ADMA	Asymmetric dimethylarginin
BMI	Body mass index
BPDDS	Bileopancreatic diversion with duodenal switch
CRP	C-reactive protein
CT	Computer tomography
CVD	Cardiovascular disease
FFA	Free fatty acids
FMD	Flow-mediated dilatation
Hs-CRP	High sensitive C-reactive protein
HT	Hypertension
IL	Interleukin
IFN	Interferon
LDL	Low density lipoprotein
LPS	Lipopolysaccharide
PAI-1	Plasminogen activator inhibitor type-1
RYGB	Roux-en-Y gastric bypass
SDMA	Symmetric dimethylarginin
T2DM	Type 2 diabetes mellitus
TNF-alpha	Tumor necrosis factor-alpha
WHO	World Health Organization

Summary

Obesity is considered a worldwide pandemic. Morbidly obese patients are at risk of developing comorbidity such as diabetes mellitus type 2, hypertension and cardiovascular disease. The mechanisms behind the development of such complications in morbidly obese patients are still not fully understood, but low-grade inflammation and visceral adiposity are both involved. Effective treatment that leads to a significant weight loss can improve and/or repeal these comorbidities.

In this prospective study we have investigated what impact lifestyle intervention followed by bariatric surgery has on markers of glucose metabolism, inflammation and coagulation. 134 morbidly obese patients (who underwent lifestyle intervention followed by bariatric surgery) and 36 lean subjects (admitted for elective laparoscopic procedures) were included in 4 different studies. The morbidly obese patients were followed one year after surgery. The 36 lean subjects served as a control group.

We have shown that markers of low-grade inflammation such as the concentration of hs-CRP, C3 and C4 in serum were significantly higher in the morbidly obese group compared to the CG at admission. One year after bariatric surgery there was a significant reduction in C3 and C4 in the morbidly obese group and there was no longer a significant difference compared to the control group. The same was seen for several other important inflammatory markers. We found that there was a positive correlation between serum lipopolysaccharide and HbA1c. Through measuring bacterial DNA load and the volume of different adipose tissue compartments, we found that there was an increasing content of bacterial DNA with increasing proximity

to the gut. We also found that monocyte activation measured by sCD14 is closely associated with obesity-related vascular dysfunction.

Finally, in a group of non-diabetic morbidly obese patients we found that 11 out of 40 patients had insulin resistance at admission and that one year after surgery this was no longer present.

These findings indicate a central role of low-grade inflammation in development of comorbidity in morbidly obese patients, and underscore the position of the gut microbiota in this process.

Sammendrag

Overvekt har blitt en global pandemi. Sykelig overvektige pasienter er i risikogruppen for utvikling av tilleggssykdommer som diabetes mellitus type 2, hypertensjon og kardiovaskulær sykdom. Effektiv behandling som fører til et signifikant vekttap kan forbedre og/eller oppheve disse tilstandene. Mekanismene bak utviklingen av tilleggssykdommer er fremdeles ikke helt kartlagt. Lavgradig inflammasjon og viseralt fettvev er assosiert med utviklingen av disse tilleggssykdommene.

I denne prospektive studien har vi undersøkt hvordan livsstilsendringer etterfulgt av overvektskirurgi påvirker markører for glukose metabolismen, inflammasjon og koagulasjon. 134 sykelig overvektige pasienter som gjennomgikk livsstilsendringer etterfulgt av overvektskirurgi og 36 normalvektige pasienter som var innlagt for elektiv laparoskopisk kirurgi ble inkludert i fire forskjellige studier. De sykelig

overvektige pasientene ble fulgt i ett år etter kirurgi. De 36 normalvektige pasientene inngikk i en kontrollgruppe.

Vi har vist at markører for lavgradig inflammasjon som for eksempel hs-CRP, C3 og C4 i serum var signifikant høyere i den sykelig overvektige gruppen ved inkludering enn den normalvektige. Ett år etter kirurgi var det en signifikant reduksjon i disse parametrene og forskjellen fra de normalvektige var ikke lengre signifikant. Det samme mønster så vi for flere andre viktige inflammatoriske markører.

Vi fant at det var en nær sammenheng mellom serum lipopolysakkarid og HbA1c. Ved å sammenligne volum av og bakteriell DNA-mengde i forskjellige fettvevskompartment med de normalvektige, fant vi et økende innhold av bakterielt DNA hos de sykelig overvektige jo nærmere man kom tarm. Vi fant også at monocytt-aktivering målt med sCD14 var nært assosiert med overvektsrelatert vaskulær dysfunksjon. Til slutt, i en gruppe ikke-diabetiske sykelig overvektige pasienter, fant vi at 11 av 40 pasienter hadde insulinresistens ved inklusjon og at denne ikke lengre var tilstede ett år etter kirurgi.

Våre funn peker på at lavgradig inflammasjon og tarmens mikrobiota kan være sentrale i utviklingen av tilleggssykdommer hos sykelig overvektige pasienter.

Publications included

I. **Nestvold TK, Nielsen EW, Lappegard KT. Bariatric surgery reduces risk factors for development of type 2 diabetes mellitus in morbidly obese, nondiabetic patients. *Metab Syndr Relat Disord.* 2013;11:441-446.**

II. **Nestvold TK, Nielsen EW, Ludviksen JK, Fure H, Landsem A, Lappegard KT. Lifestyle changes followed by bariatric surgery lower inflammatory markers and the cardiovascular risk factors C3 and C4. *Metab Syndr Relat Disord.* 2015 Feb;13(1):29-35.**

III. **Troseid M, Nestvold TK, Rudi K, Thoresen H, Nielsen EW, Lappegard KT:, Plasma lipopolysaccharide is closely associated with glycemic control and abdominal obesity: evidence from bariatric surgery. *Diabetes Care.* 2013;36:3627-3632.**

IV. **Troseid M, Nestvold TK, Thoresen H, Nielsen EW, Seljeflot I, Lappegard KT. Soluble CD14 is closely associated with markers of vascular dysfunction in bariatric surgery patients. *Metab Syndr Relat Disord.* 2015 Apr;13(3):119-24.doi:10.1089/met.2014.0111. Epub 2015 Jan 6.**

1. Introduction

1.1 Classification and epidemiology

Obesity is one of the biggest health problems the world is facing today [1]. The obesity itself is not the only problem, but obesity - and especially morbid obesity - is associated with several conditions of concern to the public health in general, such as; type 2 diabetes mellitus (T2DM), hypertension (HT) and cardiovascular disease (CVD), including myocardial infarction and stroke [1]. Morbid obesity is not only a problem in the developed part of the world, but also in developing countries [2].

Table 1. The WHO classification of obesity. Adapted and modified from [1].

Classification	BMI	Risk of comorbidities
Underweight	< 18.5	Low (but risk of other clinical problems increased)
Normal weight	18.5-24.9	Average
Overweight/ pre-obese	25.0-29.9	Increased
Obese class-1/ Obesity	30.0-34.9	Moderate
Obese class-2/ Morbid obesity	35.0-39.9	Severe
Obese class-3/ Severe morbid obesity	≥ 40.0	Very severe

WHO= World Health Organization, BMI= Body Mass Index

The classification of obesity differs in terms of vocabulary. Epidemiological studies often use the term obese-class 1-3 (Table 1), in clinical studies the term obesity,

morbid obesity and severe morbid obesity is more often used. In this thesis, the term "morbid obesity" is chosen for patients with a body mass index (BMI) ≥ 35 kg/m².

The World Health Organization (WHO) states that in 2008 35% of the world population over the age of 20 had a BMI > 25 kg/m^2 [3]. In the US Division of Health and Nutrition Examination Surveys the age-adjusted prevalence in the US population with BMI ≥40 kg/m² was 6.3% in 2011-2012, compared to 2.8% in 1988-1994. In women the age-adjusted prevalence was 8.6 % in 2011-2012, compared to 3.9 % in 1988-1994. In men the age-adjusted prevalence is lower but the trend over the same time period is the same, 4.4% in 2011-2012, compared to 1.7% in 1988-1994 [4]. In Norway the trend in age-adjusted prevalence is the same even if the prevalence is much lower than in the US. This has been shown in the HUNT study (a population study of Northern Trøndelag, a county in the middle of Norway). Comparing the years 2008 and 1986 the age-adjusted prevalence of obese-class 2 (35≤BMI<40) was 3.2% versus 0.8% in men and 5.0% versus 2.6% in women. For obese-class 3 (BMI ≥ 40) the corresponding prevalence were 0.5% vs. 0.1% in men and 1.5% vs. 0.7% in women [5].

