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# **FLUID AND NUTRITION SUPPORT BEFORE TOTAL HIP REPLACEMENT SURGERY**

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*Carpe diem!*

## ABSTRACT

Surgical trauma results in a variety of physiological reactions in the body, including transient insulin resistance. The effect of surgery on postoperative wellbeing has been linked to this insulin resistance, which can be prevented through the ingestion of carbohydrate-rich liquid before surgery. The aim of this Thesis is to improve the clinical usefulness of the intravenous glucose tolerance test (IVGTT) by shortening the sampling time from 3 hours to 75 minutes and to use the IVGTT, and two other simple tests (QUICKI and HOMA-IR), to study whether most or all of the beneficial effects of the carbohydrate drink on postoperative insulin resistance can be explained by the water component of the drink.

**Study I:** Twenty healthy volunteers underwent a 75-min IVGTT. Insulin resistance was then assessed using the hyperinsulinemic glucose clamp technique, regarded as the gold-standard method. The resultant measurements of plasma glucose and insulin concentrations during the IVGTT were combined, using a variety of algorithms, allowing the insulin resistance, as measured by the clamp, to be predicted in 2/3 and 4/5 of subjects.

**Study II:** Sixty non-diabetic patients scheduled for hip replacement surgery were randomized into preoperative fasting (control), drink flavored water (placebo) or carbohydrate-rich liquid group. An IVGTT was performed the day before surgery, immediately afterwards, and the day after the surgery. No statistically significant differences were found with regard to glucose clearance, insulin resistance, postoperative complications or wellbeing between the three study groups.

**Study III:** A double-blind clinical trial was performed in which twenty-three non-diabetic patients underwent both an IVGTT and a hyperinsulinemic glucose clamp test on the day before and two days after hip replacement surgery. Half of the patients received a carbohydrate-rich liquid before the surgery; the other half received a placebo. There was a similar development of insulin resistance in both groups, but those who received a carbohydrate-rich liquid showed an increase in  $\beta$ -cell activity.

**Study IV:** Twenty-two patients from Study III were used to compare the insulin resistance results obtained from two dynamic tests (our short 7-sample IVGTT and the glucose clamp) and two static tests (QUICKI and HOMA-IR). The static tests showed slightly weaker linearity and larger residual errors compared to IVGTT in estimating insulin resistance before and after surgery. More importantly, they greatly underestimated the degree of surgery-induced insulin resistance, suggesting that they should not be used for that purpose.

## Conclusions

The simplified IVGTT results strongly correlated with those obtained using the hyperinsulinemic glucose clamp technique, which makes its use possible in the clinical setting. There was no statistical difference in surgery-induced insulin resistance between those who had received a carbohydrate-rich beverage or flavored water prior to hip replacement surgery. In addition, similar outcomes were seen in patients who had received a pretreatment carbohydrate-rich beverage, water or fasted preoperatively with regard to well-being and complications. HOMA and QUICKI should not be used to assess surgery-induced changes in insulin resistance.

## LIST OF PUBLICATIONS

This thesis is based on the following original articles, which will be referred to by their Roman numerical.

- I. A simple intravenous glucose tolerance test for assessment of insulin sensitivity.  
Hahn R.G., Ljunggren S., Larsen F., Nyström T.  
*Theoretical Biology and Medical Modelling* 2011, 8:12
- II. Oral nutrition or water loading before hip replacement surgery; a randomized clinical trial.  
Ljunggren S., Hahn R.G.  
*Trials* 2012, 13:97
- III. Insulin sensitivity and  $\beta$ -cell function after carbohydrate oral loading in hip replacement surgery: a double-blind, randomized controlled trial.  
Ljunggren S., Hahn R.G., Nyström T.  
In press, *Clinical Nutrition* 2013
- IV. Can the intravenous glucose tolerance test or the QUICKI and HOMA algorithms predict changes in insulin sensitivity during surgery?  
Ljunggren S., Nyström T., Hahn R.G.  
In press, *Eur J Anaesthesiol* 2013

# CONTENTS

1	LIST OF ABBREVIATIONS .....	8
2	INTRODUCTION .....	9
	Osteoarthritis .....	9
	Hip prosthesis .....	11
	Surgery and metabolic stress .....	14
	Insulin and C-peptide .....	16
	Insulin resistance .....	17
	Insulin secretion and disposition index .....	18
	Measurements of insulin sensitivity and insulin secretion .....	18
	Surgery-induced insulin resistance .....	20
3	AIMS OF THE STUDIES .....	21
4	ETHICS .....	23
5	MATERIALS AND METHODS .....	24
	Participants .....	25
	Study I .....	25
	Study II .....	25
	Study III .....	26
	Study IV .....	27
	Study design .....	28
	Study I .....	28
	Study II .....	28
	Study III-IV .....	30
	Biochemical analysis .....	31
	Cortisol .....	31
	3-methyl-histidine (3-MH) .....	32
	Body fluid volumes (BIA) .....	32
	Preop <sup>®</sup> .....	32
	Insulin sensitivity and insulin secretion .....	33
	Clamp techniques .....	33
	Hyperinsulinemic euglycemic clamp .....	33
	HOMA .....	34
	QUICKI .....	35
	MINMOD .....	35
	IVGTT .....	36
	Complications .....	37
	Well-being .....	38
	W-BQ12 .....	38
	Health index (HI) .....	38
	Chalder's Fatigue Scale (FQ) .....	38
	EQ-VAS .....	39
6	CALCULATIONS .....	40
	Glucose kinetics .....	40
	MINMOD .....	40
	IVGTT .....	41

	HOMA-IR.....	41
	QUICKI.....	41
	CLAMP.....	41
7	STATISTICS .....	42
	Study I.....	42
	Study II.....	42
	Study III .....	43
	Study IV .....	43
8	RESULTS .....	44
	Study I.....	44
	Study II.....	45
	<i>Glucose and insulin</i> .....	45
	<i>Glucose kinetics</i> .....	45
	<i>Insulin</i> .....	45
	<i>Physical stress, catabolism and body fluid volumes</i> .....	46
	<i>Complications</i> .....	46
	<i>Wellbeing</i> .....	46
	Study III .....	47
	<i>Glucose and insulin at baseline</i> .....	47
	<i>IVGTT</i> .....	47
	<i>Glucose clamp</i> .....	47
	Study IV .....	48
9	GENERAL DISCUSSION.....	49
	Limitations .....	53
	Future perspectives .....	55
10	CONCLUSIONS .....	56
11	SAMMANFATTNING (SUMMARY IN SWEDISH) .....	57
12	ACKNOWLEDGMENTS .....	59
13	APPENDIX.....	60
	Komplikationer .....	60
	W-BQ12.....	61
	Hälsindex .....	62
	FQ.....	64
	EQ- VAS.....	65
14	REFERENCES .....	66

# 1 LIST OF ABBREVIATIONS

AUC	Area under the curve
$\beta$ -cell	Beta-cell
BIA	Bioimpedance
C-peptide	Connecting peptide
CL	Clearance
ECF and ICF	Extra- and intracellular fluid
ELISA	Enzyme-linked immunosorbent assays
FFA	Free fatty acids
FSIGT	Frequently sampled intravenous glucose tolerance test
GLUT-4	Glucose transporter isoform-4
HOMA	Homeostasis model assessment
IL-6	Interleukin-6
IVGTT	Intravenous glucose tolerance test
MAP	Mean arterial pressure
$M_{bw}$	Metabolism of glucose after correction for body weight
OA	Osteoarthritis
OGTT	Oral glucose tolerance test
QUICKI	Quantitative insulin sensitivity check index
$r$	Correlation coefficient
$r^2$	Square of the correlation coefficient
RCT	Randomized control study
THR	Total hip replacement
$T_{1/2}$	Half-life
VAS	Visual analog scale
$V_d$	Volume of distribution
W-BQ12	A well-being questionnaire with 12 questions
3-MH	3- methyl-histidine



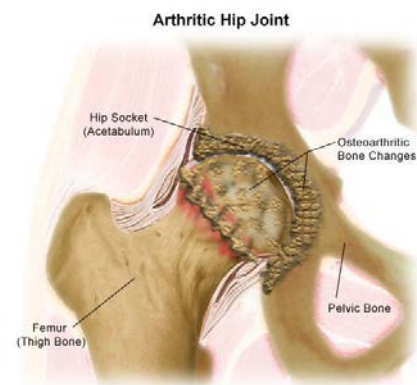
## 2 INTRODUCTION

My interest regarding hydration and nutritional support prior to elective hip replacement surgery arose as a result the extent to which patient recovery differs following the procedure. Many patients experience fatigue for several weeks after the surgery, and this is generally accepted as an expected effect of the surgery. Previous orthopedic research has mostly focused on the outcome of various implants, such as plates, screws and artificial joints. However, we easily overlook the importance of patient well-being and convalescence after surgery.

### OSTEOARTHRITIS

Joint surfaces are covered by a layer of articular cartilage a few millimeters thick. This cartilage distributes load across the joint and reduces friction during movement. The cartilage matrix can be likened to a fiber-reinforced, water retention gel with hygroscopic properties. The synovial tissue which lines the joint capsule secretes and is lubricated by a thin layer of viscous synovial fluid, which contains a high concentration of hyaluronic acid.

On loading, some water is squeezed out of the cartilage tissue, only to be sucked in again when the load decreases. It has been suggested that an optimized loading of the hip joint, through weight reduction and exercise, can slow the progression of the disease (Messier, Loeser et al. 2000)(Swedish social board),



The principle focus of the treatment of osteoarthritis is to reduce pain and improve function. Treatments techniques may be divided into basic, adjunctive and surgical. Currently, there are no drugs that directly affect the disease. In the United States, osteoarthritis is estimated to cost the society 215 billion dollars per year. In Sweden, health economists have calculated that the costs related to musculoskeletal diseases are higher than those for all the diseases that affect the brain and peripheral nervous system combined. Joint disease is the most common chronic disease in older people; more common than hypertension, heart disease or diabetes ([www.boneandjointdecade.org](http://www.boneandjointdecade.org)).

Common symptoms of joint disease are pain and functional impairment, leading to a reduced quality of life. Osteoarthritis can occur in any joint and the most commonly affected are the knee and the hip. People with osteoarthritis of the knee or hip have an increased risk of premature death from cardiovascular disease, with the mortality rate increasing in proportion to the degree of joint function impairment ([www.boneandjointdecade.org](http://www.boneandjointdecade.org)). It is therefore

important to investigate the risk-factors that can be influenced, e.g., high blood pressure, hyperlipidemia, smoking, obesity and low physical activity.

Osteoarthritis, in common with over- or under loading of a joint, changes the metabolic equilibrium, resulting in an imbalance between degradation and repair. The cells try to carry out repairs, but are not able to regenerate a functional matrix and, as a result, the shock-absorbing ability of the joint is progressively lost.

The incidence of osteoarthritis is strongly age-related, rising steeply after 50 years of age. Approximately 15 percent of men and women aged 35–55 years suffer from pain in the knee and/or the hip, often caused by osteoarthritis. In people aged 65 years or older, osteoarthritis is the most common cause of physical functional impairment. For women over 60, osteoarthritis is the fifth most common disease.

More rarely, osteoarthritis can occur in young and middle-aged people, often associated with overweight or obesity, or as a result of a previous joint injury. The risk of osteoarthritis in both knees and hips is related to the degree of excess bodyweight. Weight and previous injury are of greater importance in predicting the development of osteoarthritis than factors such as work-related loading of the joints. There is also a substantial hereditary risk of developing osteoarthritis.

The degree of joint changes and the symptoms experienced are often not directly related. This emphasizes the importance of using disease symptoms to guide diagnosis and treatment.

In cases where a precipitating cause can be identified there might be a time lapse of between 10 and 30 years before osteoarthritis can be diagnosed on X-ray. The primary radiographic criterion for a diagnosis of osteoarthritis is a decreased joint space, which, in turn, is a result of the destroyed articular cartilage. It follows that a routine X-ray examination can only diagnose advanced osteoarthritis, when the cartilage has already been destroyed.

In early stages, X-rays are essentially normal. Severe cartilage damage can, however, be seen on arthroscopy. This may be a contributing factor to the poor correlation between the radiographic signs of osteoarthritis and pain. The relationship with pain is clearer when the radiographic signs of osteoarthritis are severe.

It is pain that usually leads a patient to approach the health service. Initially, pain occurs on movement or load-bearing. Later, the pain is also experienced at rest and overnight. Recent studies have shown that the osteoarthritis, as measured by X-ray, is not always progressive and in approximately half of cases the radiographic changes may not alter for many years{Haugen, 2013}. Patients who receive a new hip joint are advised to refrain from physical activity which may place large loads on the prosthesis and instead encouraged to undertake exercise such as cycling and Nordic walking.

## HIP PROSTHESIS

Elective hip replacement surgery is a very common operation worldwide. In 2011, the number of total hip arthroplasties in Sweden was the same as in 2010. Approximately 16,000 operations were carried out; a rate of 170 per 100,000 inhabitants. The number of hemi-arthroplasties undertaken was also unchanged, with approximately 4,500 operations performed. The numbers of reoperations were 2,200 and 330 respectively.

Modern hip replacement surgery was developed by John Charnley in the late 1960's. His concept of a cemented femoral stem made of a metal alloy, with a socket of polyethylene (total hip replacement/total hemiarthroplasty) (Fig.1.). This became the dominant system used over subsequent years, and it still remains to be the main option for hip arthroplasty in Sweden. There are also another concept where only the femoral stem and head but not the acetabulum are replaced (hemi-arthroplasty)



Fig. 1.

With the help of the Swedish Hip Arthroplasty Register, which started in Gothenburg in 1980, it has been possible to examine how various implant systems perform over time. Charnley's original prosthesis has proved, over many years, to be one of the best, with a revision rate (reoperation because of prosthetic loosening) of less than 10% after 10 years. To date, a total of 23,000 operations have been entered into this registry.

According to data from the Swedish Hip Registry, there are now several newer implant systems with even lower revision rates over a 10-year period. The Swedish prosthetics market is now dominated entirely by five different models, all of which are cemented.

Even by the early 1970's, surgeons had begun experimenting with different concepts to that developed by Charnley. Initial interest focused on whether it was possible to anchor the prosthesis without using the bone cement that Charnley had used and a large number of prostheses using uncemented anchoring were developed. By the early 1980's, many surgeons believed that they could solve the anchoring problems by pushing or screwing the prostheses.

However, the Swedish Registry reveals that most of the newer systems have had a significantly poorer outcome over time, compared to cemented systems. After initially adopting the uncemented systems, most orthopedic surgeons in Sweden have now largely reverted to cemented prostheses. As a result, Sweden now has the lowest revision rate of hip prostheses in the world.

New uncemented prostheses have appeared on the market. These are coated with a material that allows bone to grow into it, resulting in better anchoring, and have shown good results over time (Yamada, Yoshihara et al. 2009). The type of fixation ultimately used is determined by the bone appearance and quality (Hailer, Garellick et al. 2010).

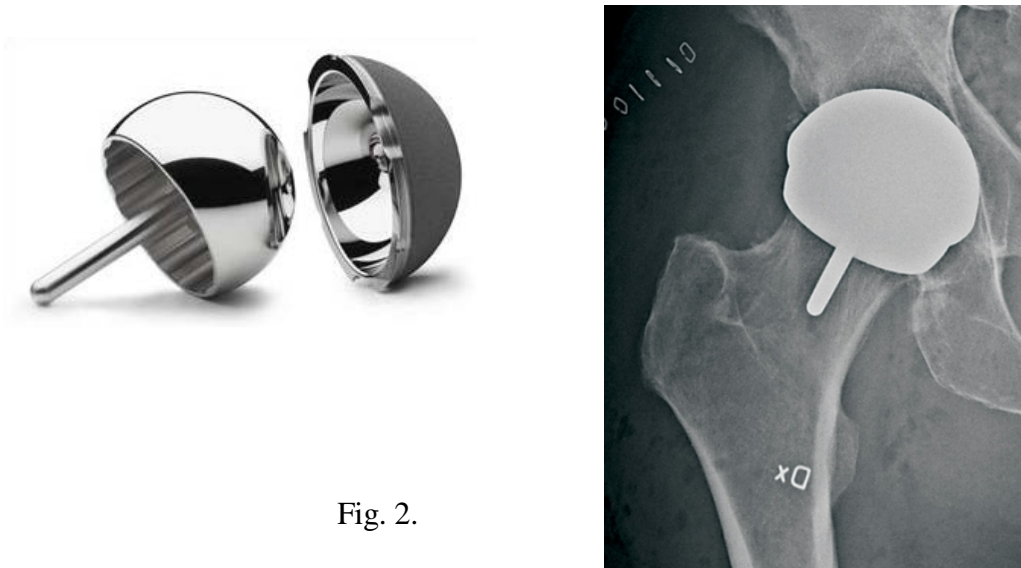


Fig. 2.

Hip resurfacing is an alternative method that is used primarily in younger and physically active patients, especially in males. The principle is that the femoral head is not replaced but the articular cartilage is removed and then replaced by a metallic coat (Fig.2.) (Westacott, McArthur et al. 2013).

Today, minimally invasive surgical techniques have been developed requiring only a small skin incision and the duration of hospital stay and period of convalescence have decreased as a result. However, the learning curve is long and there is a risk of non-optimal placement of the prosthesis due to poor overview of the operation field (De Geest, Vansintjan et al. 2013).

### **Focus on the insertion of the prosthesis**

In recent times attention has also been focused on the approach used to insert the prosthesis. Techniques which do not require osteotomy of the trochanteric part of the hip have gradually been developed (Weaver 1975).

Hardinge developed a technique in which half the gluteus medius muscle is detached from the greater trochanter (Hardinge 1982). This is a very common surgical approach and reduces the risk of backward dislocation. The problem with this technique is that patients may initially develop a Trendelenburg limp. Furthermore, there is evidence that the reinserted gluteus medius sometimes detaches, giving rise to a persistent limp.

Over the same time period, a technique was developed in which the short external rotators at the back of the hip joint are loosened. The technology was not, in itself, new (Gibson 1950), but had not previously been used for the insertion of the prosthetic hip. It can result in minor postoperative problems with paralysis resulting in a slightly higher risk of implant dislocation. This is the result of a reduced backwards stability so that the prosthesis can dislocate in certain

situations, e.g., when the patient raises up from a low chair and then bends the trunk forward while bending the hip (Weeden, Paprosky et al. 2003).

There are a large number of studies that compare the results of the front and rear approaches in hip replacement, and it is not possible to firmly conclude which is the better technique. The most important consideration is, of course, that the surgeon should be familiar with the technology that they are using.

No description can be found in the literature concerning how long a conventional incision should be. There is considerable variation, depending on the patient height and weight. Most orthopedic surgeons would probably use an incision of approximately 20 cm for the insertion of a hip prosthesis in the conventional way.

The average length of stay for a patient undergoing total hip replacement is largely dependent on the patient's overall state of health. An otherwise healthy 70-year-old patient would, on average, have a hospital stay of 3–5 days, while an 85-year-old patient with other diseases might have a hospital stay of twice that. There are also local variations regarding treatment times. In healthier patients, the information that they receive preoperatively can also help to shorten the length of their stay. The average operation time for conventional hip replacement surgery is about 90 min.

## **SURGERY AND METABOLIC STRESS**

Transient insulin resistance is one of the most basic reactions to injury (Thorell, Nygren et al. 1999). The neuroendocrine and inflammatory systems become activated within minutes and put the body into a metabolic state of stress (Kratzing 2011, Vigano, Cereda et al. 2012). This state involves a cascade of reactions including reduced insulin sensitivity (similar to type 2 diabetes) (Brandi, Frediani et al. 1990, Ljungqvist, Nygren et al. 2000), increased stress hormone secretion, and increased muscle breakdown (Hedstrom, Ljungqvist et al. 2006, Ljungqvist, Soop et al. 2007). The stress-response arrests anabolic processes and initiates a catabolic state, enabling the release of reserves of fuel and building blocks from all bodily stores (fat, carbohydrate, and protein) with the production of acute phase proteins and increased tissue healing (Thorell, Nygren et al. 1999, Ljungqvist 2010). A central feature of this massive change in metabolism is the development of insulin resistance.

Insulin is the body's most important anabolic hormone but its effectiveness is reduced in catabolic states (Ljungqvist, Thorell et al. 1994, Thorell, Efendic et al. 1994). In studies by Thorell et al. (Thorell, Efendic et al. 1993, Thorell, Nygren et al. 1999) the degree of insulin resistance was found to be related to the magnitude of the surgery. This change in metabolism may last for up to 2 weeks, even after a moderate surgical stress (Thorell, Nygren et al. 1999). Studies suggest that insulin resistance is one of the key mechanisms triggering several common surgical complications (Ljungqvist, Nygren et al. 2000).

Traditionally, insulin resistance has been regarded as the way the body responds to stress, and it was usually believed to be beneficial (Ljungqvist, Nygren et al. 2000). Indeed, studies of certain severely stressful situations have shown insulin resistance to be a key to survival (Robinson and van Soeren 2004). This is true in cases of acute hemorrhage, where insulin resistance is necessary to mobilize glucose to assist in fluid movement and plasma replacement (Ljungqvist, Sandberg et al. 1989, Ljungqvist and Alibegovic 1994). However, recent studies of elective surgical patients have suggested that in modern surgical practice insulin resistance is harmful and prolongs recovery (Thorell, Nygren et al. 1999). Occasionally, nausea and fatigue may persist for several weeks of the convalescence period (Hausel, Nygren et al. 2005, Ashby, Grocott et al. 2008, Lauwick, Kaba et al. 2009). Hence, these studies suggest that insulin resistance is an important parameter to monitor after surgery because it might influence the course of the patients' convalescence (Wiklund and Romanus 1991, Ostendorf, van Stel et al. 2004, Riediger, Doering et al. 2010).

Several studies have shown that transiently reduced insulin sensitivity may be limited, or prevented, by giving insulin (Brandi, Frediani et al. 1990), a preoperative intravenous infusion (Ljungqvist, Thorell et al. 1994, Nygren, Thorell et al. 1998) or oral administration of glucose (Nygren, Soop et al. 1999, Ljungqvist 2010).

A recently published meta-analysis (Li, Wang et al. 2012), based on 13 trials comparing a carbohydrate drink with placebo (flavored drinking water), included three randomized studies with patients undergoing total hip replacement (Soop, Nygren et al. 2001, Soop, Nygren et al.

2004, Aronsson, Al-Ani et al. 2009). A smaller increase in glucose level was observed at the end of surgery in those patients who had been given a carbohydrate drink, compared with placebo, but there were no statistical differences in insulin level at the end of surgery or in glucose levels at the induction of anesthesia. The overall result of this meta-analysis, which also included colorectal surgery, laparoscopic cholecystectomy, and cardiac surgery, could not make a definitive conclusion regarding the usefulness of a carbohydrate drink in hip surgery, as it only included a small number of such studies. In addition, the quality of the evidence obtained for most outcomes was relatively low. It is not clear whether a carbohydrate drink can affect postoperative catabolism, although insulin resistance has been correlated with catabolism and intracellular dehydration following hysterectomy (Strandberg and Hahn 2005). The benefit of a nutritional carbohydrate drink with regard to overall postoperative wellbeing was, when I began my studies, still unknown (Wiklund and Romanus 1991, Ostendorf, van Stel et al. 2004).

## INSULIN AND C-PEPTIDE

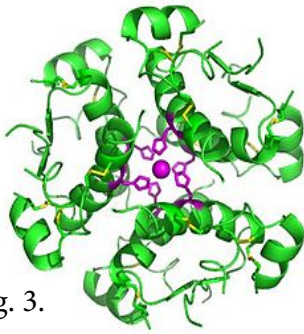


Fig. 3.

Insulin (Fig. 3.) is an anabolic hormone which is secreted by the pancreatic  $\beta$ -cells in response to increased circulating levels of glucose and amino acids. It is essential for maintaining glucose homeostasis and prevents hyperglycemia by reducing hepatic glucose output, by decreasing gluconeogenesis and glycogenolysis, and by increasing the rate of glucose uptake, primarily into skeletal muscle, adipose tissue and the liver (Thorell, Nygren et al. 1999). Glucose is then stored, as glycogen, in the liver and skeletal muscle. In muscle and fat cells, the clearance of circulating glucose depends on the

insulin-stimulated translocation of the glucose transporter iso-form-4 (GLUT-4) to the cell surface (Fig. 4.) (Shulman 2000). In skeletal muscle, GLUT-4 is the rate-controlling step for insulin-stimulated muscle glycogen synthesis (Shulman 2000).

Insulin also profoundly affects lipid metabolism, increasing lipid synthesis in liver and fat cells and attenuating free fatty acid (FFA) release from triglycerides (Kahn 2003). The action of insulin is initiated through binding to, and activation of, the cell surface receptor (Saltiel and Kahn 2001). The receptor then undergoes a series of intra-molecular reactions, stimulated by insulin, resulting in glucose uptake, through the activation of GLUT-4. The body reacts to surgical stress with a change in metabolism to a catabolic state. This, in turn, can affect these intra-molecular reactions, resulting in insulin resistance. This is further aggravated by the fasting state commonly found preoperatively.

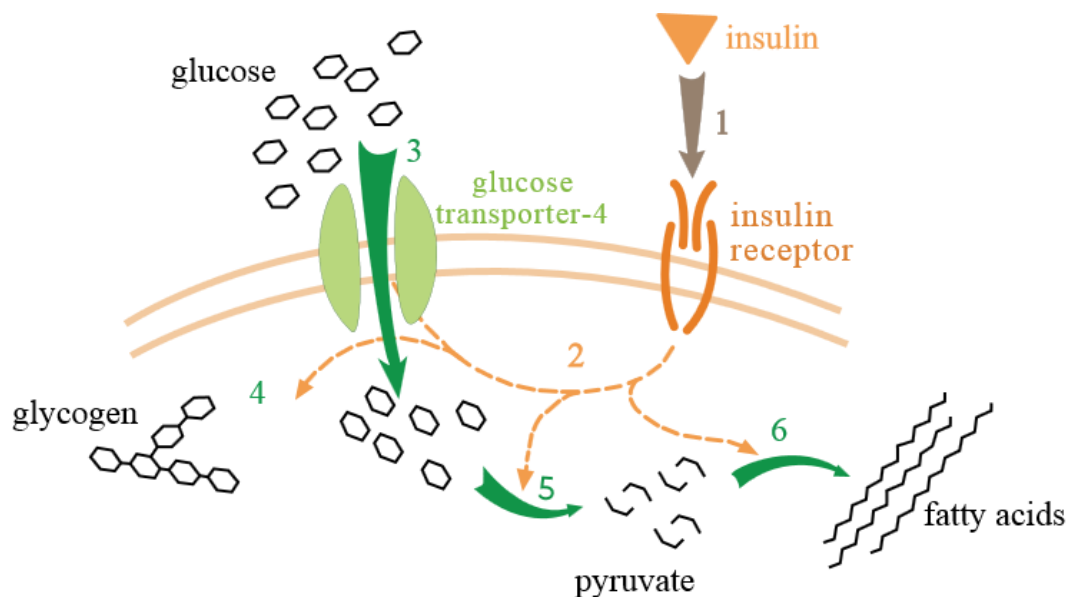


Fig. 4.



C-peptide is a byproduct of insulin production and is cleaved off during storage in the  $\beta$ -cells in the pancreas secretion granules (Fig. 5.). Exogenous insulin does not contain C-peptide, making it possible to assess endogenous insulin production, which is important in diabetes care (Hope, Jones et al. 2013). Unlike insulin, C-peptide is not taken up by the liver. The uptake of insulin by the liver is about 50% of the total insulin secretion under basal conditions, but varies between individuals and is influenced by factors such as liver disease and other metabolic conditions (Pacini and Mari 2003). The C-peptide concentration can therefore reflect the secretion of insulin in a more precise way than insulin concentration measurements can. It is eliminated through kidney glomeruli and metabolized mainly in the tubules (Pietropaolo 2013).

**Proinsulin molecule**

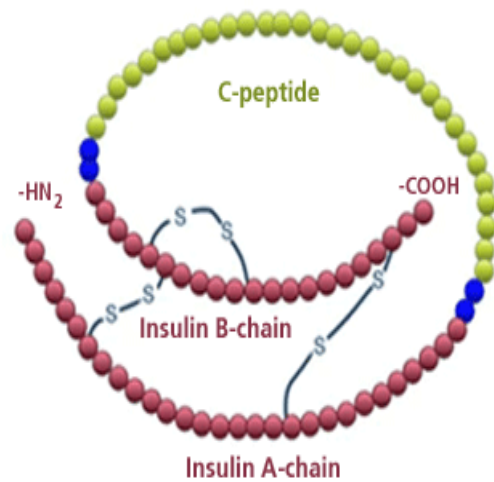


Fig. 5.

## INSULIN RESISTANCE

The opposite of insulin sensitivity is insulin resistance. In this condition more insulin is required to slow sugar production by the liver and to stimulate the uptake of sugar into the muscles, i.e., the normal amount of circulating insulin is insufficient to regulate glucose uptake by the cells (Reaven 1997).

Insulin resistance is defined as an impaired glycemic response to either exogenous or endogenous insulin action. Initially, the pancreatic cells ( $\beta$ -cells) manage to compensate for insulin resistance through an increase in insulin secretion, often seen in clinical practice as an increase in plasma insulin concentrations (Hunter and Garvey 1998, Saltiel 2000).

This might occur in response to some kind of stress (such as surgery), infection, intake of cortisone, too little exercise and if there are high blood lipid levels.

High blood lipid levels increase the deposition of lipids in the liver and muscles, which in turn slows insulin action (insulin resistance) (Fellander, Nordenstrom et al. 1994, Reaven 1997, Bacha, Lee et al. 2010). Exercise, i.e. muscle work, has the effect of reducing the amount of insulin required to maintain normal blood sugar levels, which is comparable with enhanced insulin sensitivity (Wallberg-Henriksson, Rincon et al. 1998).

It has been observed that some risk factors for type 2 diabetes and cardiovascular disease (coronary heart disease, stroke, peripheral vascular disease) can interact with each other, increasing the overall risk of morbidity or mortality (Reaven 1997, Laakso 1999). This accumulation of risk factors is usually called “the metabolic syndrome”. This syndrome can be

defined in many different ways, but no internationally accepted definition exists today. Many scientists also query whether the syndrome exists at all, but most agree that the presence of several of the syndrome criteria increases the risk of developing type 2 diabetes and cardiovascular disease (Kahn, Buse et al. 2005). The risk factors included in the metabolic syndrome: are abdominal obesity (increased waist circumference: for men >102 cm and for women >88 cm), elevated blood lipids, increased blood pressure, and elevated fasting plasma glucose (Borai, Livingstone et al. 2007, Muniyappa, Lee et al. 2008, Bacha, Lee et al. 2010).