There is no reason to believe this development is slowing down. Finkelstein et al have used a regression model to estimate the prevalence of obese individuals (obese-class 1 and 2) and severe obese individuals (obese-class 3) in 2030. The estimate predicts a prevalence of 42% and 11% respectively [6].

1.2 Mechanisms behind the development of morbid obesity

The development of morbid obesity is caused by a variety of factors; environmental (the society), genetic predisposition and human behavior (the individual itself), the environmental factor being the most important [7-9]. Economic development tends to reduce the quantity of physical activity and increase the intake of refined energy-dense-, high-fat food leading to a discrepancy between energy intake and energy expenditure [1;9]. Several environmental factors contribute. Availability of cars and public transportation [9] and the use of internet and television lead to a sedentary lifestyle [10]. The food industry, food commercials and easy access to shops affect our choice and consume [7;11]. On the other hand there is growing evidence that common genetic variants or single-nucleotide polymorphisms may play a role in the obesity pandemic [12]. In addition there are some rare single gene mutations that lead to monogenic obesity such as leptin receptor deficiency [13].

In a minority of patients several other diseases lead to obesity and morbid obesity [8]. Examples are Cushing syndrome, hypothalamic disorders, insulinoma, growth hormone deficiencies, bulimia and binge eating disorders. Drugs such as tricyclic antidepressants, litium, and glucocorticoids can also contribute.

The dramatic increase in the number of morbidly obese subjects over the last three decades has encouraged researchers to search for causal factors. One of the hypotheses is the influence of the gut microbiota composition. Both in mice and humans, a significant difference in gut the bacterial composition has been shown when comparing lean and morbidly obese individuals. This has led to the theory that there

are mechanisms linking the gut microbiota to energy harvesting, storage and expenditure [14-16]. Another hypothesis is the role of the brain-gut axis. There is a complex collaboration between the brain and the gastrointestinal tract. Leptin and insulin influence hypothalamus and releasing hormones such as glucagon-like peptide-1(GLP-1), peptide YY3-36, cholecystokinin (CCK) and ghrelin regulate hunger and satiety. Chronic imbalance between hunger and satiety signals can lead to increased food intake and body weight gain [17;18].

1.3 Comorbidity

In this thesis the terms "comorbidity" and "comorbid diseases" cover diseases frequently observed in morbidly obese patients.

Both obesity and increased waist circumference are factors associated with development of comorbidity (Table 1 and 2 respectively), such as T2DM, [19], hypertension and CVD [20].

Table 2. Adapted and modified from [1].

	Waist circumference	
	Women	Men
Moderate risk of comorbidity	≥ 80 cm	≥ 94 cm
Increased risk of comorbidity	≥ 88 cm	≥ 102 cm

The observation that a significant proportion of patients with CVD and T2DM present with a number of known risk factors simultaneously has led to the introduction of the term ”metabolic syndrome”. The term indicates the concomitant presence of at least three out of five pre-specified risk factors (Table 3) [21;22].

Table 3. Criteria for Clinical Diagnosis of the Metabolic Syndrome (adapted and modified from [22]).

Measure	**Cateorical cut points**
Elevated waist circumference	Population- and country-specific definitions
Elevated triglycerides (or treated)	≥ 1.7 mmol/L
Reduced HDL-C (or treated)	1.9 mmol/L in males and 1.3 mmol/L in females
Elevated blood pressure (or treated)	Systolic blood pressure ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg
Elevated fasting glucose (or treated)	≥ 5.6 mmol/L

The presence of at least three out of five risk factors should lead the physician to intensify treatment of these risk factors. The physician can give lifestyle intervention advise or treat the patients with e.g. lipid lowering medication or antidiabetic medication if needed, and thereby lower the risk of CVD development [23].

The incidence of comorbid diseases increases with increasing BMI [1] and waist circumference (Table 2) [24-26]. Waist circumference reflects intra abdominal fatty tissue amount and thereby the mesenteric fat. The mesenteric fat is central in the

transport of metabolites from the intestine to the portal vein and to the liver - especially metabolites active in the humoral immune response [19;24;27].

The cut-off values for waist circumference regarding risk of development of comorbidity are different depending on population and racial background. Table 3 shows the cut-off for the Caucasian population. Waist circumference measurements depend on the accuracy of the personnel performing the procedure. Thus, in a clinical setting the personnel involved have to be aware of this and perform the procedure accordingly. WHO has suggested a method whereby waist circumference measurement should be done, i.e. midway between the lateral lower ribs and the iliac crest with the patients in an upright position [28].

In some populations, important genetic factors lead to obesity and its comorbidity, such as among the PIMA indians where obesity and T2DM are endemic [29]. On the other hand, a genetic factor in developing obesity may not be completely determining. In the western population the genetic disposition is present both in lean and obese subjects [30-32].

Morbid obesity is not only a state that leads to somatic diseases as discussed above, but the patients also carry a higher risk of developing sleep apnea [33], arthritis, infertility, gastro esophageal reflux disease and several types of cancer [34;35]. Morbid obesity is also associated with increased mortality [36;37] as well as reduced quality of life [38;39], unemployment [40] and infertility [41]. In all, morbid obesity and its comorbidities lead to a significant socioeconomic burden [42;43].

1.3.1 Mechanisms for developing comorbid diseases

There are several etiological factors for the development of comorbidities in morbidly obese subjects, among them altered gut microbiota, low-grade inflammation, dyslipidemia and hypertension - these factors cross-talk through complex mechanisms [44-47].

Gut microbiota and low-grade inflammation

Low-grade chronic inflammation is a key mechanism that in obese individuals can lead to both atherosclerosis [48-50] and T2DM [51]. Altered gut microbiota may have an important role in that respect [52]. Lipopolysaccharide (LPS) – an endotoxin in gram-negative bacteria cell wall that is translocated from the intestine through the mesentery fatty tissue to the systemic circulation - initiates a cascade reaction of the innate immune system. This mechanism was first seen in mouse models [15;53;54], and later confirmed in humans [16;55;56]. LPS promotes inflammation mainly through Toll-like receptor 4 (TLR4) on macrophages and monocytes - the first line component of the innate immune system [57].

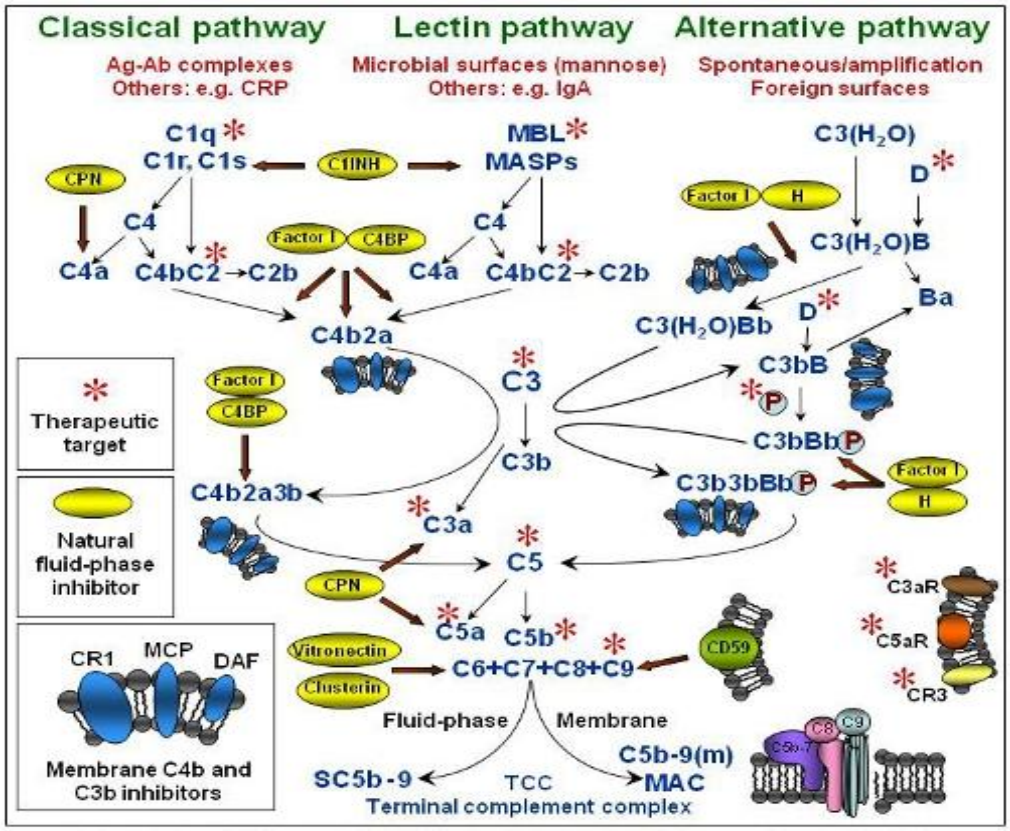
CD14 plays a central role by transferring LPS to the TLR4 receptor complex [57]. Soluble CD14 (sCD14) is secreted to the circulation mainly by activated monocytes and macrophages upon stimulation with LPS and other microbial products.