## INSULIN SECRETION AND DISPOSITION INDEX

The assessment of pancreatic  $\beta$ -cell function in humans is challenging because of the complex interplay between insulin sensitivity, insulin secretion, and hepatic insulin extraction, which interact with each other in complicated closed loop systems (Ahren and Pacini 2004, Cobelli, Toffolo et al. 2007, DeFronzo 2009). Basically, the relationship between insulin sensitivity and insulin secretion can be described by an approximate hyperbola (Fig. 6), (Kahn, Prigeon et al. 1993, Ferrannini and Mari 1998, Muniyappa, Lee et al. 2008) with the product of the two variables being constant for individuals with the same degree of glucose tolerance, i.e. disposition index (Kahn 2003, Faerch, Bruns et al. 2010).

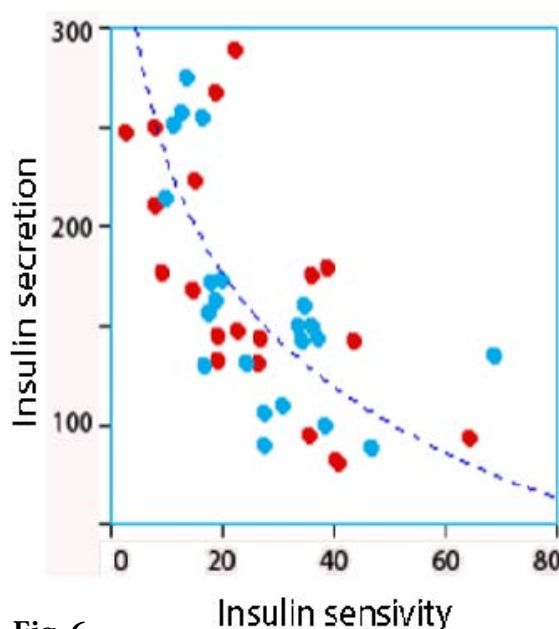


Fig. 6

Several techniques can be used to estimate pancreatic  $\beta$ -cell function, such as data derived from the frequently sampled i.v. glucose tolerance test (FSIGT), the oral glucose tolerance test (OGTT), the glucose-dependent arginine stimulation test or the euglycemic hyperinsulinemic clamp technique, in combination with a test of insulin secretion.

## MEASUREMENTS OF INSULIN SENSITIVITY AND INSULIN SECRETION

A problem encountered when assessing insulin sensitivity is that the measurements are demanding and complex. The test widely used, and regarded as the gold standard, is the *hyperinsulinemic euglycemic clamp* (DeFronzo, Tobin et al. 1979).

The principle behind this glucose clamp test is to maintain a predetermined glucose level during a constant insulin infusion. Assessment of insulin secretion can be made by the *hyperglycemic glucose clamp*. Here, the insulin secretion is measured in a situation of steady

state hyperglycemia. In both cases, measurements of the plasma insulin and C-peptide concentrations (allowing prehepatic insulin secretion to be calculated) are made while the infusion rate of glucose is governed by frequent measures of plasma glucose on site. In this test multiple samples of insulin and C-peptide concentrations are analyzed.

A conventional *glucose tolerance test* gives a good indication of insulin resistance but is difficult to implement during surgery, being more suitable to a laboratory environment. It is therefore natural that a number of methods have been developed to study  $\beta$ -cell function and insulin sensitivity in vivo.

Some of these tests are easy to perform and can be used as a clinical examination, for example, QUICKI and HOMA-IR, which are static tests. These are based on the plasma glucose and insulin concentrations at baseline and do not take account of any variation – “the dynamics”. These tests were originally designed for population studies in diabetic research (Beard, Bergman et al. 1986, Katz, Nambi et al. 2000) but their usefulness in capturing surgery-induced changes in insulin resistance is unclear.

Other tests are more invasive. The *intravenous glucose tolerance test* (IVGTT) is, together with the glucose clamp method, denoted “dynamic tests”. The IVGTT estimates insulin resistance from the balance between plasma insulin levels over time (stimulus) and the half-life of glucose (effect) throughout the metabolism of an intravenous glucose load. The endogenous insulin secretion can also be used as a measure of the  $\beta$ -cell function which of course, can not be used during euglycemic glucose clamp (Pacini and Mari 2003).

The *oral glucose tolerance test* (OGTT) is considered as a simple test for estimating both insulin sensitivity and  $\beta$ -cell function. It is used to diagnose glucose intolerance and diabetes. The effects of the oral glucose load are affected by, in addition to insulin secretion and insulin sensitivity, other factors such as gastrointestinal glucose absorption. This is probably the reason that the method has a relatively poor reproducibility (25% intra-individual variation) (Matsuda and DeFronzo 1999, Pacini and Mari 2003). The calculated indices do not bear simple relationships with glucose and insulin data, and the insulin sensitivity and  $\beta$ -cell function are determined using equations that have an empirical basis. The validity of these indices clearly depends on the validity of the assumptions made in determining them; this is the main weakness of the OGTT method (Borai, Livingstone et al. 2007). For this reason we refrained from using this method.

The QUICKI and HOMA-IR tests, in contrast, reflect the balance between glucose and insulin in the absence of any metabolic challenge, and are believed to reflect hepatic insulin resistance (Abdul-Ghani, Jenkinson et al. 2006). The clamp test represents insulin resistance in peripheral tissues and in reaction to surgery.

We wished to develop an IVGTT with a reduced number of data points, without compromising measuring accuracy, which would only marginally affect a patient's physiology and plasma volume. This improvement would make it possible to use this test in a clinical setting, such as surgery, to measure a patient's insulin sensitivity.

## **SURGERY-INDUCED INSULIN RESISTANCE**

Hormones, such as catecholamines, cortisol, glucagon, and growth hormones, cause insulin resistance (Thorell, Nygren et al. 1999). Epidural anesthesia blocks catecholamine release which reduces the degree of postoperative insulin resistance (Uchida, Asoh et al. 1988). Cytokine release may also cause insulin resistance. In addition, the degree of postoperative insulin resistance following simple elective procedures is correlated with interleukin-6 (IL-6) levels (Thorell, Loftenius et al. 1996). This release of hormones during surgery pushes the body into a catabolic state, with increased metabolism and a diversion of nutrients away from their previous uses. It also leads to the suppression of immune function through atrophy of lymph nodes, decreased lymphocytes and IgA production, and inhibition of cellular immunity (Gunerhan, Koksall et al. 2009). The result of this suppression is an increased risk of infection. While these mediators may not entirely explain surgery-induced insulin resistance, several studies have suggested the involvement of stress hormones and cytokines in the development of this condition.

Insulin resistance develops in both skeletal muscle and adipose tissue in response to surgery (Nygren, Thorell et al. 1997). There is also a small but significant increase (10–15%) in glucose production after surgery (Nygren, Thorell et al. 1997).

A recent meta-analysis that included a variety of methods of assessing insulin sensitivity suggested that preoperative oral carbohydrates reduce surgery-induced insulin resistance (Awad, Varadhan et al. 2013). Some authors have also shown a beneficial effect on surgery-induced insulin resistance through the use of the same techniques as developed here (Nygren, Soop et al. 1998, Soop, Nygren et al. 2001). However, not all studies have demonstrated that preoperative oral carbohydrate loading has a beneficial effect on insulin resistance (Vigano, Cereda et al. 2012).

It is still unclear whether the amount of fluid in the carbohydrate drink can, in itself, affect the post-surgical reaction. Glucose has the effect of translocating fluid into cells, and this may counter the dehydration that occurs during surgery (Stranberg, Hahn 2005, Berndtson, Olsson et al. 2008).

We wanted to study whether the quantity of fluid found in a commonly used carbohydrate drink (preOp<sup>®</sup>) contributes to, or can fully explain, the beneficial effects of the carbohydrate drink on surgery-induced insulin resistance. As a further study, we wanted to compare similar groups of patients who had received a hip prosthesis to see if a carbohydrate drink affects insulin sensitivity and  $\beta$ -cell function, as measured using the glucose clamp technique and IVGTT, respectively.

### **3 AIMS OF THE STUDIES**

The overarching goal of this project was to improve the wellbeing of patients undergoing hip replacement surgery through modification of fluid and nutritional therapy.

The first step in this process, and also the general aim of the present Thesis, was to increase the opportunities to assess insulin resistance through the use of methods that can be applied in the surgical setting.

The specific individual study aims:

#### **Study I**

To find a simple intravenous glucose tolerance test (IVGTT) that can be used to monitor insulin sensitivity in the surgical setting.

#### **Study II**

To study whether some of the effects of the carbohydrate drink on insulin resistance and wellbeing following elective hip replacement surgery can be attributed to the fluid volume component (1.2 L) of the drink.

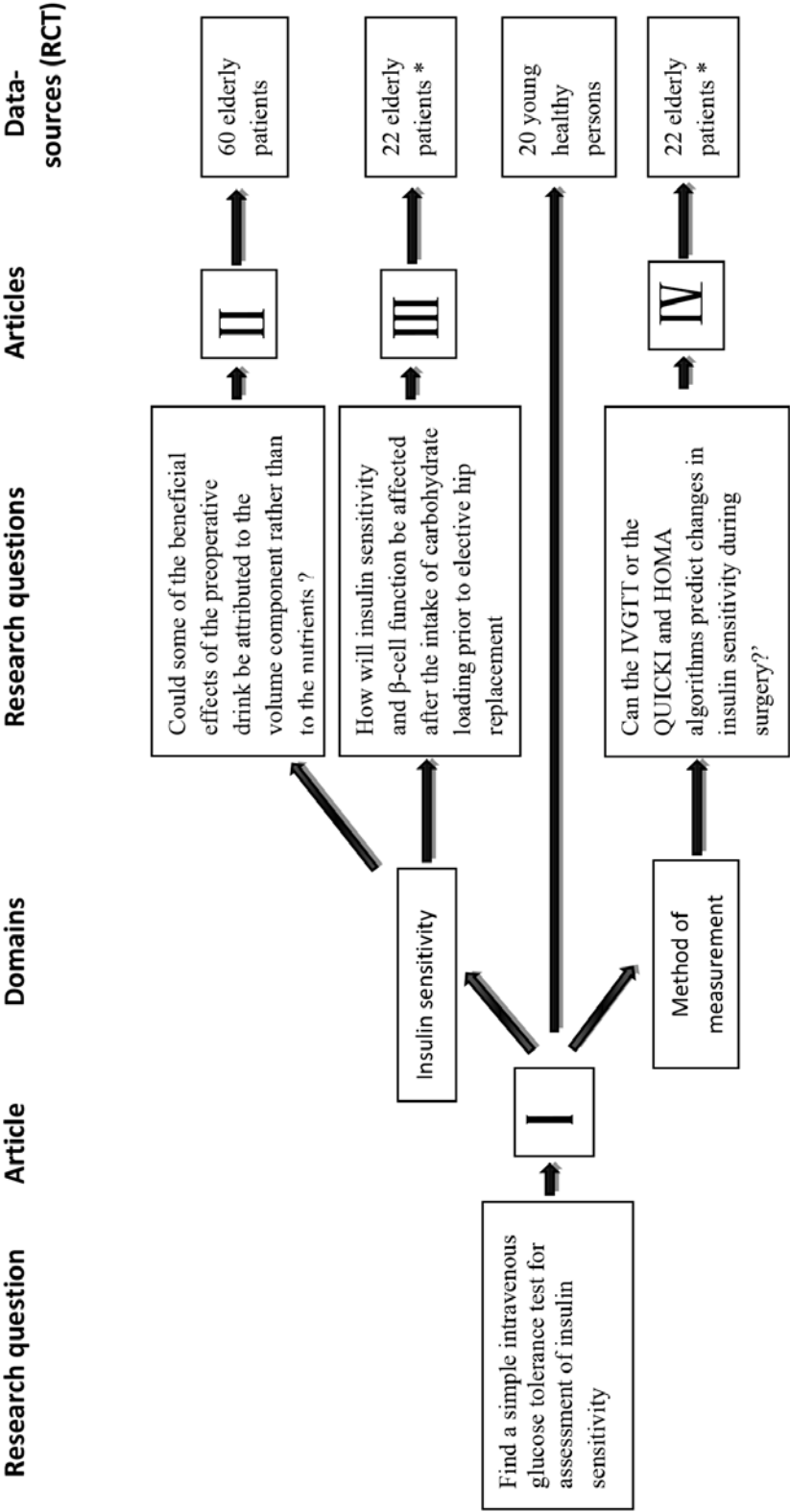
#### **Study III**

Due to the results of Study II, this study investigated the effects of carbohydrate loading on insulin resistance following elective hip replacement surgery using both the IVGTT and the euglycemic hyperinsulinemic clamp methods.

#### **Study IV**

To compare the effectiveness of commonly used methods (IVGTT, QUICKI and HOMA) in assessing surgery-induced development of insulin resistance.

Overview



\* same patient group

## 4 ETHICS

All human studies were conducted according to the Helsinki declaration and approved by the Ethics Committee of the Karolinska Institutet. The applications have the following identification numbers:

2007/1628-31/4,  
2007/1670-31,  
2011/1141-31/3.

In all studies (I–IV), each volunteer/patient gave their written consent to participate.

The studies were also registered at Clinical Trial Gov.  
Study II (NCT 01211184) and  
Study III–IV (NCT 01774084)

## 5 MATERIALS AND METHODS

Apart from in Study I, all patients were scheduled for elective hip replacement surgery and received both oral and written information concerning their participation in the study.

An overview of study design and methods is presented in Table 1.

Study	Study population	No. of	Design	Data collection	Methods of analysis
I	Healthy volunteers 2008 -2009	20	Methodological study	<ul style="list-style-type: none"> <li>• P-glucose,-insulin, -HbA1c,</li> <li>• B-Hb,</li> <li>• S-creatinine,-Na,-K</li> </ul>	Simple/multiple linear regression analysis, Friedman's test
II	THR surgery 2008-2009	57 (3 excluded)	RCT (open)	<ul style="list-style-type: none"> <li>• S-cortisol, -creatinine</li> <li>• P-glucose, -insulin</li> <li>• U-creatinine,-3-MH</li> <li>• Bioelectrical impedance</li> <li>• MAP</li> <li>• Reg. complications and well-being</li> </ul>	ANOVA, Kruskal-Wallis test, Wilcoxon's matched-pair test, Chi-square, Cronbach's alpha coefficient
III	THR surgery 2011-2012	23 (23 excluded)	RCT (double-blind)	<ul style="list-style-type: none"> <li>• P-glucose,-insulin, -C-peptide</li> </ul>	Paired/unpaired t-test, Wilcoxon's matched-pair test, Mann-Whitney U-test
IV	THR surgery 2011-2012	22 (24 excluded)	Observational study	<ul style="list-style-type: none"> <li>• P-glucose,-insulin</li> </ul>	Simple linear regression, Wilcoxon's matched-pair test



## PARTICIPANTS

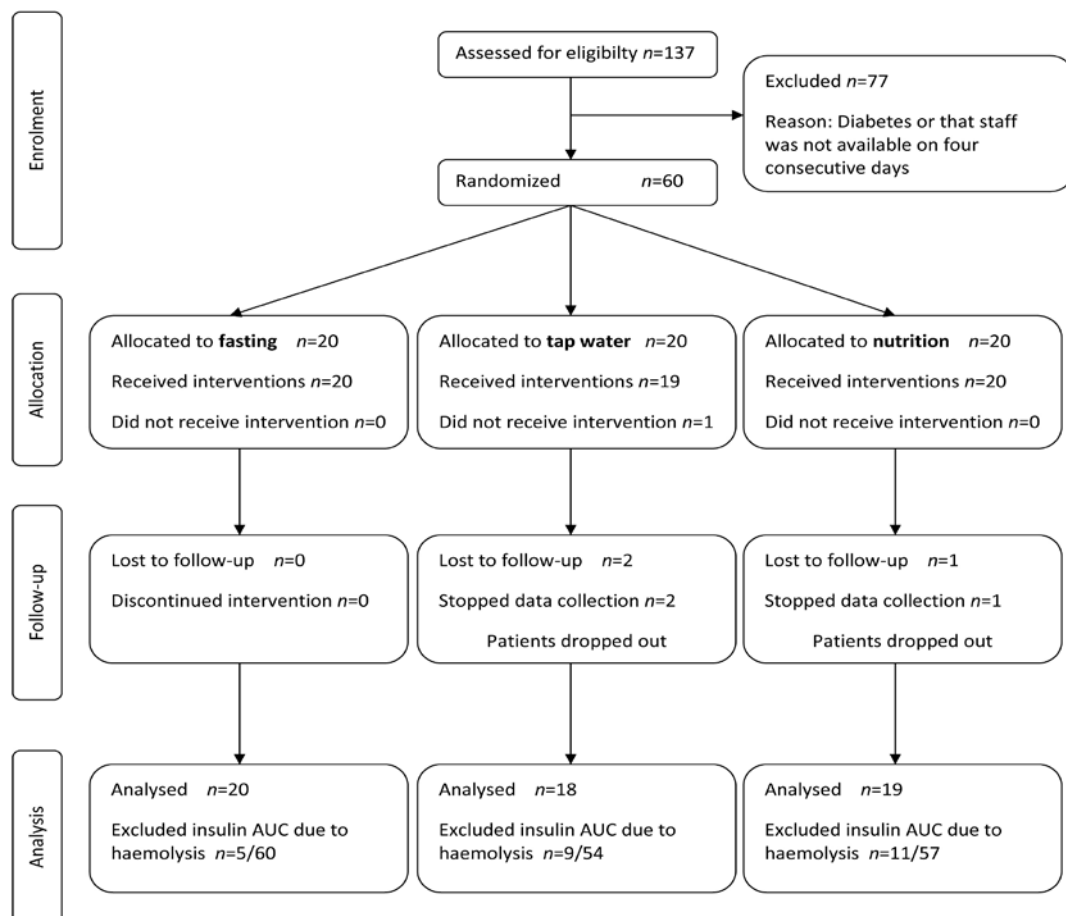
### Study I

Between May 2008 and May 2009, 20 volunteers (8 females and 12 males), aged between 18 and 50 (mean 28) years, were studied. Exclusion criteria were the presence of endocrinological disorders. Routine blood chemistry was used to confirm the absence of metabolic disease.

### Study II

Between May 2008 and September 2009, 60 patients (41 females and 19 males), aged between 44 and 89 years of age (mean age, 69 years), were studied in an open randomized clinical trial (RCT) while undergoing elective total hip replacement under spinal anesthesia.

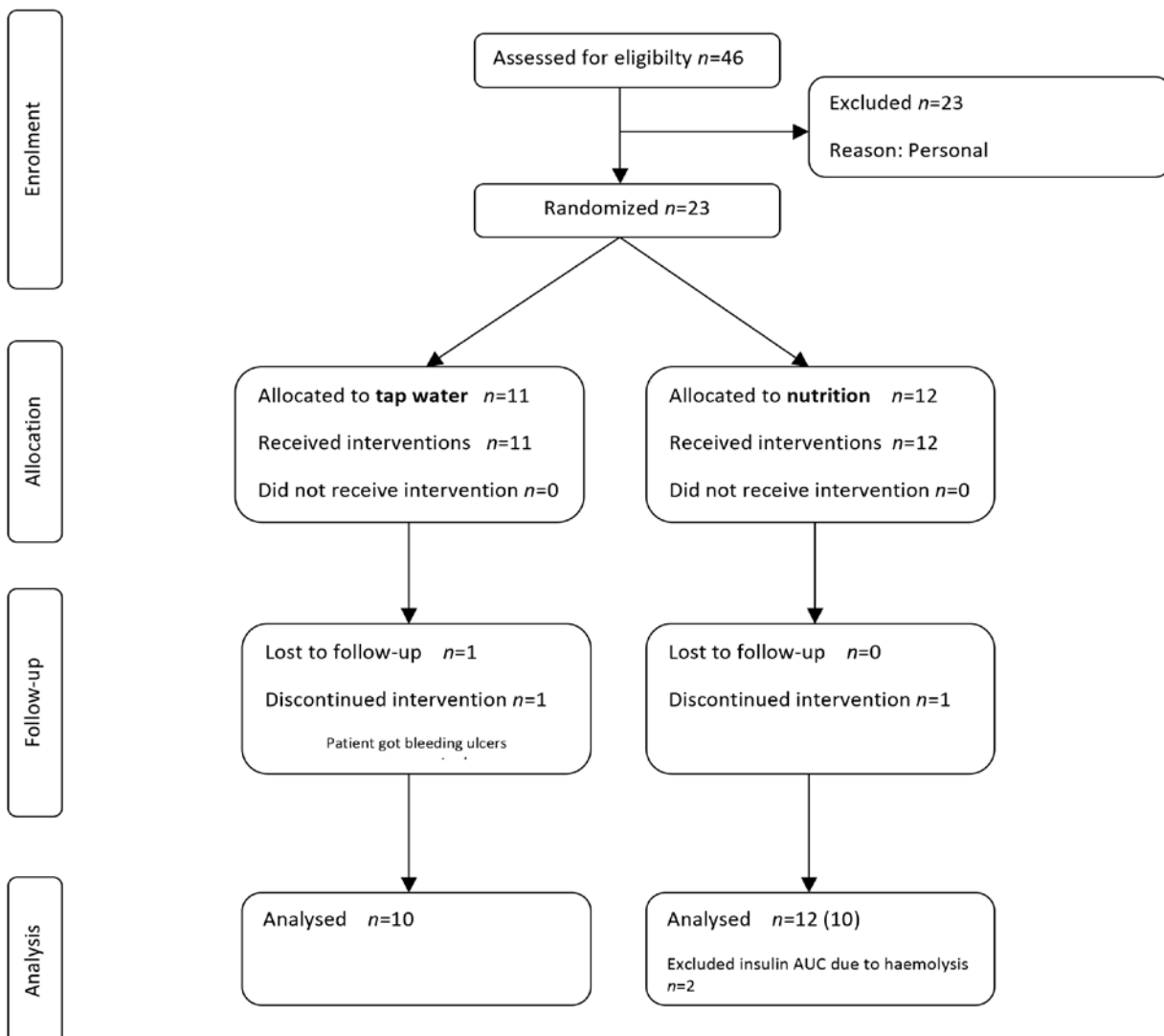
Exclusion criteria were the presence of endocrinological disorders, including diabetes, and treatment with cortisone. Each patient gave their informed consent to participate. The most common diseases affecting the patients included hypertension ( $n=17$ ), previous thrombosis ( $n=4$ ), chronic obstructive pulmonary disease ( $n=3$ ), and previous cardiovascular surgery ( $n=3$ ).



## Study III

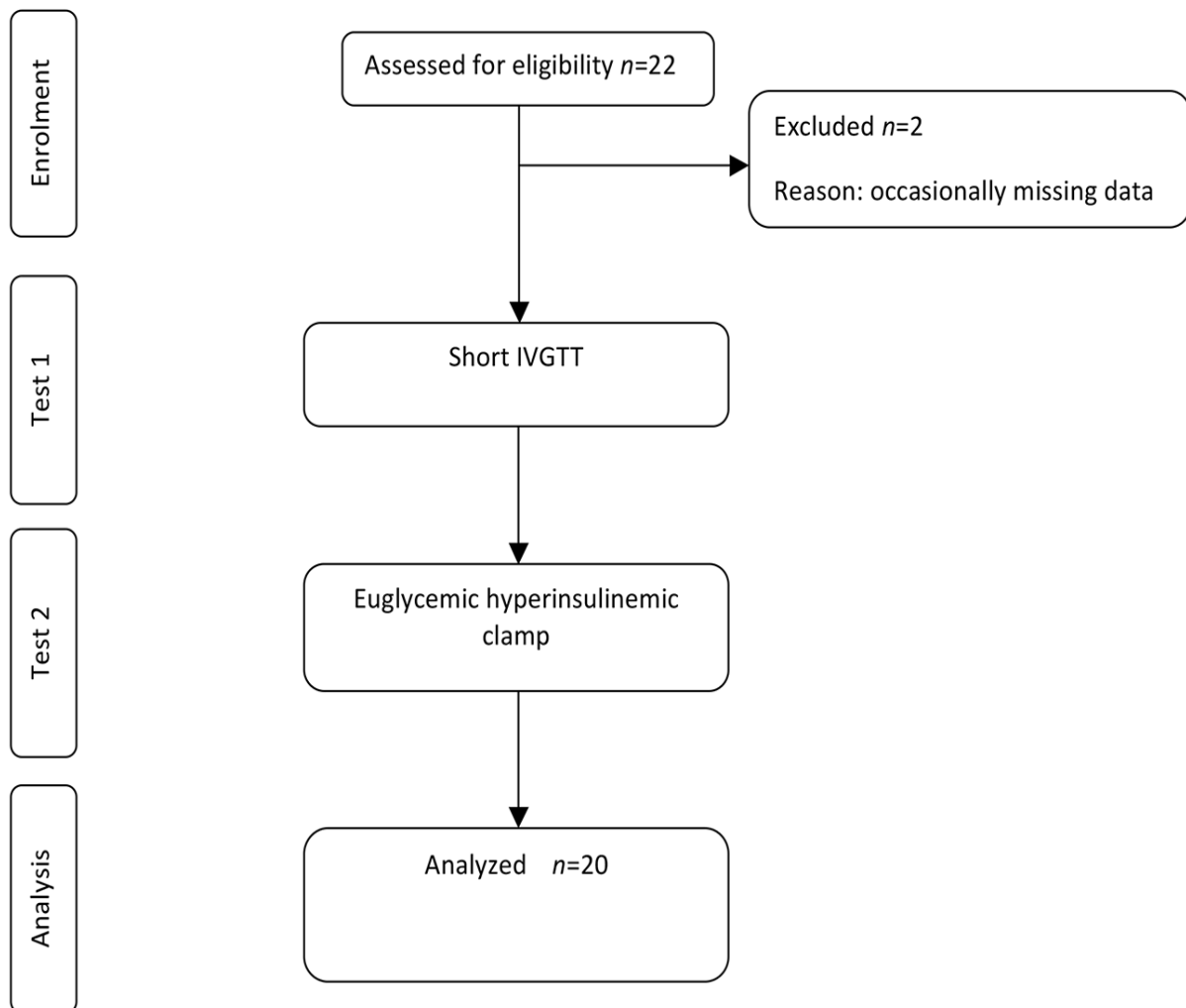
Between December 2011 and November 2012, 46 individuals were asked to participate in this study. Twenty-three subjects (16 females and 7 males), aged between 57 and 76 years (mean: 68 years), agreed to take part. All patients were scheduled to undergo elective total hip replacement surgery at the Orthopedic Departments at Södersjukhuset, Stockholm, Sweden and/or Södertälje hospital, Södertälje, Sweden.

Exclusion criteria were known diabetes, renal impairment (serum creatinine  $>150$   $\mu\text{mol/L}$ ), liver disease, blood hemoglobin (Hb) concentration  $<110$  g/L, severe ongoing infection, malignancy and mental illness. After enrolment, one patient in the placebo group decided not to participate.



## Study IV

This used the same patients as in Study III. Twenty-two non-diabetic patients (15 females and 7 males), aged between 57 and 76 years (mean 68 years), were studied between December 2011 and November 2012. Each patient was scheduled to undergo elective total hip replacement surgery at the Orthopedic Departments at Södersjukhuset, Stockholm, or Södertälje Hospital, Södertälje. Exclusion criteria were known diabetes treated with oral medication or insulin, renal impairment (serum creatinine  $>150 \mu\text{mol/L}$ ), liver disease, severe ongoing infection, malignancy, and mental illness. This study was part of a larger project assessing the intake of a nutritional drink just prior to surgery (Study III).



## **STUDY DESIGN**

### **Study I**

This was a methodological study. The volunteers, after 12 h of fasting, reported to the metabolic laboratory at Södersjukhuset between 7.30–8.00 AM. Baseline blood samples were drawn for measurement of plasma insulin and glucose. A hyperinsulinemic euglycemic clamp was then initiated for 120 min. The plasma glucose was measured every 5 min.

On the second occasion, separated by 1–2 days from the clamp study, and after 12 h of fasting, a regular IVGTT was performed. Blood was sampled at 0, 2, 4, 6, 8, 10, 20, 30, 40, 50, 60, and 75 min.

### **Study II**

After all baseline parameters had been measured, each patient was randomly assigned to one of three study groups, using the sealed envelope method. This took place in the orthopedics department on the day before the surgery. Prior to the start of the study, 60 blank envelopes were prepared. The treatment group number was written on a blank piece of paper, in the proportion 1:1:1 and one placed in each envelope. The envelopes were mixed together in a large bucket and randomly drawn and opened by the research nurse in the presence of the patient.

The groups were:

- Fasting: no food or water from midnight before the surgery (control).
- Tap water: 800 mL by mouth, 2 h before entering the operating room.
- Nutrition: a carbohydrate drink (50 kcal/100 mL; preOp®, NutriciaNordica AB, Stockholm, Sweden) 800 mL in the evening before the surgery (Day 0) and 400 mL 2 h before entering the operating room (Day 1).

Cemented total hip replacements were performed under spinal anesthesia (cement was avoided in three middle-aged patients).

Patient monitoring consisted of pulse oximetry, non-invasive blood pressure and electrocardiogram tests.

At the end of the surgical procedure, patients with anything more than minimal bleeding were given 1 g of tranexamic acid intravenously (Cyklokapron, Meda AB, Solna, Sweden).

Postoperative pain was managed using an epidural catheter with a continuous infusion of levobupivacaine and sufentanil ( $n=22$ ) or a wound catheter with ropivacaine and ketorolac ( $n=25$ ; the hospital practice changed during the study period). All patients were also given oral paracetamol. Oral oxycodone served as the rescue pain reliever.

Measure	Day 0	Day 1	Day2	Day 3	Day 14
Glucose tolerance test	X	X	X		
Surgery		X			
Urine collection (cortisol, 3-MH)		→X	→X		
Bioimpedance	X	X	X		
Complications by protocol				X	
Well-being	X X X X			X	X X X X
Randomization	X				

A short IVGTT was performed in the fasting state on Day 0, 2 to 3 h after surgery on Day 1, and before breakfast on Day 2. Blood samples were taken at baseline and at 10, 20, 30, 45, 60, and 75 min.

Bioelectrical impedance analyses were carried out and the mean of three successive recordings, based on the preoperative body weight, was used.

The serum cortisol concentration was measured 2 to 3 h after the surgery ended.

Urine samples were collected from the catheter in the urinary bladder, just before the operation started and up to the morning of Day 2. A second urinary collection program began on the morning of Day 2 and ended on the morning of Day 3.