In obese patients with insulin resistance a chronic low-grade endotoxemia is measured by plasma LPS or LPS-binding protein [58-60]. In this respect an important

component is the epithelial barrier (skin and the mucosal surface of the gastrointestinal and respiratory tracts) [61]. In morbidly obese patients the mucosal surface of the gastrointestinal tract may be less resistant to bacterial translocation, caused by increased availability of LPS and its cotransport of chylomicrones [62;63]. High fat meals can be an important factor in that respect, as shown in mouse models [52;64;65]. Although this is seen related to high fat intake, the gut microbiota can be changed due to other components of nutrients. Recently, also a connection between non-caloric artificial sweeteners and development of glucose intolerance through alterations in the intestinal microbiota was demonstrated – both in mice and humans [66]. This may – at least in part – explain the observation that morbidly obese patients don`t achieve the expected weight loss when changing from regular to diet soft drinks.

The complement system is a central constituent of innate immunity, defending the host against pathogens, coordinating various events during inflammation, and bridging innate and adaptive immune response [67] (Figure 1).

Figure 1. The complement system. From Mollnes T.E [68] (Permission to use this figure is granted from Elsevier Limited 16.10.2014)



C3 and C4 are acute phase proteins that are key complement factors in all three complement activation pathways - the alternative, the lectin and the classical pathways. In the classical and the lectin pathways C3 serves as an important contributor to the cascade but does not initiate the reaction [69-71]. In the alternative pathway however, there is a constant, spontaneous activation of C3 which is controlled by soluble and membrane bound inhibitors. Foreign surfaces lack these inhibitors, resulting in C3 activation. C3 is an important factor in the process of developing the membrane attack complex (MAC), which creates pores in the cell surface and induces cell lyses. During this process C3adesArg is produced. C3adesArg is important in triglyceride synthesis in fat storing cells and glucose transport [50;72;73]. C3 has been

shown to be an important risk factor for T2DM and CVD development [74]. The synthesis of these proteins increases in response to inflammation and infections, and inflammation is associated with T2DM and CVD [75-78]. Complement factor C3 production is regulated by interleukin (IL)-6 and IL-1ß and C4 is regulated by interferon (IFN)-γ [50]. Both these complement factors are mainly synthesized in the liver, but adipose tissue is also a contributor [50;71;79]. Adipose tissue is also an important humoral organ synthesizing and releasing several pro-inflammatory cytokines of the innate immune system, such as IL-1 and tumor necrosis factor (TNF)-alpha [45;47;80]. Both fat cells and non-fat cells in the fatty tissue can contribute in this respect [51;81;82]. Inflammatory cytokines induces the production of IL-6, which in turn stimulates the production of acute phase reactants such as C-reactive protein (CRP) and fibrinogen in the liver [83]. CRP is central in the activation of the complement cascade and is also an opsonin for various pathogens [84]. Plasminogen activating inhibitor-1 (PAI-1) is another acute phase reactant that is increased by the induction of TNF-alpha, IL-1 and IL-6. PAI-1 is released from fatty tissue, endothelium and liver and has both thrombogenic and proinflammatory effects [85].

Dyslipidemia

In obesity, high levels of triglycerides and low levels of high density lipoproteins (HDL) and normal to high levels of low density lipoproteins (LDL) are often seen due to postprandial hyperlipidemia [86;87]. The dyslipidemia leads to reduced triglyceride lipolysis and impaired free fatty acids (FFA) trapping. This in turn leads to increased transport of FFA from the adipocytes to the liver and other tissues and thereby to

increased formation of small dense LDL [73;87]. Postprandial increase of insulin stimulates to lipoprotein lipase activity which in turn induces triglyceride lipolysis in the circulation, and regulates FFA mobilization from fatty tissue and chylomicrones. LDL can migrate to the sub-endothelial space and into the monocytes and macrophages. This mechanism leads to endothelial dysfunction – the first step towards the process of hypertension and at a later stage – atherosclerosis.

Endothelial dysfunction

The endothelium is important in modulating vascular function and structure. Nitric oxide is produced by endothelial cells, its function is to exert vasodilating effects and induce other protective actions against the development of atherosclerosis in the vessel wall. In obesity endothelial dysfunction develops, especially when insulin resistance and T2DM are present. The increased production of adipokines (especially leptin and adiponectin) and pro-inflammatory cytokines induces oxidative stress leading to reduced nitric oxide availability [88;89].

In clinical studies, endothelial dysfunction is commonly assessed by flow-mediated dilatation (FMD), which is a direct assessment of the vascular function. Several studies have shown impaired FMD in obesity and related comorbidities, although results on the effect of bariatric surgery on FMD have been conflicting [90-92]. Endothelial cells often express TLR4 as a response to pro-atherogenic stimuli, and a link between LPS-induced TLR4 activation of endothelial cells and coronary artery disease has previously been reported [93]. To assess vascular dysfunction plasma levels of

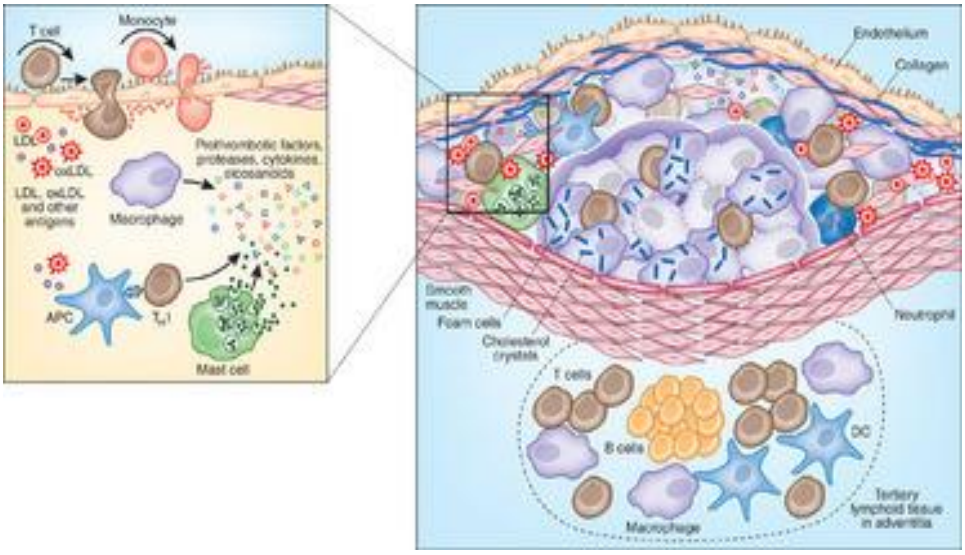
asymmetric dimethylarginine (ADMA) and its stereoisomer symmetric dimethylarginine (SDMA) can be measured. ADMA contributes to impaired endothelial function through its inhibitory effect on nitric oxide synthase [94]. SDMA does not inhibit nitric oxide synthase, but is regarded as a novel marker of vascular dysfunction and renal disease [95].

Pedersen et al recently showed that the plasma level of sCD14, but not LPS, is independently associated with both ADMA and SDMA in HIV-infected patients [96], but this relationship has not previously been evaluated in obese subjects.

Atherosclerosis

Atherosclerosis is a slowly progressing chronic inflammatory disorder of large and medium-sized arteries. It is a complex process caused by lipid-containing macrophages together with T-lymphocytes that infiltrate the endothelium from the blood and gradually accumulate to “fatty streaks” (Figure 2). These lesions are initially asymptomatic, but more complex, atherosclerotic plaques can develop, containing apoptotic and necrotic cells, cell debris and cholesterol crystals [48].