The breakdown of muscular protein (3-MH) was quantified in the urine collected on Day 2 and Day 3. All patients were served lacto-vegetarian food from the day before surgery (Day 0) and throughout their hospital stay to prevent any confounding effects from the ingestion of exogenous meat on the excretion of 3-MH.

The data on complications were collected in three different ways and four scales previously used and validated in healthcare assessed life quality. The patient filled in these forms in the afternoon on the day before surgery. A research nurse interviewed the patients, and re-completed the forms, 2 weeks post-surgery. The health index (HI) was also applied on the second postoperative day.

The Swedish text used for these scales is shown in the Appendix.

## **Studies III–IV**

The patients reported at the metabolic laboratory at 8 AM on the day before and two days after surgery. They underwent an IVGTT (a bolus of glucose of 300 mg/kg in a 30% solution) to test  $\beta$ -cell function, followed, approximately 15 min later, by a euglycemic hyperinsulinemic clamp to quantify peripheral insulin sensitivity. On both occasions, the patients had fasted since midnight

Upon completion of the first day's measurements, the patients were randomized using the sealed envelope method to receive either tap water (placebo) or the carbohydrate beverage (preOp<sup>®</sup>). The sealed envelope was opened by staff at the laboratory. The research leader and the patient were unaware of the randomization result.

The patients received the fluid in identical containers. They were instructed to ingest one bottle containing 800 mL of fluid at bedtime, no later than midnight, and another bottle containing 400 mL approximately 2 h before surgery. Aside from this fluid, the patients did not receive any other beverage or food from midnight until the start of the surgery.

Ringer's acetate could be given for volume replacement upon the induction of spinal anesthesia. This was administered to all except three patients, who received general anesthesia. Infusion fluid during the surgery was provided at the discretion of the anesthetic team. The total hip replacement method used was totally cemented, partially cemented or totally cementless.

Postoperative care followed established clinical routines and included free intake of food and drink, as well as rehabilitation with a physiotherapist.

Two days after the surgery, the patients returned to the laboratory for the second insulin sensitivity measurement, which was performed using the same procedure as used for the preoperative measurement.

## BIOCHEMICAL ANALYSIS

### Cortisol

One way of quantifying physical stress is to measure the cortisol formed in the adrenal cortex, which is derived from cholesterol. This is the body's main stress hormone.

The synthesis and secretion of cortisol is stimulated by ACTH (adrenocorticotrophic hormone), which is formed in the pituitary gland. The concentration of cortisol in plasma follows ACTH's diurnal variation, with the highest values in the morning and lowest at midnight. Elevated cortisol levels in the blood inhibit the secretion of ACTH and vice versa.

In the plasma, cortisol is transported primarily bound to transcortin. Approximately 75% of the cortisol in circulation is bound to transcortin with the rest bound to serum albumin (Sandberg, Woodruff et al. 1964). Only the free fraction (about 10%) is biologically active. Trauma (such as surgery), burns, infections, hypoglycemia and other forms of stress will normally greatly increase ACTH and cortisol secretion.

The majority of cortisol is metabolized in the liver and excreted as various inactive derivatives in the urine. Normally only small amounts of free cortisol are found in the urine. The binding capacity of transcortin will be exceeded if serum cortisol levels are markedly increased, resulting in an increase in the excretion of free cortisol in the urine.

The analysis of serum and/or urinary cortisol levels are essential in investigations of suspected adrenal cortex disorders (Turpeinen and Stenman 2003). Elevated values occur in Cushing's syndrome, during treatment with ACTH, cortisol and cortisone, as well as during stress. Cortisol also affects carbohydrate and fat metabolism.

Liquid chromatography-tandem mass spectrometry was used for the cortisol analysis (Roche-Hitachi Modular E170). (Vogeser 2003, Vogeser and Parhofer 2007).

### **3-metyl-histidine (3-MH)**

3-MH is an amino acid residue of muscle catabolism. It cannot be synthesized or metabolized in the body and any 3-MH excreted via the urine must therefore be derived from muscle catabolism.

The concentration of this amino acid was measured in daily urine collections using a Biochrom 30 amino acid analyzer (Biochrom Ltd., Cambridge, UK) (Bisgaard, Kristiansen et al. 2004, Hahn, Ljunggren et al. 2011). The ratio of 3-MH to urinary creatinine was used to compensate for incomplete urine samples (Elia, Carter et al. 1981, Sjolín, Hjort et al. 1987).

The ingestion of meat can cause errors in these measurements and intake of meat was therefore prohibited over the first two days for those participating in the study (Elia, Carter et al. 1981, Sjolín, Hjort et al. 1987).

### **Body fluid volumes (BIA)**

Intracellular volume was measured using multi-frequency bioimpedance (BIA), with a Xitron 4000B Spectrum Analyzer (Xitron Technologies Inc., San Diego, CA, USA).

Two stickers were placed on the patient's hands and feet, and a series of approximately 50 direct currents of various frequencies transmitted through the body. The patient does not feel these currents. Electrical current is conducted at different speeds by cells and non-cellular items, allowing the calculation of relative volumes (Johnson, Virk et al. 1992, De Lorenzo, Andreoli et al. 1997) (Jaffrin and Morel 2008).

### **PREOP<sup>®</sup>**

preOp<sup>®</sup> (NutriciaNordica AB, Stockholm, Sweden), is a clear, non-carbonated, lemon flavored, iso-osmolar carbohydrate drink. It contains (per 100 mL); 50 kcal (215kJ) , 0 g fat, 0 g protein, 12.6 g carbohydrate (100 E%) in the form of maltodextrin and fructose.



## INSULIN SENSITIVITY AND INSULIN SECRETION

### Clamp techniques

Insulin affects the circulating plasma glucose by inhibiting glucose release from the liver and by stimulation of glucose uptake in the liver and peripheral target organs (skeletal muscle, adipose tissue)

Any quantification of insulin-mediated glucose uptake therefore requires that the glucose release from the liver should be negligible, requiring a total inhibition of hepatic glucose production.

Additionally, the circulating insulin level should be entirely determined by the experimental conditions. Through the use of various combinations of an insulin–glucose infusion, oral glucose levels and/or the circulating insulin levels can be controlled under experimental conditions. The clamp technique allows key parameters to be studied, despite the complex inter-relationships normally seen between the factors governing glucose turnover. A clamp implies that one parameter is deliberately maintained at a controlled level while other physiological parameters are changed. The so-called *hyperinsulinemic euglycemic clamp* allows quantitative estimation of insulin-mediated glucose uptake for a given insulin concentration.

### Hyperinsulinemic euglycemic clamp

The hyperinsulinemic euglycemic clamp is the most accurate method for quantifying insulin-induced glucose uptake and peripheral insulin sensitivity.

In our studies, the clamp was initiated approximately 15 min after the IVGTT had ended. During the course of the 120 min test, insulin  $20 \text{ mU BSA} \cdot (\text{min} \cdot \text{m}^2)^{-1}$  (Human Actrapid, NovoNordisk A/S, Bagsverd, Denmark) was infused, along with 20% dextrose (Fresenius Kabi, Uppsala, Sweden). A superficial dorsal hand vein was cannulated in the retrograde direction with a small three-way needle and kept patent through repeated flushing with a saline solution. The hand and lower arm were kept warm with a heating pad (Copeland, Kenney et al. 1992) during the period of intermittent sampling of arterialized venous blood for glucose determination.

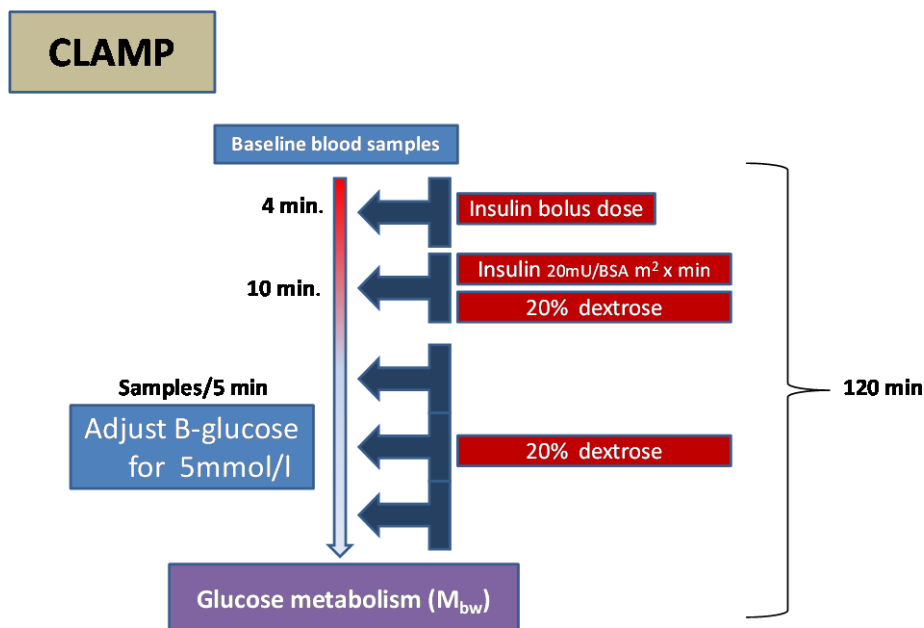
Baseline blood samples were drawn, and the euglycemic hyperinsulinemic clamp was initiated through infusion of a bolus dose of insulin for 4 min, followed by a step-wise increase in glucose for 10 min. The plasma glucose was measured every 5 min using the glucose oxidase method on a YSI 2300 STAT Plus™ (Yellow Springs Instruments, OH, USA). The results were used to adjust the insulin rate to keep the subject's blood glucose levels constant at 5 mmol/L (DeFronzo, Tobin et al. 1979).

Plasma insulin levels and C-peptide concentrations were measured every 15 min. Plasma glucose was measured using a Modular P (Roche Diagnostics, Tokyo, Japan), and plasma insulin and C-peptide on a Roche-Hitachi Modular E170 (Hitachi, Tokyo, Japan).

The infusion rate of the glucose during the last 30 min, after correcting for body weight, was taken to represent the metabolism of the glucose ( $M_{bw}$ ) (DeFronzo, Tobin et al. 1979, Ferrannini and Mari 1998, Muniyappa, Lee et al. 2008).

This method causes a rapid increase in the circulating levels of insulin to a steady state of at least 400 pmol/L .

The method ensures that the concentration of glucose is maintained at a normal level through an intravenous infusion of glucose which rate is adjusted in response to frequent determinations of plasma glucose levels in the arterialized blood. The resultant degree of hyperinsulinemia almost completely inhibits hepatic glucose output, and the amount of glucose added experimentally is therefore equivalent to the total glucose uptake in peripheral tissues. (M value: mg glucose/kg  $\times$  min), the higher the M-value, the greater insulin sensitivity.



## HOMA

HOMA, which is an abbreviation for HOMeostasis Model Assessment, is a mathematical model for the assessment of  $\beta$ -cell function and insulin sensitivity, based solely on the determination of basal glucose and insulin concentrations in plasma.

(Wallace, Levy et al. 2004, Muniyappa, Lee et al. 2008, Borai, Livingstone et al. 2011). Using a computerized nomogram, the relative degree of  $\beta$ -cell dysfunction, specifically with regard to insulin resistance, can be calculated graphically.

Subject to certain given assumptions regarding the parameters, insulin resistance can be calculated as:

$$\text{Insulin resistance} = \text{P-insulin} \times \text{P-glucose}$$

The calculation of  $\beta$ -cell function and insulin sensitivity using to this theoretical model has a relatively good correlation with more advanced techniques. However, the reproducibility of the method is not said to be particularly impressive, with a variation of over 30% (Matthews, Hosker et al. 1985).

## **QUICKI**

QUICKI is the inverse of the logarithm of the product of plasma glucose and plasma insulin at baseline which is one of the HOMA-equations (Katz, Nambi et al. 2000) (Wallace, Levy et al. 2004, Muniyappa, Lee et al. 2008).

## **MINMOD**

Another less costly method for the determination of insulin sensitivity and insulin secretion in vivo is the minimal method (MINMOD) technology (Bergman 1989).

This method is based on frequent determinations of plasma glucose and insulin levels following IVGTT. Mathematical models are used to generate a so-called 'insulin sensitivity index' from computer processed plasma glucose and insulin kinetics results. This represents glucose uptake related to variations in insulin levels (Pacini and Bergman 1986, Nittala, Ghosh et al. 2006).

The method also gives an indication of early and late insulin-secretion patterns. The main disadvantage of this method, in addition to the fact that it is based on a series of theoretical assumptions, is that it requires a relatively well preserved insulin response. This complicates the use of the method in cases of type 2 diabetes. For this reason, a modified version has been developed which maximally stimulates the insulin response through the combined administration of glucose and sulphonylurea (tolbutamide) (Saad, Steil et al. 1997).

## IVGTT

IVGTT is one of the first methods developed for the assessment of peripheral insulin sensitivity *in vivo*. In this method, insulin is injected intravenously, and the rate at which the plasma glucose concentration falls is measured. The faster the plasma glucose levels drop the greater the insulin sensitivity.

Two main procedures are used; regular IVGTT (Nadon, Little et al. 1964) and modified FSIGT (frequently sampled intravenous glucose tolerance test) (Lin, Chen et al. 2013). In the former, a bolus injection of glucose (0.3g/kg) is given intravenously over 30–60 seconds and blood samples are collected for 3–4 h. FSIGT is used for subjects with insufficient insulin response. In this test an additional insulin injection, or short infusion, (0.03–0.05 U/kg) is administered after 20 min. Glucose, insulin and C-peptide concentrations are measured, as they were in our study. The advantage of the IVGTT is that it is relatively easy to perform and there good correlations are found between estimates of insulin sensitivity using the insulin tolerance test and results from the clamp.

After an intravenous bolus injection of glucose a biphasic insulin response is seen (Fig. 7). Initially, there is a rapid and pronounced insulin rise (early phase) which reaches peak levels 1–3min following administration of glucose (endogenous glucose production continues) and is completed after about 10 min (DeFronzo, Tobin et al. 1979).

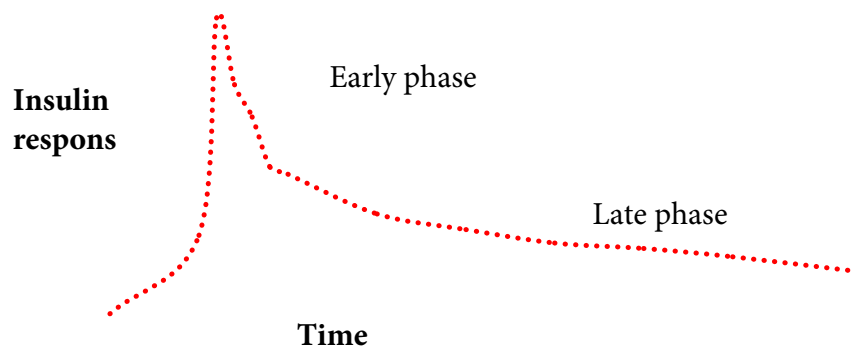
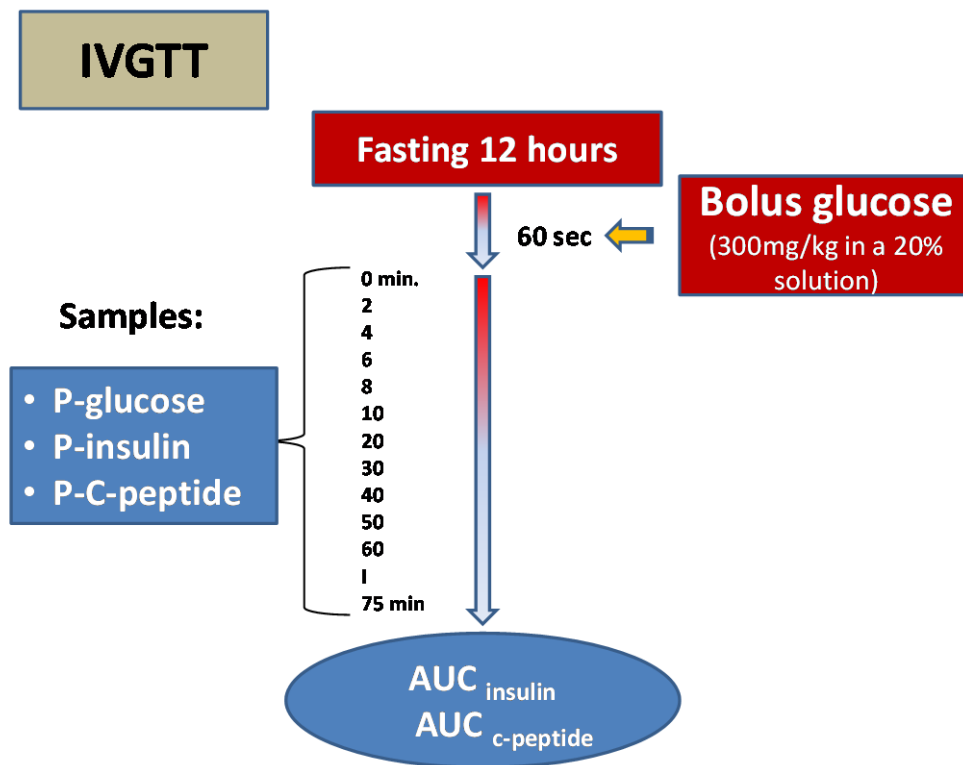


Fig. 7.

After basal sampling, glucose (20% or 30 % glucose solution) is injected over a 1-min period. Samples (plasma glucose, insulin, and C-peptide) are collected at frequent intervals over the first 10-min period to monitor the early insulin response. Thereafter, samples are taken every 5–15 min for up to 75 min.

In our study the plasma glucose was measured using the glucose oxidase method on a Modular P system (Roche Diagnostics, Tokyo, Japan). The plasma insulin and C-peptide concentrations were measured using ELISA kits (Modular E170, Roche Diagnostics).



The calculation of the early and late insulin response was performed by determining the area under the insulin curve ( $AUC_{\text{insulin}}$ ) over the period of 0–10 min (early response) and 10–75 min (late response) (Tura, Sbrignadello et al. 2010). In subjects with type 2 diabetes, the early response is usually missing, while the late release of insulin is preserved. Normally, the half-life of glucose is 15–30 min. In type 2 diabetics it is about 40 min.

## COMPLICATIONS

Complications were dealt with using 3 different approaches. The handwritten medical records from the beginning of the intraoperative period up to when the patient left the postoperative care unit were used to calculate average systolic blood pressure. The second approach relied on a checklist of 18 complications administered by a research nurse. This was based on two published schemes (Bennett-Guerrero, Welsby et al. 1999, Brandstrup, Tonnesen et al. 2003) and used to register all postoperative adverse events up to Day 3. Finally, an orthopedic surgeon, blinded to the randomization process listed the complications seen during the entire hospital stay as noted by the hospital staff in the digital medical records. The Swedish text used for this checklist is shown in the Appendix.

## **WELL-BEING**

### **W-BQ12**

The wellbeing questionnaire (W-BQ12) consists of 12 items designed to collect information concerning different aspects of psychological wellbeing (Wredling, Stalhammar et al. 1995, Pouwer, Snoek et al. 2000). The scale measures 'energy' as well as 'negative' and 'positive' wellbeing using four statements to evaluate each factor; the patient can respond using one of four levels. Higher values denote a better general wellbeing.

### **Health index (HI)**

The HI questionnaire is a Swedish questionnaire used to assess a self-reported health status. It is comprised of 10 items, measuring energy, temper, fatigue, loneliness, sleep, vertigo, bowel function, pain, mobility and one final item measuring general health status (Nordstrom, Nyman et al. 1992). The responses are scored on a 4-grade scale ranging from 10 to 40. A high value indicates a high level of self-reported health.

The HI has been tested for reliability in different patient populations with satisfactory results (Cronbach's alpha 0.77–0.85). When used on the day following surgery, the questions refer to the previous 12 h. Answers can be given on one of four levels in which: 1=very poor; 2=rather poor; 3=rather good; and 4=very good. The highest possible total HI score is 40 and the lowest is 10. This test has been used to study patient's self-perceived health status after ileal conduit urinary diversion surgery (Nordstrom, Nyman et al. 1992) and following laparoscopic cholecystectomy (Barthelsson, Anderberg et al. 2008).

### **Chalder's Fatigue Scale (FQ)**

The FQ scale consists of 14 questions covering both the physical and mental aspects of fatigue (Chalder, Berelowitz et al. 1993). A 'yes' answer to a question about fatigue is graded as 1 and a 'no' as 2. Uncertain answers are graded as 1.5. The scale has been used to study fatigue in cancer (Chalder, Berelowitz et al. 1993) and also in other fields, such as in population surveys (Kocalevent, Hinz et al. 2011).

The translation of the HI from English to Swedish was checked by back-translation. The wording was virtually identical except in one question where 'sleepy or drowsy' back-translated to 'tired or lethargic'.

## **EQ-VAS**

This is a ruler or scale-based method which allows patients to rate their self-perceived health; it is the last item of the EQ-5D instrument for the measurement of health outcomes (Group 1990, Ostendorf, van Stel et al. 2004).

(The Swedish text used for these scales are shown in the Appendix.)

## 6 CALCULATIONS

### GLUCOSE KINETICS

The first study (Study I) was designed to develop a simpler method of measuring insulin sensitivity compared to the more widespread IVGTT which, using the original method, involves measurements made over a 240 min period (Bergman, Ider et al. 1979, Bergman, Prager et al. 1987).

The pharmacokinetics of the glucose load were analyzed using a one-compartment open-model (Sjostrand and Hahn 2003). In this model, the plasma concentration ( $G$ ) at any time ( $t$ ) resulting from infusing glucose at the rate  $R_o$  is calculated using the following differential equation:

$$\frac{d(G - G_b)}{dt} = \frac{R_o}{V_d} - \frac{CL}{V_d} * (G(t) - G_b)$$

where  $G_b$  is the baseline glucose,  $G(t)$  is the plasma concentration of glucose at any given time ( $t$ ),  $V_d$  is the volume of distribution,  $CL$  the clearance and  $CL/V_d$  the slope of the glucose elimination curve. The half-life ( $T_{1/2}$ ) of the exogenous glucose load was obtained from the equation  $\ln 2V_d/CL$ . Best estimates for the unknown parameters in these models were individually calculated for each experiment using non-linear least-squares regression, using the mathematical program Matlab (MathWorks, Natick, MA, USA).

### MINMOD

Glucose and insulin data were also analyzed through the application of the “minimal model” (MINMOD) developed by Bergman et al. (Bergman, Ider et al. 1979, Nittala, Ghosh et al. 2006). This quantifies insulin sensitivity based on the action of insulin in a remote physiological compartment, meaning that the insulin exerts its effects following a delay following the measurement of the plasma concentration. The MINMOD analysis was applied to the data in Study I. This was attempted for Study III but as the estimates necessary for this model were not very precise in this study the results are not reported. Our opinion is that MINMOD requires more data than those provided by the 7-sample IVGTT in order to be reliable.



## IVGTT

In order to estimate insulin sensitivity we used glucose kinetic parameters and the  $AUC_{\text{insulin}}$ , indicating plasma insulin, above baseline during the IVGTT to find equations that best described the outcome of the clamp procedure.

The AUC for plasma insulin was calculated by using the linear trapezoid method;

$$\text{Insulin sensitivity} = {}^{10}\log \left[ \frac{CL \cdot 10^6}{V_d \cdot AUC_{\text{ins}}} \right] \quad \text{"Key algorithm I"}$$

$$\text{Insulin sensitivity} = \left[ \frac{1}{{}^{10}\log(T_{1/2} \cdot AUC_{\text{ins}})} \right] \quad \text{"Key algorithm II"}$$

## HOMA-IR

The HOMA-IR (Homeostasis Model of Assessment – Insulin Resistance) model expresses insulin sensitivity only as the product of the baseline concentrations of plasma glucose and insulin only.

## QUICKI

Insulin sensitivity was also quantified using the QUICKI method, which is the inverse of the logarithm of the product of plasma glucose and plasma insulin at baseline; one of the HOMA-equations (Katz, Nambi et al. 2000) (Wallace, Levy et al. 2004, Muniyappa, Lee et al. 2008).

$$\text{QUICKI} = 1 / (\log \text{P-glucose} + \log \text{P-insulin})$$

## CLAMP

Insulin-induced glucose uptake during the last 30 min of the test, divided by bodyweight, was taken as the insulin sensitivity and is denoted by the symbol  $M_{\text{bw}}$ .

## 7 STATISTICS

The results are presented here as the mean and standard deviation (SD) and, in the case of a skewed distribution, as the median (25–75th percentile range). In Study IV the results are given only as the median (25–75th percentile range). In all the studies,  $P < 0.05$  was considered to be statistically significant. The statistical approaches and tests used in each of the four studies are specified below.

### STUDY I

Simple or multiple linear regression analyses, in which  $r^2$  is the coefficient of determination, were used to express “linearity” when studying the relationship between the  $M_{bw}$  of the glucose clamp (control) and various algorithms for insulin sensitivity, derived from data collected during the IVGTT.

The error in the prediction of  $M_{bw}$  associated with each regression analysis was obtained as [100% (fitted – measured)/measured]. The change in the prediction error obtained by restricting the analysis period from 75 to 40 and 30 min was tested by using Friedman’s test.

### STUDY II

This study was powered so as to be able to detect a difference in the  $CL$  for glucose of 30% between any of the study groups and the control group with a certainty of 90%.

The reference data were obtained from both preoperative and postoperative studies. In non-diabetic healthy humans,  $CL$  has been reported as 0.72 (mean, SD 0.18) (Sjostrand, Edsberg et al. 2001) and 0.60 (0.26) (Hahn, Ljunggren et al. 2011). During laparoscopy,  $CL$  has been measured as 0.21 (0.05) (Sjostrand, Edsberg et al. 2001) and, on the second postoperative day in hysterectomy patients, 0.42 (0.08) (Strandberg and Hahn 2005).

The mean of these values yields a standardized difference of 1.05 which was entered into a nomogram. This showed that 18 patients per group were required (Altman 1980) for this study. The ability of the study to detect postoperative differences would be greater than that in the preoperative situation due to less expected scattering of those data. The means (SD) of differences between the groups were evaluated using the variance of analysis (ANOVA).

Differences were studied with the Kruskal–Wallis test when a skewed distribution was found. Changes during the study were evaluated using the Wilcoxon matched-pair test. The incidence data were studied with the chi-square test.

Cronbach’s alpha coefficient was used to test the reliability of the wellbeing tests.

Cronbach's alpha coefficient is the most widely used method for measuring reliability. This method uses the degree to which each item in the test is associated with any other item. A good degree of reliability can be inferred if similar readings are obtained each time the same thing is measured. In practical terms, there should be a correlation dimension of at least 0.7, ideally about 0.9, in order for a test to be considered to have good reliability.

### **STUDY III**

The size of the study was chosen to detect a difference in the uptake of glucose of 10% at the  $P < 0.05$  level, with a power of 80%. The calculation was based on a previous study showing an intra-individual coefficient of variation for repeated glucose clamps of 5.8% (Soop, Nygren et al. 2000).

The  $AUC_{\text{insulin}}$  and the C-peptide concentrations were calculated using the linear trapezoid method.

Comparisons between the paired samples were made with the paired  $t$ -test or Wilcoxon's matched-pair test, depending on the distribution.

Similarly, unpaired data were evaluated by the unpaired  $t$ -test or the Mann–Whitney  $U$ -test.

### **STUDY IV**

$M_{bw}$  was calculated using the same methods as in Study I. Simple linear regression analysis, where  $r$  is the correlation coefficient, was used to express linearity for the relationships between the insulin resistances derived from the various approaches.

Differences were studied using the Wilcoxon matched-pair test.

## 8 RESULTS

### STUDY I

When we performed the glucose clamp we found that  $M_{bw}$  varied 7-fold. Between 2/3 and 4/5 of this variation could be predicted by linear regression, based on indices of glucose and insulin turnover from the data collected during the IVGTT. During the IVGTT, the glucose kinetics in three experiments could only be analyzed up to 40 min due to rapid elimination leading to mild hypoglycemia.

#### *IVGTT versus glucose clamp*

We tested different mathematical algorithms when trying to express  $M_{bw}$  using the data obtained during the IVGTT. The principle used in this testing was to make a ratio of the “pressure” to decrease plasma glucose, which was taken as the  $AUC_{insulin}$ , and the “effect”, which was the half-life of the exogenous glucose load. One useful algorithm contained the  $^{10}\log$  of the product of  $T_{1/2}$  for the exogenous glucose load and  $AUC_{insulin}$ . Various modifications of the algorithm correlated with  $M_{bw}$  with a linearity of  $r^2=0.63-0.68$ . Another algorithm inserted glucose kinetic parameters and the  $AUC_{insulin}$  in a multiple regression equation, which yielded a maximum linearity of  $r^2=0.83$  for the relationship between the IVGTT and  $M_{bw}$ . This means that up to 83% of the variability in insulin resistance, as obtained by the glucose clamp, could be explained by the result of the short IVGTT in these volunteers.

#### *QUICKI and MINMOD versus the glucose clamp*

We also compared  $M_{bw}$  with QUICKI, HOMA, Bergman's minimal model analysis (MINMOD) and an equation recently published by Tura et al (Tura, Sbrignadello et al. 2010). This algorithm has the same construction as QUICKI, which uses only the baseline values of plasma glucose and insulin. The original QUICKI equation correlated with  $M_{bw}$  with a linearity of only  $r^2=0.41$ , which was still slightly stronger than for other similar expressions, such as HOMA-IR ( $r^2=0.35$ ) and the G/I ratio ( $r^2=0.39$ ) (Muniyappa, Lee et al. 2008).

## STUDY II

Of the 60 patients initially enrolled, 57 completed the study. Thirty of the 171  $AUC_{\text{insulin}}$  could not be used due to occasional hemolysis.

### *Glucose and insulin*

Baseline values for P-glucose and insulin were quite similar in the different study groups. P-glucose was 16% higher on Day 1 and another 10% on Day 2.

### *Glucose kinetics*

Over the three days, IVGTT showed an increase in P-glucose (8.3, 9.7, and 9.9) and a decrease in CL, with no difference between the groups.  $T_{1/2}$  increased on both days but mostly on Day 1.

### *Insulin*

The mean plasma insulin response to the IVGTT decreased on Day 1, but showed an increase of 18% on Day 2. AUC changed from -36% to +51% between Day 1 and Day 2. The same pattern was found in all three groups.