Figure 2. Immune components of the atherosclerotic plaque. Adapted from Hansson GK [48]. (Permission to use this figure is granted by Nature Publishing Group 16.10.14)



Inflammatory cells infiltrate and pro-inflammatory mediators and enzymes are produced in the centre of an atheroma. This process can lead to occlusion and stenosis of the vessel wall, resulting in ischemia in the surrounding tissue [97]. Furthermore, unstable plaques can rupture. This leads to exposure of thrombogenic material through the core of the plaque followed by platelet aggregation, humoral coagulation and thus to formation of a thrombus. If a thrombus, or part of one, is detached from the vessel wall, an embolus is formed which in turn can migrate to other sites distally of its origin, e.g. in the coronary arteries leading to a myocardial infarction or in the carotid arteries leading to a cerebral infarction [48].

1.3.2 Type 2 diabetes mellitus

In obesity T2DM is the most frequent comorbid disease. Many obese individuals have an undiagnosed T2DM [98;99]. T2DM can be defined by blood sample measurement of glycosylated hemoglobin (HbA1c), fasting glucose or Oral Glucose Tolerance Test. The criteria for the diagnosis of T2DM are listed in Table 4.

Table 4. Adapted and modified from European Society of Cardiology and European Association for the Study of Diabetes 2014 [100].

Diagnose/measurement	**WHO 2011 Can be used**	**ADA Recommended**
Diabetes		
HbA1c	≥ 6.5%	≥ 6.5%
Fasting plasma glucose	≥ 7.0 mmol/L	≥ 7.0 mmol/L
2 h plasma glucose	or ≥ 11.1 mmol/L	or ≥ 11.1 mmol/L
Impaired glucose tolerance		
Fasting plasma glucose	< 7.0	< 7.0 (not required)
Oral glucose tolerance test	≥ 7.8 - <11.0 mmol/L	≥ 7.8-<11.0 mmol/L (if measured)
Impaired fasting glucose		
Fasting plasma glucose	6.6-6.9 mmol/L	5.6-6.9 mmol/L
Oral glucose tolerance test	If measured < 7.8 mmol/L	-

WHO= World Health Organization, ADA= American Diabetes Association

Globally the age adjusted prevalence of T2DM in adults has increased from 8.3% in men and 7.5% in women in 1980 to 9.8% in men and 9.2% in women in 2008 [101]. The number of people having diabetes mellitus in the world has increased from 153

million in 1980 to 347 millions in 2008 [101]. The increasing prevalence of T2DM is the main reason for the doubling of individuals having diabetes mellitus in total, as the incidence and prevalence of T1DM is rather stable in comparison. When comparing the increased prevalence of obesity and T2DM from 1980 until 2003 the trend is similar [99]. The morbidly obese patients tend to get T2DM earlier in life than lean patients. The development of impaired glucose tolerance is important to detect and treat in order to avoid the development of T2DM [100]. Up to 70% of morbidly obese patients who have prediabetes (defined as impaired glucose tolerance (IGT) and/or insulin resistance) will develop T2DM during their lifetime [102]. Insulin resistance can be assessed by calculating Homeostatic model assessment-insulin resistance (HOMA-IR) score, using fasting glucose and insulin or C-peptide concentration [103].

In obese subjects lifestyle intervention leading to weight loss has been shown to reduce the risk of developing T2DM substantially [100]. T2DM and its complications have severe implications on the patients in terms of CVD, kidney, eye and neurological diseases. The longer T2DM persists, the greater risk for complications, and the more difficult for complications to resolve [104].

1.3.3 Cardiovascular disease

CVD is a group of disorders of the heart and blood vessels such as coronary heart disease (e.g. angina pectoris, myocardial infarction), cerebrovascular disease (e.g. stroke) and peripheral arterial disease [105]. The term CVD can be confusing as some articles only includes ischemic heart disease in the definition. In this thesis the above

written definition is used. CVD is the leading cause of mortality in most countries, middle- and low-income countries in particular [105]. It is estimated that 17.5 million people died from CVD in 2012, representing 31 % of all global deaths [105]. In Norway it is estimated that 33 % of all deaths are caused by CVD [106]. Important factors for CVD are smoking, T2DM, dyslipidemia and hypertension. Morbid obesity is another contributing factor, while overweight and light obesity can be protective [107;108]. Obese patients with CVD can even have a better prognosis than their leaner counterpart with the same CVD – "the obesity paradox". Explanations for this mechanism may be early detected HT leading to a more aggressive medical treatment, increased muscle mass and muscular strength, lower age at presentation and lower prevalence of smoking [107]. In morbidly obese patients this "obesity paradox" is not present [107]. Even though the treatment of T2DM, dyslipidemia and hypertension has improved, the number of morbidly obese patients is increasing contributing to a persisting risk of CVD [107;108]. Smoking is associated with development of CVD, insulin resistance and metabolic syndrome. Heavy smokers tend to be less physical active and have a less beneficial diet that may lead to weight gain [109].

1.4 Treatment of morbid obesity

Morbid obesity can be treated in several ways e.g. through lifestyle intervention, medication and surgical intervention. Lifestyle change is however necessary whatever treatment approach chosen.

1.4.1 Lifestyle intervention

Obesity and its complications has been one of the main topics in medical research for the last decade. Many studies have focused on finding a non-surgical solution for the treatment of morbid obesity such as different lifestyle intervention programs [110] and development of different medications to try to find targets dealing with inflammatory processes and/or decreasing energy uptake from the gastrointestinal tract) [111].

In Norway, lifestyle intervention programs (non-surgical treatment) through the health care system are several, such as outpatient clinics (in groups or individual guidance) and residential intermittent programs (in rehabilitating institutions) [112].

Patient compliance is vital for achieving a significant weight loss that does not result in weight regain over time [113].

Treatment programs normally include one or several of the following components:

- nutritional and physical activity advice
- behavioural treatment
- decreasing sedentary activities, and increasing physical activities
- social and/or psychological support.

To achieve permanent weight loss the patients need to be followed up closely by experienced health care workers [114;115].

1.4.2 Bariatric surgery

Bariatric surgery has been performed for many years [116]. Earlier it was looked upon as a purely surgical treatment of obesity, so information and education of lifestyle intervention was not emphasized. In recent years however, bariatric surgery is combined with lifestyle intervention that includes focus on diet, eating behavior and physical exercise. The patients who are admitted for bariatric surgery have to go through a thorough investigation, not only medically but also mentally, in order to be prepared for a life with altered gastrointestinal tract and weight loss after surgery [112]. The history of bariatric surgery started in 1953 when Richard L. Varco (US) performed the first jejunoileal bypass on an obese patient. Since then, there has been a development both in the technical aspect but also concerning the knowledge of how bariatric surgery influences the function of the gastrointestinal tract [116]. In this thesis the surgical procedures performed are gastric bypass (GBP) (Figure 3) and biliopancreatic diversion with duodenal switch (BPDDS) (Figure 4). The effect of these procedures are both restrictive (small ventricle volume) and malabsorptive (the intestine is divided in three limbs; alimentary limb, biliopancreatic limb and common limb). The lengths of the limbs are different according to the procedure chosen.

As previously mentioned, the significant weight loss achieved after metabolic/bariatric surgery leads to improvement and even resolution of comorbidities (T2DM, CVD and sleep apnea) [117-119].

Figure 3. Gastric bypass (Adapted from Neff. KJ et al [120] The illustration is the property of Johnson & Johnson and Ethicon Endo-Surgery (Europe). It is reproduced here with their kind permission.