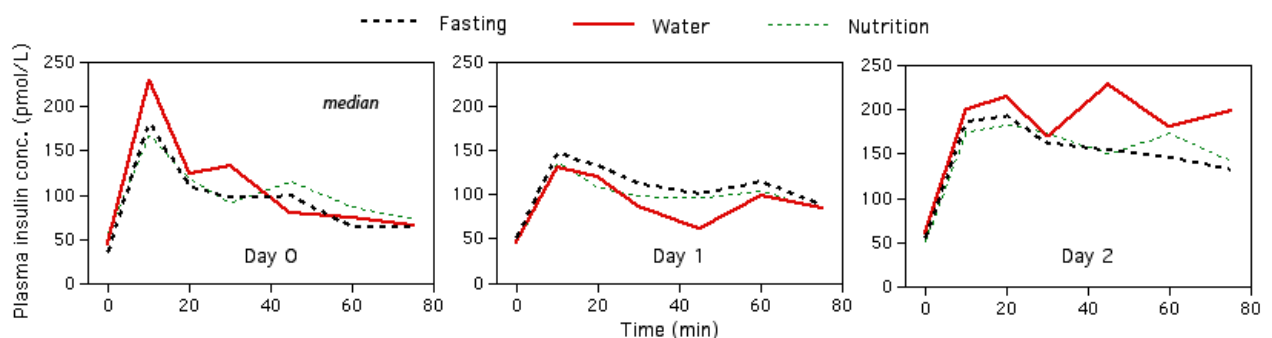


Fig. 8.

$M_{bw}$  fell on Day 2 but was essentially unchanged on Day 1. When we compared the two methods of calculation, i.e., IVGTT against QUICKI, the decline was significantly greater with the IVGTT, -48% vs. -8% (Fig. 8).

### *Physical stress, catabolism and body fluid volumes*

There were no statistical differences between the groups with regard to plasma-cortisol postoperatively. U-cortisol decreased by 26% between Day 1 and Day 2. There were no differences in muscle breakdown (U-3MH/creatinine ratio) between the groups, although the excretion rate generally decreased between Day 2 and Day 3.

ECF was unchanged by the surgery but ICF tended to increase slightly.

### *Complications*

Pain, nausea and hypotension were the most common complications. The nurse follow-up showed that a mean of 1.5 complications had occurred per patient by Day 3. A mean of only 0.3 complications per patient was recorded in the digital journal. There was no hemodynamic difference between the groups before or after the operation. There was no statistical difference in the length of hospital between groups.

### *Wellbeing*

No statistically significant difference was seen in general well-being, which improved slightly after surgery. Cronbach's alpha coefficients for the responses to the W-BQ12, HI, and FQ tests before surgery were 0.80 (mean of the three parts), 0.85, and 0.87, respectively. Two weeks after surgery, the corresponding values were lower: 0.72, 0.71, and 0.71.

### STUDY III

Of the 23 patients enrolled in the study, one patient dropped out due to personal reasons. Hemolysis was detected in the insulin samples from two patients, meaning that their AUC<sub>insulin</sub> could not be calculated. Their results are, however, reported with respect to other parameters, including the glucose clamp measurements. There were no statistically significant differences with respect to demography, preoperative morbidity and operation details.

#### *Glucose and insulin at baseline*

The baseline values for plasma glucose concentration were higher after, compared with before, surgery (+9%,  $P<0.01$ ). The baseline values for plasma insulin concentration were only higher in the nutrition group (31%,  $P<0.01$ ). The baseline C-peptide concentrations tended to be higher after surgery in the nutrition group (+28%), but the difference from the preoperative value did not reach statistical significance ( $P=0.11$ ).

#### *IVGTT*

The half-life of exogenous glucose injected during IVGTT was longer after surgery (mean 29 vs. 37 min,  $P<0.02$ ), and the total AUC of plasma insulin and C-peptide larger ( $P<0.01$ ). The early phase (0–10 min) of the AUC for plasma C-peptide was larger after the operation ( $P<0.05$ ), but not for plasma insulin.

Compared with the preoperative measurement, the second IVGTT in the nutrition group was followed by significantly higher plasma insulin concentrations compared with the corresponding changes in the control group. This was the case for both the first and second phases of insulin secretion, as well as for the total AUC ( $P<0.05$ ). There was a similar trend with respect to the AUC for the plasma C-peptide, but the differences between the groups were not statistically significant. However, the differences between these groups were significant when the C-peptide concentrations were corrected for plasma glucose. Hence, the ratio of AUC for C-peptide and plasma glucose concentrations changed by +46% (2–70) in response to the surgery in the nutrition group, whereas there was no such change after the surgery in the control group where the difference was –5% (–13 to +16,  $P<0.03$ ).

#### *Glucose clamp*

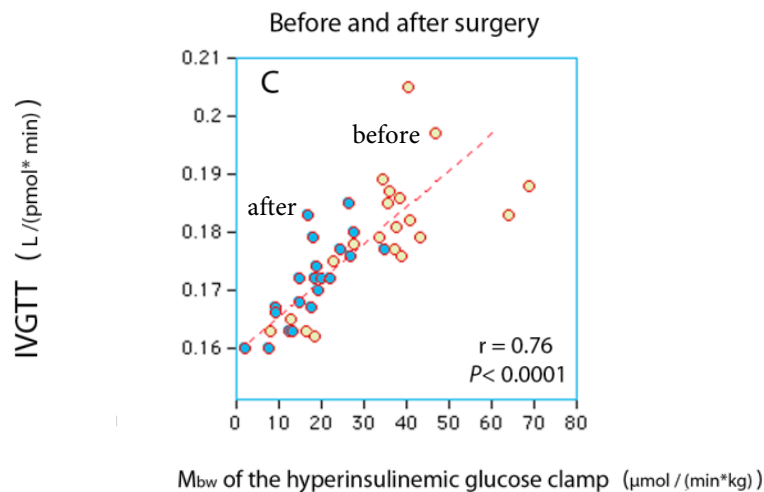
The glucose clamp showed that insulin sensitivity had decreased after surgery ( $P<0.01$ ). The median decrease was 45%. There was no statistically significant difference between the groups with regard to the degree of insulin resistance that developed in response to the surgery.

## STUDY IV

Missing data reduced the number of evaluable patients to 20. The plasma insulin concentrations after 90, 105 and 120 min (when the insulin sensitivity was measured) were 555 pmol/L (483–651) before surgery and 455 pmol/L (390–531;  $n=63$ ) afterwards, which confirms that maximum insulin stimulation was achieved. The baseline concentration was 60 (42–93) pmol/L.

The glucose clamp showed a 10-fold spontaneous variation in  $M_{bw}$ . A highly statistically significant linear relationship was observed between the insulin sensitivity measured by the IVGTT and by the glucose clamp before and after surgery. The pre- and postoperative measurements were also pooled to obtain the correlation regardless of point in time (Fig.9).

Fig. 9.



Similarly, statistically significant correlations were found between the QUICKI and HOMA methods and the glucose clamp.

The precision with which the three algorithms could predict the  $M_{bw}$  obtained by the clamp before and after surgery was better for the IVGTT (median absolute prediction error 18%) compared to QUICKI (29%;  $P < 0.03$ ) and HOMA (31%,  $P < 0.001$ ). Surgery-induced insulin resistance amounted to 45% (glucose clamp), 26% (IVGTT), 4% (QUICKI), and 3% (HOMA). Despite reasonably good linear correlations, the static tests grossly underestimated the degree of insulin resistance that developed in response to surgery.



## 9 GENERAL DISCUSSION

### INSULIN RESISTANCE

All forms of physical trauma induce a variety of complex mechanisms designed to cope with the strain it places on the body. Surgery creates physical stress, triggers a cascade of reactions, including a transient reduced insulin sensitivity (Ljungqvist, Nygren et al. 2000), increases stress hormone secretion, and increases muscle breakdown (Hedstrom, Ljungqvist et al. 2006, Ljungqvist, Soop et al. 2007). These reactions may induce nausea and fatigue, and additionally affect convalescence for several weeks after surgery (Hausel, Nygren et al. 2005) (Ashby, Grocott et al. 2008, Lauwick, Kaba et al. 2009).

Several studies have shown that the transiently reduced insulin sensitivity, which is similar to type 2 diabetes, might be limited or prevented by providing exogenous insulin (Brandi, Frediani et al. 1990), a preoperative infusion (Ljungqvist, Thorell et al. 1994, Nygren, Thorell et al. 1998) or oral administration of glucose (Nygren, Soop et al. 1999). The studies demonstrating a reduction in surgery-induced insulin resistance have encouraged many surgical units around the world to implement a carbohydrate drink in their daily patient care.

A prerequisite for Study II was the idea that ingestion of a carbohydrate-rich drink before surgery would alleviate the subsequent development of insulin resistance. We questioned whether this effect could be attributed only to the carbohydrates and hypothesized that the effect could, alone or in part, be due to the fairly large volume of water in the drink (1.2 L).

The reason to why water could work is that glucose translocates fluid into the cells. This fluid shift might counteract the dehydration that seems to initiate surgery-induced catabolism (Häussinger 1995, Strandberg and Hahn 2005). Providing tap water also prevents dehydration of the cells, which is an effect that can *not* be achieved by the electrolyte solutions (such as Ringer's acetate) routinely given for intravenous hydration in the perioperative period.

Our results show that preoperative fasting and ingestion of the nutritional drink were followed by the same degree of urinary cortisol excretion, muscle catabolism, and length of hospital stay. Neither the preoperative intake of tap water nor of a nutritional drink alleviated the reduction of glucose clearance that occurs after elective hip replacement surgery. Moreover, there was no evidence that these treatments affected insulin sensitivity, hemodynamics, the incidence of postoperative complications, or patient wellbeing.

Previous researchers have claimed that carbohydrate drinks improve wellbeing as well as reducing nausea and vomiting (Ljungqvist, Thorell et al. 1994, Nygren, Thorell et al. 1998,

Wang, Wang et al. 2010). These results have not been consistent (Henriksen, Hesselov et al. 2003, Bisgaard, Kristiansen et al. 2004), and our Study II could not confirm this claim.

This result was a little bit surprising, considering what other studies had shown. Some authors had even shown a beneficial effect on surgery-induced insulin resistance by using the same techniques that we have (Nygren, Soop et al. 1998, Soop, Nygren et al. 2001). However, not all studies have demonstrated a beneficial action on insulin sensitivity following preoperative oral carbohydrate loading (Vigano, Cereda et al. 2012). A meta-analysis by Awad et al (Awad, Varadhan et al. 2013), including 21 RCTs, examines the effects of preoperative carbohydrate treatment on clinical outcomes in patients undergoing elective surgery. The only group that showed a clear result, when it came to the reduction of length of hospital stay, was patients undergoing major abdominal surgery. Furthermore no differences were found with regard to the occurrence of postoperative complications following preoperative carbohydrate treatment, despite the reduction in postoperative insulin resistance (Awad, Varadhan et al. 2013).

As the 7-sample IVGTT that we used in Study II had not been compared with the gold standard (the hyperinsulinemic glucose clamp) in the age group undergoing hip replacement surgery we could not be certain that our measurements accurately showed the effect of the carbohydrate drink on insulin sensitivity. To further investigate the surprising lack of a difference in insulin sensitivity between the fasting and carbohydrate-loaded patients in Study II, we did compare insulin sensitivities as measured by *both* the glucose clamp and the IVGTT in Study III.

Study III had basically the same structure as Study II, i.e. we performed an IVGTT the day before surgery but added a clamp test shortly afterwards (Tripathy, Wessman et al. 2003, Laakso, Zilinskaite et al. 2008). The patients were randomized to receive either flavored water (control) or carbohydrate-rich beverage (preOp<sup>®</sup>). Two days after surgery, the subjects underwent the same tests again. The results confirmed the anticipated decline in insulin sensitivity after hip replacement surgery, but there was no statistically significant difference in the surgery-induced change in insulin sensitivity between the groups regardless of whether the preoperative drink consisted of carbohydrates and water or only water. This finding supported the result of Study II.

The relative reduction in insulin sensitivity in this study is in line with other recent studies (Nygren, Soop et al. 1998, Soop, Nygren et al. 2001). There was a trend for less of a reduction in insulin sensitivity in the control group (39%) compared to the nutrition group (51%). The reason for this is not clear; however we cannot entirely rule out that some of the beneficial effects seen in the carbohydrate oral fluid treatment studies (Nygren, Soop et al. 1998, Nygren, Thorell et al. 2001) could be attributed to the volumetric component of the drink rather than the nutrients (Nygren, Thorell et al. 1998). We earlier found (Study II) no difference in intracellular volume expansion between groups receiving carbohydrate loading or water ingestion prior to elective hip replacement surgery. A slightly increased intracellular volume expansion was demonstrated, in both groups, compared the patients being in the fasting state.

We also demonstrated in Study III, by performing the IVGTT, that endogenous insulin production increased in conjunction with preoperative administration of carbohydrate-rich beverage. The assessment of pancreatic  $\beta$ -cell function in humans is challenging because of the complex interplay between insulin secretion, insulin sensitivity and hepatic insulin extraction; comprising the disposition index (Kahn 2003). Thus, in individuals without diabetes, the insulin response to an IVGTT is greater than that seen in an insulin-resistant subject, whereas the insulin response is less in insulin-sensitive subjects, according to the disposition index (Kahn 2003).

In view of this, the insulin response was measured in our study, using IVGTT, and compared to changes in insulin sensitivity (M-value). The first fast-phase of insulin secretion (over the initial 0–10 min) is particularly important in terms of  $\beta$ -cell function. As the glucose kinetics were similar between the groups, the increased insulin response to preOp<sup>®</sup>, compared to the control group, seems to be driven by the reduction in the induced insulin resistance evoked by surgery. This might be explained by over-compensation in insulin secretion, something observed in the early stages of insulin resistance.

Earlier studies also demonstrated that a preoperative carbohydrate drink reduces complications (Nygren, Soop et al. 1999, Svanfeldt, Thorell et al. 2005, Ljungqvist, Soop et al. 2007, Li, Wang et al. 2012). We did not find any such reduction in complications in Study II, but previous studies have based their conclusions on larger groups of patients (Sato, Carvalho 2010 et al.).

## METHODS

One problem when assessing glucose metabolism and insulin resistance is that the measurements are demanding and complex. The principle tests used are the hyperinsulinemic euglycemic clamp (DeFronzo, Tobin et al. 1979), the gold standard in measuring insulin sensitivity, and the IVGTT (Borai, Livingstone et al. 2007, Muniyappa, Lee et al. 2008) which measures  $\beta$ -cell activity, i.e., insulin secretion, but can also be used to calculate insulin sensitivity (Pacini and Mari 2003). The clamp procedure has such a large effect on the body's metabolism that this test cannot be repeated in a surgical setting without altering the physiology it aims to study. From this point of view, the IVGTT is less problematic. However, the traditional IVGTT is still labor-intensive as it requires the analysis of a very large number of blood samples.

For these reasons we wished to develop an IVGTT based on a reduced number of data points, without compromising measuring accuracy, and only marginally affecting patient physiology and plasma volume (Study I). This improvement would make it possible to use the IVGTT during clinical work, such as surgery, to assess the patient's insulin sensitivity.

When comparing a short IVGTT with the hyperglycemic glucose clamp in 20 volunteers, we found that reducing the sampling time from 75 min to 40 min, or even 30 min, had only minimal effects on our quality measures, i.e., linearity and prediction errors. Our short IVGTT was also more reliable than QUICKI and HOMA-IR methods. These tests are based on plasma

glucose and insulin concentrations at baseline and do not take the dynamics of a process into account. These two tests were originally designed for population studies in diabetic research (Beard, Bergman et al. 1986, Katz, Nambi et al. 2000) but their usefulness for capturing surgery-induced changes in insulin resistance has long been unclear.