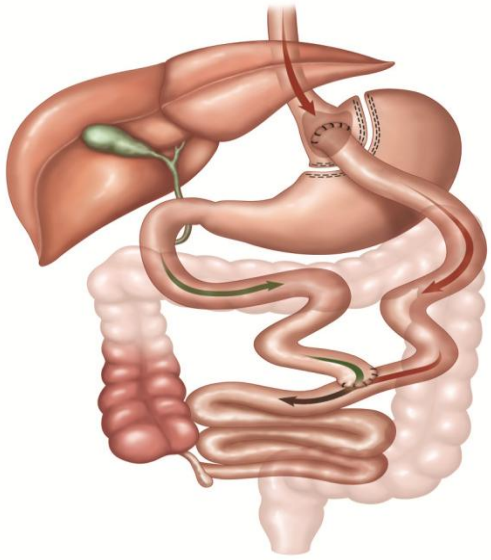
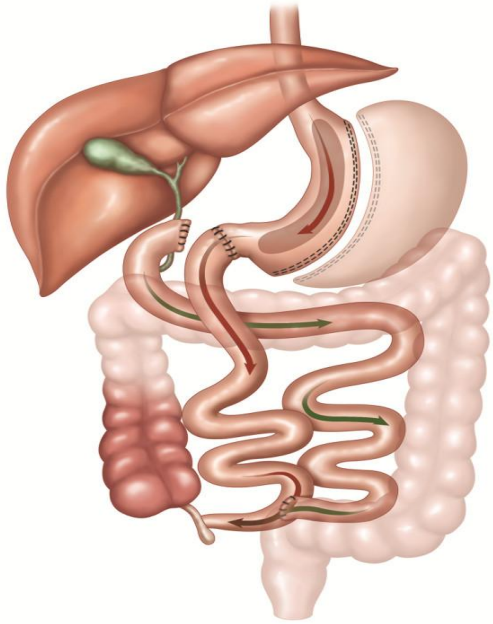


Figure 4. Duodenal switch (Adapted from Neff KJ et al [120]) The illustration is the property of Johnson & Johnson and Ethicon Endo-Surgery (Europe). It is reproduced here with their kind permission.



Postoperatively, the BPDDS, is very demanding for the patient due to the necessary changes in eating habits. If the patient is not able to follow the advice given by the operating centre i.e. avoiding food rich in glucose and fat, the patient will experience profound diarrhea and malnutrition.

1.4.3 Effect of surgical treatment for morbid obesity

As mentioned above, the aim of bariatric surgery is both restriction and malabsorption. The volume of the ventricle will be significantly smaller. The brain/gut axis is altered, changing the sensations of hunger and satiety, reducing the craving for sweets, salty or fatty food [121-123]. Postoperatively, the absorption of nutrients depends on the surgical procedure. For example procedures involving the intestine (GBP and BPDDS) differ between them and also from purely restrictive procedures such as gastric banding where the intestine is left unchanged [124]. Bariatric surgery leads to increased hepatic insulin sensitivity induced by energy restriction, and the beta cell function is improved due to exaggerated postprandial GLP-1 secretion because of altered transit of nutrients [125]. Triglycerides decrease after bariatric surgery. The significant weight loss leads to improvement and even resolution of comorbidities (T2DM, CVD and sleep apnea). The endothelial function improves and thereby reduces the risk of hypertension and CVD [72;126]. Both in animal models and in humans the gut microbiota changes after GBP [127;128], leading to the theory that changing the gut microbiota may impair the development of comorbidity related to morbid obesity. After GBP and BPDDS the patients need lifelong vitamin and calcium

supplementation. This is due to reduced uptake of these substances when food bypasses the duodenum and proximal jejunum. To avoid development of vitamin, calcium and other malabsorptive deficiencies these patients demand specific attention.

The aim of treatment of morbid obesity in a clinical setting is weight loss, improvement and/or resolution of comorbid conditions and improvement of quality of life. For the individual patient quality of life and being able to work play more important roles than resolution or improvement of comorbidities [40;129;130]. Treatment of morbidly obese patients has been shown to be beneficiary not only to the individual patient but also to the society. A morbidly obese patient is more likely to take sick leaves and more likely to obtain disability benefits [131]. The health care costs in general are much higher in the morbid obesity population, including pharmaceutical costs, physician visits and outpatient and inpatient visits [132;133]. Studies have shown that metabolic/bariatric surgery is the most effective treatment [72;134;135]. Lifestyle intervention programs have a positive impact that is not to be overlooked [113;136;137], and it is important to emphasize that lifestyle intervention also is a crucial part of any surgical approach.

2. Aims of the study

2.1 Overall aim

To investigate the effect of lifestyle intervention followed by bariatric surgery on parameters important for the development of comorbidity in morbidly obese patients.

2.2 Specific aims

2.2.1 Paper I

To investigate if preoperative lifestyle intervention followed by bariatric surgery could reduce risk factors for development of T2DM in morbidly obese, non-diabetic patients.

2.2.2 Paper II

To investigate the effect of lifestyle intervention followed by bariatric surgery in morbidly obese patients on levels of C3 and C4 as well as several other markers of inflammation, coagulation and glucose metabolism.

2.2.3 Paper III

To investigate if there were differences concerning the quantity of bacterial DNA in different adipose tissue compartments, and if any such differences were correlated with serum LPS. As LPS initiates the inflammatory cascade reaction, we investigated whether circulating plasma LPS would be associated with the amount of intra-

abdominal adipose tissue and HbA1c, and if a reduction of LPS after lifestyle intervention followed by bariatric surgery would correlate with improved glycemic control.

2.2.4 Paper IV

To investigate potential associations between LPS and soluble CD14 on markers of endovascular dysfunction in a cohort of morbidly obese patients undergoing lifestyle intervention followed by bariatric surgery.

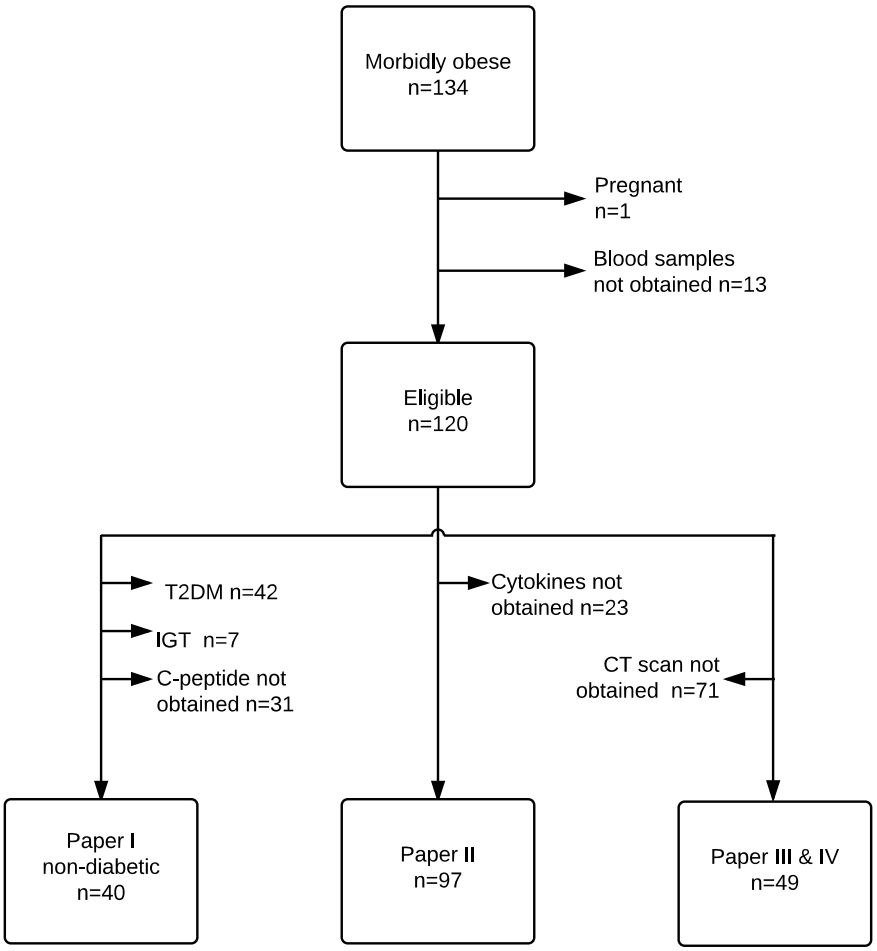
3. Patients, material and methods

3.1 Study subjects and design

3.1.1 Morbidly obese group

The four prospective studies included morbidly obese patients admitted to the Regional centre for treatment of morbid obesity at Nordland Hospital, Bodø. They were referred from general practitioners. The patients were included from December 2006 to June 2009. All the participants fulfilled the criteria for treatment of morbid obesity according to the Norwegian guidelines [112]. Inclusion criteria for the morbidly obese group were BMI ≥35 kg/m² with associated comorbidity such as hypertension, T2DM or sleep apnea, or BMI ≥40 kg/m². The patients had to be over the age of 18. Inclusion was done continuously at first admission to the centre.

Figure 5. Morbidly obese patients included in the four studies:



3.1.2 Control group

In Paper II, III and IV we included a normal weighted control group. The patients in the control group were admitted to the Department of gastrointestinal surgery, Nordland Hospital, Bodø, to receive treatment for gall stone disease or reflux disease (laparoscopic cholecystectomy or laparoscopic fundoplication, respectively). The participants were recruited at admission to surgery. The inclusion criteria for the control group were; BMI≤25 (in Paper II) (Figure 6) or BMI≤ 28 (in Paper III and IV)

(Figure 7), and absence of infection or other chronic conditions that could lead to systemic inflammation.

Figure 6. Control group included in Paper II.

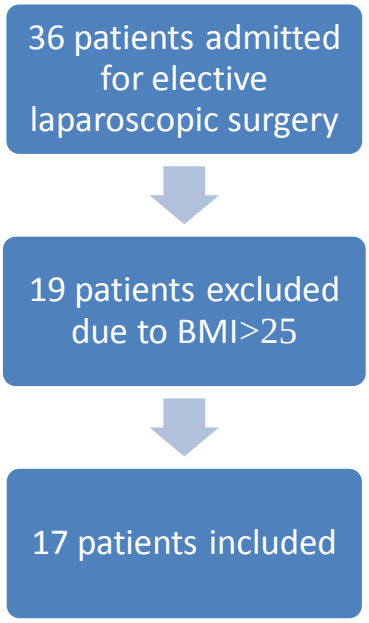
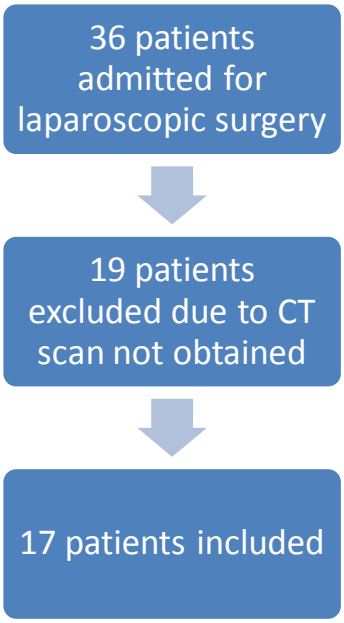


Figure 7. Control group included in paper III and IV.



The studies were performed according to the Helsinki Declaration and were approved by the Regional Ethics Committee of Northern Norway. Written informed consent was obtained from all participants.

3.2 Anthropometry

In the morbidly obese group height, weight, and calculated BMI were obtained from all included patients at three time points; at first admission, the day before surgery (after 3 months of lifestyle intervention) and one year after surgery. Waist circumference was measured as recommended by WHO [28] at admission and one year after surgery. In the control group the anthropometric measurements were obtained before surgery.

3.3 Blood sampling

In the morbidly obese group fasting blood samples were obtained by standard venipuncture on three occasions: at first admission, the day before surgery (after 3 months of lifestyle intervention) and one year after surgery. In the control group blood samples were obtained before surgery. Routine blood analyses were performed on the day of sampling at the laboratory of Nordland Hospital. Serum, citrate plasma, EDTA plasma and EDTA whole blood and were frozen in aliquots at -80℃ and analyzed in batch at the end of the study.

In Paper I the analyses included oral glucose tolerance test at first visit and HbA1c at all three occasions. In addition fasting glucose and C-peptide was measured at first admission and after one year. HOMA-IR score was calculated using the following HOMA-IR equation: [103].

plasma c-peptide x fasting plasma glucose/22.5

In Paper II, C3 and C4 were analyzed in serum, D-dimer and fibrinogen in citrate plasma. Cytokines, PAI-1, insulin and leptin were analyzed in EDTA plasma using a magnetic bead-based multiplex assay according to the manufacturer`s instructions.

In Paper III and IV LPS and soluble CD14 were determined in EDTA plasma. LPS was analyzed by limulus amebocyte lysate colorimetric assay according to the manufacturer`s instructions. Soluble CD14 was analyzed by an ELISA method according to the manufacturer`s instructions. The inter-assay coefficient of variation (CV) was <10%.

In Paper IV, ADMA and SDMA were determined in EDTA plasma by high performance liquid chromatography and precolumn derivatization with o-phthaldialdehyde as described in details elsewhere with minor modifications [94]. The inter-assay CV's were < 5% for both analyses.

3.4 Lifestyle intervention

Patients had to undergo lifestyle intervention preoperatively. The aim of preoperative lifestyle intervention was to prepare the patients for a life with altered gastrointestinal tract after GBP or BPDDS. At admission patients received personal guiding on eating habits and increased physical. This information was repeated by phone consultations with a nutritionist or specially educated nurse every two weeks preoperatively. The patients were informed to have a meal every three to four hours, for a daily total of three main meals and three to four small meals in between. They were advised to an energy restricted diet of 2000 kcal for males and 1500 kcal for women. That included a fat energy content of less than 30 %, complex carbohydrates energy content of less than 40-50 % and protein energy content of less than 20 %. A mandatory two-day course focused on lifestyle intervention and on how bariatric surgery would affect daily life was held. Information and education followed the Norwegian guidelines for treatment of morbidly obese patients [112] and Norwegian guidelines for nutrition, both given by the Norwegian ministry of health [138]. The patients were not accepted for surgery until they had achieved a 10% weight loss. Prior to surgery – included in the 10 % weight loss - the patients had to go through a low caloric diet of 900 kcal a

day for three weeks. In average, it was 12 weeks between admissions at the centre to surgery.

3.5 Surgery

All operations were performed by two experienced bariatric surgeons, and two surgical methods were used:

1.Roux-en-Y gastric bypass (Figure 3), using a standardized laparoscopic procedure by creating a small ventricular pouch of 30 ml, a biliopancreatic limb of 50 cm and an alimentary limb of 100 cm. This method was used for patients with BMI $\leq$ 50.

2. BPDDS (Figure 4), using a standardized laparotomy procedure. We created a gastric sleeve using a 32 French probe to measure the diameter of the gastric tube, an alimentary limb of 150 cm with a common limb of 100 cm.

The latter method was chosen for patients with a BMI > 50. One patient received a Laparoscopic Roux–en-Y gastric bypass despite a BMI of 51.7. Another patient received a BPDDS despite a BMI of 46.7. This patient had lost 14.7 kg prior to the first admission, so the initial BMI was 51.7.

3.6 Adipose tissue sampling

In Paper III adipose tissue biopsies were used. Biopsies from the mesenteric, omental and subcutaneous adipose tissue compartments were obtained during the surgical

procedure. The biopsy specimens were stored on ice and snap-frozen at -80°C until analysis.

3.6.1 Quantification of bacterial load in adipose tissue

Approximately 0.5 g adipose tissue was excised from the biopsy samples. DNA was purified and the bacterial load was determined by quantitative PCR targeting generally conserved regions of the gene encoding small units of ribosomal RNA. The ratio between bacterial and human DNA was used as a measure of the bacterial load as described in detail in Paper III.

3.6.2 Quantification of adipose tissue volumes

Computer tomography (CT) was obtained at admission. The CT scan was performed with the subjects in supine position and with their arms stretched above their head. It was obtained with a slice thickness of 10 mm ; in women at the level 5 cm above the L4/L5 intervertebral space and in men 10 cm above the L4/L5 level. The attenuation interval for adipose tissue calculations was -30 to-190 Hounsfield units. The subcutaneous and intra-abdominal adipose tissue compartments were manually traced, and the inner abdominal muscular wall separated the compartments. All CT scans were examined by the same radiologist.

3.7 Statistics

In Paper I and II numerical data was presented with mean. Repeated measures ANOVA were used for comparing the longitudinal effect of lifestyle intervention followed by bariatric surgery on the different variables. One sample paired t-test was used for comparing variables from baseline to the time of surgery and from the time of surgery to one year after surgery. In Paper II unpaired student t-test was used to calculate the differences between the morbidly obese group and the control group. The Shapiro-Wilks test was used to test for normality distribution. Correlation between variables was calculated with Pearson's correlation test, as the distribution was normal. Graphs and analyses in Paper I and II were done in PRISM 6 (GraphPad Software Inc, La Jolla, CA, USA).

In Paper III and IV differences between the morbidly obese group and the control group were evaluated with a paired t-test, Mann-Whitney U test or independent samples t-test for continuous data, and with Chi squared or Fischer`s exact test for categorical data as appropriate. Wilcoxon test for paired observations was used to evaluate changes from baseline compared with preoperative and postoperative evaluations. Correlation analyses were performed using Spearman`s rho or the Pearson correlation, as appropriate. ANOVA was used for comparison of tertiles

In Paper IV, given the small sample size, multivariate linear regression models included only age and gender as covariates. Models including a third covariate were created, adding variables one at a time based on significant associations in the univariate analyses or potential impact on vascular dysfunction. Statistical assumptions

for the use of the linear regression model were satisfied. The graphs and analyses in Paper III and IV were performed with SPSS software, version 19.0 (SPSS Inc, Chicago, USA).

A two-tailed significance level of 0.05 was used in all four papers. Different statistical methods were used in the papers due to the assumption of whether normality was fulfilled or not. The corresponding authors chose different statistical data software; GraphPad Prism and SPSS, respectively. Detailed descriptions are given in the papers.

4 Summary of results

4.1 Paper I

We investigated the effect of lifestyle intervention followed by bariatric surgery on anthropometric and glucose metabolism parameters in 40 non-diabetic morbidly obese patients (32 women and 8 men). Lifestyle intervention resulted in a mean weight reduction of 14.3 kg. One year after bariatric surgery the patients had a mean total reduction in weight of 50.5 kg. Mean waist circumference was reduced from 136.5 cm to 100.7 cm from admission to one year after surgery. At admission all 40 patients had a waist circumference >100 cm; one year after surgery 18/40 did. At baseline 11 out of 40 had insulin resistance (as defined by a homeostasis model score >3.99), whereas one year after surgery none of the patients did.

There was a statistically significant correlation between change in waist circumference and change in insulin resistance ($p<0.02$), and between HbA1c and weight loss ($p<0.002$).

4.2 Paper II

We investigated the effect of lifestyle intervention followed by bariatric surgery in 97 morbidly obese patients and a control group of 17 normal weighted subjects on anthropometric, inflammatory, coagulation and glucose metabolism parameters. At admission, the morbidly obese group had significantly elevated levels of C3 and C4 compared to the lean control group ($p<0.0001$). Levels of C3 and C4 dropped significantly in the morbidly obese group over time ($p<0.0001$), and one year after the operation levels were comparable to those of the control group.

The same changes were seen for markers of inflammation (hs-CRP, TNF-alpha, IFN-γ, IL-1ra, IL-6 and IL-13), coagulation (fibrinogen and PAI-1) and glucose metabolism (leptin and insulin). There was a positive correlation between changes in C3 and body mass index, weight, coagulation parameters, inflammatory parameters and leptin, respectively.

4.3 Paper III

Investigating morbidly obese patients undergoing lifestyle intervention followed by bariatric surgery, we examined the translocation of microbial products to adipose

tissue and the circulation and its potential impact on glycemic control. Plasma levels of lipopolysaccharide (LPS) were elevated in obese individuals compared to control group ($p<0.001$), and were reduced after bariatric surgery ($p=0.010$). LPS levels were closely correlated with HbA1c ($r=0.56$, $p=0.001$) and intra-abdominal fat volumes ($r=0.61$, $p<0.001$), but only moderately with subcutaneous fat volumes ($r=0.33$, $p=0.038$). Moreover, there was a decreasing gradient (two-fold) in bacterial DNA levels going from mesenteric via omental to subcutaneous adipose tissue compartments ($p=0.041$). Finally, reduced LPS levels after bariatric surgery were directly correlated with a reduction in HbA1c ($r=0.85$, $p<0.001$).

4.4 Paper IV

We investigated the potential impact of lipopolysaccharide (LPS) and subsequent monocyte activation measured by sCD14 on markers of vascular dysfunction. The cohort included 49 morbidly obese patients undergoing lifestyle intervention followed by bariatric surgery and a control group of 17 normal weighted subjects. Plasma levels of LPS, sCD14, ADMA and SDMA were obtained. Levels of ADMA were significantly higher in the morbidly obese group compared to the control group, but were not significantly reduced one year after bariatric surgery. In the morbidly obese group at baseline, there was a significant trend to increasing levels of ADMA and SDMA through tertiles of sCD14, and decreasing levels of both markers through tertiles of LPS. In multivariate linear regression models, sCD14 but not LPS remained independently associated with ADMA and SDMA. For every 10% age- and gender-

adjusted increase in sCD14, ADMA increased 0.031 µM (5.6%), whereas SDMA increased 0.039 µM (10.8%).

5. Methodological considerations and limitations

5.1 Study design

In these four papers the patients included are morbidly obese patients referred to the Regional centre of treatment of morbid obesity at our hospital. The prospective longitudinal design and the fact we have included a control group in Papers II, III and IV strengthens the studies.

Blood samples and adipose tissue biopsies were obtained and stored at -80˚C until they were analysed in batch. Routine blood parameters were analysed using standardized methods at our hospital routine laboratory. The analysing equipment is validated and routines for calibration are followed according to the manufacturers' instructions.

There are several limitations; the studies include a small number of patients which increases the risk of statistical type II errors, whereas type I errors are less likely. Fatty tissue specimens and CT scans were obtained only at admission. Thus, even though we have demonstrated a significant reduction in circulating LPS and the fact that changes in weight and BMI strongly suggest a reduction in fat volume, we are unable to verify changes in the various fat compartments as well as any possible changes in bacterial DNA content.

From the control group we obtained blood samples, fatty tissue specimens and CT scans only at one time point. It would have strengthened the studies if we had samples after one year, as well as if we had a control group of morbidly obese patients that received lifestyle intervention treatment for one year. Unfortunately, this was not possible in our institution.

5.2 Selection bias

In Paper III and IV we set a BMI cutoff of ≤ 28 in the control group due to the limited numbers of patients having performed the CT scan. In Paper II we retrospectively set a BMI cutoff of < 25 due to the definition of normal weight individuals (Table 1). Including patients with 25<BMI≤ 28 does not, however, influence on the results in Paper III and IV (Data not shown).

In the study population all patients were consecutively included regardless of comorbidity – both in the morbidly obese group and control group, so no selection bias was present in that respect. The cohort running from admission to one year after surgery included 134 patients. The patients were included in the four different studies due to the study design (Figure 9).

In Paper III and IV the sample size was determined by the number of CT scans obtained both in the morbidly obese group and in the control group. The CT scanner available for these studies could not be used if the patient had a waist diameter > 50 cm, excluding a number of patients.

There was no random selection concerning surgical procedure. In the inclusion period 26 patients were admitted for BPDDS. BPDDS was chosen if BMI ≥ 50, otherwise GBP was chosen. The results in the morbidly obese group were not divided despite of 2 surgical methods being used due to the limited sample size in the BPDDS group. The calculations have, however, been done and no differences between the surgical methods concerning glucose metabolism and inflammatory markers were found (Data not shown).

5.3 Measurement bias

The adipose tissue volume quantification was performed by one radiologist to minimize measurement bias. It was not possible to perform a blinded randomized CT scan investigation, due to the obvious difference in fatty tissue volume in the morbidly obese group compared to the control group.

Standardized anthropometric measurements were obtained by trained nurses working at the Regional centre for treatment of morbid obesity.

6. General discussion

To simplify the discussion, paper I and II will be discussed separately, followed by a common discussion of paper III and IV.

6.1 Effect of lifestyle intervention followed by bariatric surgery on risk factors for development of type 2 diabetes mellitus in morbidly obese, non-diabetic patients.

In Paper I we demonstrated how lifestyle intervention followed by bariatric surgery led to a significant weight loss and reduced risk factors for development of T2DM in morbidly obese, non-diabetic patients. Risk factors that were evaluated were weight, BMI, waist circumference, fasting glucose, HbA1c and HOMA-IR score. We identified patients with insulin resistance [102]), using C-peptide and fasting glucose, which enabled us to calculate HOMA-IR score. HOMA-IR score is a simple tool of recognizing insulin resistance and is comparable to hyperinsulinemic euglycemic clamp [24;103;139] HOMA-IR score ≥ 3.99 indicates insulin resistance and thereby identifies patients at risk of T2DM. In our study, 11 of 40 patients had HOMA-IR score ≥ 3.99 at admission whereas none one year after bariatric surgery.

HbA1c has now become a diagnostic criteria for T2DM [140]. It is also used in evaluation of treatment in T2DM. In our cohort, a 10% preoperative weight loss resulted in a small but statistically significant change in HbA1c. One year after surgery an even further reduction in HbA1c was seen. In line with this, the Swedish obesity study showed that bariatric surgery was more efficient than usual health care in the prevention of T2DM in obese subjects [141]. However, this is in contrast to the findings of Jorgenson et al. were there was no significant change in HbA1c in the non-diabetic group one year after GBP [119]. In our study, however, patient weight and HbA1c at baseline were higher, weight range was wider, and the patients lost more weight during the study period compared to patients in the study by Jorgensen et al. Also of importance; in our study, several patients received the more extensive

BPDDS-procedure because of baseline BMI > 50. The BPDDS- procedure leads to a greater weight loss [142]. In our study 11 out of 40 patients had baseline BMI > 50. However, even if we exclude patients with baseline BMI > 50 from in our study, we still observed a higher weight loss one year after surgery than did Jorgenson et al. In our study a higher weight loss was correlated with a higher reduction in HbA1c. As discussed in the paper, HbA1c correlates to weight reduction one year after surgery ($p<0.002$). Comparing these two studies one can hypothesize that a certain amount of weight loss is necessary to achieve a significant reduction in HbA1c. A randomized trial comparing different levels of weight loss to changes in HbA1c could probably answer this. Waist circumference reflects the amount of intra-abdominal fatty tissue and is a good predictor (of risk) for development of both T2DM and CVD [19;24;25;27]. Andersson DP et al. showed that reduction waist circumference can predict reversal of insulin resistance following weight loss after bariatric surgery [139]. In our paper all 40 patients had waist circumference >100 cm at admission, one year after surgery 22 patients had waist circumference <100 cm. In that respect they reduced their waist circumference significantly and thereby the risk of T2DM. There was a significant positive correlation between changes in waist circumference and HOMA-IR score ($p<0.02$).

6.2 Effect of lifestyle intervention followed by bariatric surgery on selected anthropometric measures and inflammatory markers.

In paper II we demonstrated that there were significant differences in several serological markers known to be risk factors for CVD and T2DM between morbidly

obese patients and lean subjects. In this paper we included markers of inflammation (C3, C4 and hs-CRP), coagulation (fibrinogen, PAI-1 and D-dimer) and glucose metabolism (leptin and insulin). We found a significant difference ($p<0.0001$) between the groups at baseline for all parameters except for D-dimer. One year after bariatric surgery, however, the differences between the morbidly obese group and control group (at baseline) were no longer statistically significant except for leptin (even if the decrease in leptin concentration in the morbidly obese group was significant over time). Our findings concerning reductions of the parameters over time are consistent with other studies [50;143]. Low grade systemic inflammation is an important contributor in the development of comorbidity in morbidly obese patients [69;71;72;144;145]. Bariatric surgery has been shown to have a positive effect not only on weight loss and reduction of waist circumference [126;131;144;146], but also in reducing risk factors for cardiovascular events [117] and improvement or resolution of T2DM [145;147].

Complement proteins can be synthesized in adipose tissue [79], and recent data demonstrate a close association between adipose tissue volume and C3 levels [50]. To our knowledge this is the first study to compare levels of complement factors between morbidly obese patients and a lean control group.

In this study 32 out of 97 morbidly obese patients suffered from T2DM at admission, whereas 1 year after surgery only six patients received treatment for T2DM. In addition; seven patients had impaired glucose tolerance at admission, one year after surgery that was no longer present in any of these patients.

Leptin is a hormone responsible for hypothalamus-induced appetite reduction and increased energy expenditure [148]. Leptin is mainly produced in fatty tissue and has an indirect as well as a direct effect on blood pressure regulation through proliferation of endothelial cells leading to endothelial dysfunction. In morbidly obese patients, the leptin level is increased - an apparent contradiction when we look at its function. It is, however known that in addition to insulin resistance, morbidly obese patients also develop leptin resistance [13;17]. Thus, the finding that leptin levels were reduced following bariatric surgery in the same manner as insulin and HbA1c is well in accordance with the theory of increased leptin sensitivity. Changes in leptin levels were significantly and positively correlated to changes in weight, BMI, waist circumference, C3 and PAI-1, indicating a close relationship between metabolism, inflammation and thrombosis.

6.3 Effect of lifestyle intervention followed by bariatric surgery on markers associated with gut microbiota and endothelial dysfunction

In Paper III and IV we have shown that levels of circulating LPS and soluble CD14 are significantly higher in morbidly obese patients compared to a lean control group ($p<0.001$). LPS is a potential trigger of T2DM [149]. In paper III we found that reduced LPS correlated with improved glycemic control. Our findings are in line with other studies [150]. We were not able to demonstrate any significant effect of lifestyle intervention, suggesting that a 10% weight loss was insufficient to achieve this effect. It is however important to emphasize that LPS was not correlated to BMI reduction one year after surgery, so factors beyond weight loss are likely to be involved [128]. A

possible link between gut microbiota and obesity is widely discussed [151;152]. We know that a high fat diet changes the gut microbiota which in turn increases the endotoxemia [56]. By measuring bacterial DNA in fatty tissue we found that there was a decreasing bacterial quantity from mesenteric to omental and subcutaneous adipose tissue specimens, respectively. Such findings have previously been seen in mouse models [16], but to our knowledge not in humans. Our findings support a hypothesis of translocated gut bacteria as a potential trigger of obesity and diabetes, and suggest that the anti-diabetic effects of bariatric surgery might be mechanistically linked to, and even the result of a reduction in microbial translocation.

In Paper IV the main finding was a close association between sCD14 and both ADMA and SDMA, indicative of a potential role of monocyte activation in obesity-related vascular dysfunction. We found that an age- and gender-adjusted increase in CD14 of 10% was associated with an increase of 0.031 μM in ADMA-levels. For comparison, in the Framingham offspring study, a 0.13 μM increase in ADMA was associated with 21% increased risk of death [153]. Others have previously shown that increased ADMA-levels are associated with obesity and other risk factors for CVD [154;155]. The novel finding of this study is the potential role of monocyte activation in this process, and our findings fit well with a previous report of association between sCD14 and aortic stiffness in a population-based study [156]. Furthermore, Pedersen et al have recently reported similar findings in an HIV-infected population, with sCD14 but not LPS levels, being independently associated with ADMA and SDMA [96]. Our results suggest that monocyte activation as measured by sCD14 is closely associated

with obesity-related vascular dysfunction, whereas potential upstream triggers including microbial products should be investigated in future studies.

7. Conclusion

7.1 Paper I

Our study shows that, in morbidly obese non-diabetic individuals, lifestyle intervention followed by bariatric surgery leads to a significant weight loss, a reduction in waist circumference, HbA1c and HOMA-IR score and thereby reduces the probability of developing T2DM.

7.2 Paper II

This prospective study shows that several established risk factors involved in the low-grade inflammation seen in morbidly obese subjects are not only susceptible to change, but can in fact be normalized as the result of significant weight loss due to lifestyle intervention followed by bariatric surgery.

7.3 Paper III

In morbidly obese patients plasma levels of circulating LPS were closely correlated with HbA1c and intra-abdominal adipose tissue volume. There was an increasing bacterial content in adipose tissue compartments with increasing proximity to the gut. After lifestyle intervention followed by bariatric surgery reduction of LPS levels were associated with improved glycemic control. Furthermore, our results suggest that the anti-diabetic effects of bariatric surgery might mechanistically be linked to, and may even be a result of, a reduction in microbial translocation.

7.4 Paper IV

This study showed that in morbidly obese patients monocyte activation measured by sCD14 was independently associated with markers of vascular dysfunction. Our results support a role for monocyte activation in development of vascular dysfunction and subsequent increased cardiovascular risk in obesity.

8. Future studies

In my research work many questions has arisen concerning comorbid diseases in morbidly obese patients. The mechanisms behind are still not clear so prolonged research in this respect it will be of importance. So far I have studied serological markers for low grade inflammation – it would have been very interesting to do the same analyzes in fatty tissue biopsies and compare the different fatty tissue compartments. This to confirm or refuse our theory concerning increased inflammatory activity close to the gut because of altered gut microbiota.

One or more studies where markers used in paper II-IV were measured in morbidly obese patients going through a lifestyle intervention program but no surgery would be interesting to perform. These patients should be followed over at least one year. Furthermore, a control group of lean subjects followed over one year could be included, in order to confirm our findings in the control group in paper II-IV.

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