Analysis of the 7-sample IVGTT used endogenous insulin secretion in response to an exogenous glucose bolus to quantify the balance between plasma insulin over time (stimulus) and the half-life of glucose (effect) throughout the disposition of an intravenous glucose load.

The QUICKI and HOMA methods, in contrast, reflect the balance between glucose and insulin in the absence of any metabolic challenge and are believed to reflect hepatic insulin resistance (Abdul-Ghani, Jenkinson et al. 2006). The benefits are that they can be used in clinical medicine and can be repeated, however, they do not perfectly correlate with the glucose clamp (Study I and IV).

In Study IV we investigated the accuracy of the QUICKI and HOMA algorithms and the simplified IVGTT in quantifying insulin resistance, as determined by the glucose clamp, when applied to the clinical situation. We used the data from Study III, from the baseline samples and the IVGTT at 0, 10, 20, 30, 40, 50, 60, and 75 min for the measurement of the plasma glucose (P-glucose) and insulin (P-insulin) concentrations and compared them with the glucose clamp.

We used the calculations that I described in the Calculations section above, and found that our 7-sample IVGTT, QUICKI and HOMA all correlate reasonably well with the result of the glucose clamp for single points in time, although the linearity and precision were slightly poorer for the QUICKI and HOMA than for the 7-sample IVGTT. However, the QUICKI and HOMA did not capture the severity of the insulin resistance that developed in response to surgery. These algorithms indicated a change of only 3–4% while the glucose clamp showed an increase in insulin resistance of 45%. Consequently, these algorithms cannot be used to assess changes in insulin resistance that occurs during surgery. The IVGTT reflected the surgery-induced changes more accurately, but it still did not completely correlate with the clamp.

We concluded that if we sampled data more frequently, so as to capture the early insulin response seen during the first 10-min after injection of the exogenous glucose, we would yield a 6% larger  $AUC_{\text{insulin}}$  and that the IVGTT would agree more closely with results obtained by the glucose clamp. The reader may note that the first 10-min was included in Study I. However, this improvement of the IVGTT suffers from the practical problems involved in taking blood samples every 2 min between 2 and 8 min. The 7-sample IVGTT that we suggest uses samples taken from 10 min onward.

## LIMITATIONS

Study II, compared to Study III, was non-blinded as blinding was considered difficult to carry out for the fasting group. The study protocol was also based on the assumption that the nutritional drink would preserve insulin sensitivity and reduce the incidence of complications, so that the focus was on postoperative comparisons between the groups. This fact explains why cortisol and 3-MH were not measured preoperatively. As the study was powered to discover differences in glucose clearance, conclusions with regard to insulin sensitivity, postoperative complications and wellbeing would probably require larger study groups for firm conclusions to be safely drawn. However, the lack of even a trend towards benefits as a result of the intervention is striking.

The clamp technique, regarded as the gold standard, was not used to measure insulin sensitivity in Study II, although intravenous glucose tolerance is a widely accepted alternative.

The relatively sparse sampling scheme in Study II, which was motivated by ethical considerations regarding maximum blood sampling volume, has been validated against the hyperglycemic glucose clamp used in Study I. The sparse sampling scheme used for the IVGTT in all studies also made it difficult to apply Bergman's MINMOD kinetics method with confidence. The MINMOD set of equations were programmed in the Matlab environment but yielded parameter estimates with confidence intervals too wide to be useful.

Hemolysis in some insulin samples made the reported values too uncertain to be included in calculations of the AUC. As a result, 18% of the AUC's for insulin had to be deleted, which resulted in insulin sensitivity being based on less data than originally intended.

One should note that we did not use a tracer technique (Dalla Man, Piccinini et al. 2013) with the clamp to quantify the endogenous glucose production. However, the endogenous glucose production is strongly inhibited by high insulin concentrations, and should be very small when the insulin sensitivity is assessed at the end of the glucose clamp procedure. Moreover, the glucose uptake elicited by maximal insulin stimulation was measured with an insulin dose lower than that originally suggested by DeFronzo (DeFronzo, Tobin et al. 1979). The lower dose is routinely used in our metabolic laboratory since it is safer and the plasma insulin concentrations hardly differ between the two (400-500 pmol / l for the higher one) (Nystrom, Gutniak et al. 2004)). Accordingly we used different doses of glucose in the Study II (0.2 g/kg) compared with Study III-IV (0.3 g/kg).

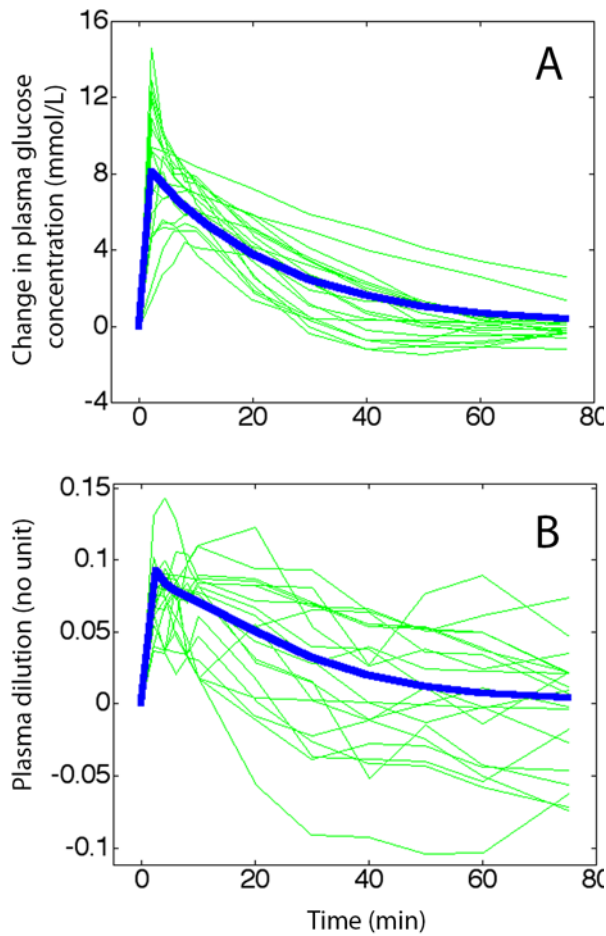


Fig. 10

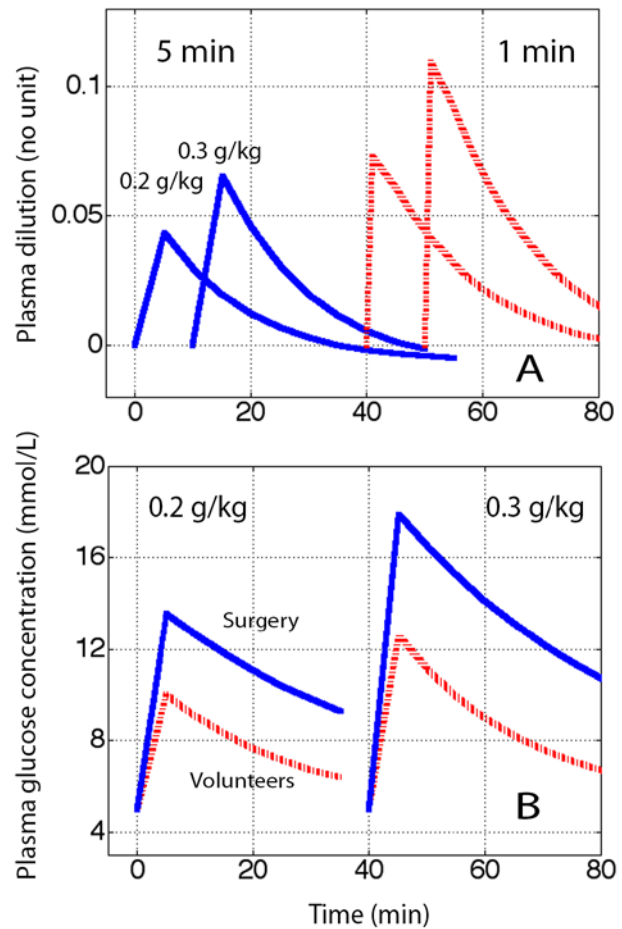


Fig. 11

A theoretical problem when using the IVGTT is that the peak plasma glucose concentration will exceed the renal limit for glucose conservation (Fig. 10 A), and some of the injected glucose will therefore appear in the urine. Moreover, the hypertonic nature of the injected glucose will translocate fluid from the cells to the plasma, causing plasma volume expansion that is far greater than the volume of the injected glucose solution (9%, see Fig. 10 B). These problems are of little consequence in young volunteers as shown in the Fig. 10 which is derived from data in Study I. However, in the elderly subjected to surgery, the hyperglycemia might be more pronounced and the plasma volume expansion have hemodynamic consequences.

Before Study II was initiated, we performed computer simulations based on glucose and volume kinetic data from Study I to find out whether a smaller dose of glucose injected over a longer period of time than 1 minute could minimize these problems. The results are not included in the present Thesis but published elsewhere (Hahn & Nyström 2011). It was found that the extending the injection time from 1 to 4 minutes and reducing the glucose dose from 0.3 to 0.2 g/kg could markedly alleviate the both the hyperglycemia (Fig. 11 A) and the sudden plasma volume expansion hyperglycemia (Fig. 11 B) resulting from the IVGTT.

## **FUTURE PERSPECTIVES**

Our 7-sample IVGTT is fairly simple to implement and might have a place in clinical medicine. Currently, relatively little is known about insulin resistance and its relation to surgery. Using our method it might, for example, be possible to follow what happens with insulin resistance during and after gastric bypass surgery for obesity, which is of great interest to many people nowadays. Furthermore, it may be useful in cases of severe illness, patients with ASA grade  $\geq$ III, diabetes or in those undergoing emergency surgery. Furthermore, it would be interesting to compare the different measurement methods in patients who also have diabetes or other metabolic diseases.

## 10 CONCLUSIONS

- It is possible to shorten the IVGTT to enable measurement of insulin resistance under clinical conditions (Study I).
- We could not demonstrate any differences between preoperative fasting, water ingestion or a carbohydrate-rich drink with respect to hemodynamics, postoperative complications or wellbeing after elective hip replacement surgery (Study II).
- There were no statistically significant differences between water and a carbohydrate-rich drink with regard to the reduction of glucose clearance. These treatments were followed by the same decrease in insulin sensitivity after the surgery (Study II and Study III).
- Our short seven-sample IVGTT shows better correlation with the gold standard, the euglycemic hyperinsulinemic clamp, than QUICKI and HOMA-IR (Study IV).



## 11 SAMMANFATTNING (SUMMARY IN SWEDISH)

Det är välkänt att kirurgiskt trauma startar en rad olika fysiologiska reaktioner i kroppen, bl.a. en övergående insulin resistens liknande typ 2 diabetes, stress hormon sekretion (cortisol) och sannolikt en obalans i kroppsvätskefördelningen. Det katabola tillstånd som triggas igång medför också en nedbrytning av muskulatur. Den påverkan på allmäntillståndet som uppträder efter kirurgi har kopplats till en övergående insulinresistens. Man har tidigare visat i studier att man kan förebygga detta genom att ge patienten kolhydratrik vätska innan kirurgi.

Vi funderade på om man det var den tillförda vätskemängden i sig som påverkar insulinresistensen än kolhydrattillförseln. För att kunna mäta detta i kliniskt bruk så var vi tvungna att ta fram ett mer förenklat test att mäta insulinresistensen än vad de tester som fanns att tillgå, som var mer för mätning i laboriemiljö än för kliniskt bruk.

**Studie I;** en metodstudie, med 20 friska frivilliga försökspersoner, där vi önskade att förenkla ett 120 minuters intravenöst glukostolerans test (IVGTT). Vi gjorde jämförelser mellan olika kinetiska parametrar med insulinkänsligheten ( $M_{bw}$ ) mätt med "gold standard"- hyperinsulinemisk euglykemisk clamp. Plasma glukosvärdet användes för att beräkna distributionsvolymen ( $V_d$ ) och clearance (CL) för den tillförda intravenösa bolusdosen av glukos. Plasma insulin svaret kvantifierades genom att mäta arean under kurvan för insulin ( $AUC_{ins}$ ), vilket återspeglar "styrkan" av svaret. Vi prövade olika algoritmer baserade på glukoselimineringsskurvans lutning i kombination på  $AUC_{ins}$ .

Vi fann att vi kunde reducera mätpunktsintervallet från 180 minuter till 30-40 minuter med vårt förenklade IVGTT, till 7 mätpunkter, med kvarstående stark korrelation, mellan 2/3 och 4/5, mot clampen.

**Studie II;** en randomiserad kontrollerad studie (RCT) med 60 patienter, utan diabetes, som var planerade för höftprotesoperation. De randomiserades till att fasta (kontroll), dricka smaksatt vatten (placebo) eller kolhydratrik vätska (preOp<sup>®</sup>) före operation. Vi utförde ett IVGTT dagen innan operation, direkt efter operationen på den postoperativa avdelningen, samt dagen efter operationen.

Vi beräknade glukos-clearance och insulinresponsen ( $\beta$ -cellsfunktionen), mätte urin- och plasmacortisol samt muskelnedbrytning (urin-3-methylhistidin). Vi registrerade eventuella komplikationer samt patienterna fick fylla i olika välbefinnandeformulär vid olika tidpunkter. Vi fann ingen statistisk skillnad beträffande glukosc Clearance, insulinsensitiviteten, postoperativa komplikationer eller välbefinnande mellan vatten och den kolhydratrika vätskan.

**Studie III;** en dubbelblind RCT med 23 patienter utan diabetes som var planerade för en höftprotesoperation. De randomiserades till antingen smaksatt vatten (placebo) eller kolhydratrik vätska (preOp<sup>®</sup>) före operation. Vi beräknade insulinrespons (IVGTT) och insulinsensitivitet ( $M_{bw}$ ) dagen före samt två dagar efter operation.

Vi fann en signifikant men liknande minskning av insulinsensitiviteten mellan grupperna, men kolhydratgruppen visade en ökad  $\beta$ -cellsaktivitet vilket kan bero på en möjlig överkompensation i insulinsekretionen. Detta har man tidigare kunnat se tidigt i förloppet vid insulinresistens.

**Studie IV;** en observationsstudie med 22 patienter från studie III där vi jämförde två dynamiska test (vårt korta 7-provs IVGTT och euglykemisk hyperinsulinemisk clamp) med två statiska test ("QUICKI" och HOMA-IR). De senare testerna baseras endast på utgångsvärdena av plasma-glukos och -insulin. Clampen fungera som kontroll-metod (=rätt svar).

Vi fann att det förelåg en svagare linjäritet och ett större residualfel vid användning av QUICKI och HOMA-IR än vid IVGTT när vi uppskattade insulinresistensen före och efter operation. Dessa två underskattade också grovt graden av insulinresistensen vilket för tanken till att kirurgiinducerad insulinresistens kan bero på förändringar hos de perifera vävnaderna, såsom muskel och fettvävnad, men inte på leverns.

### **Slutsats**

Vi kan med vårt förenklade 7-provs IVGTT mäta den insulinresistens som uppkommer i samband med kirurgi med kvarstående stark korrelation till ursprungsmetoden (clamp) vilket gör det möjligt att använda metoden i klinisk miljö. Vi har även funnit att det inte föreligger någon statistisk skillnad mellan intag av vatten eller kolhydratrik dryck innan operation beträffande minskningen av insulinsensitiviteten. Vi fann heller ingen uppenbar skillnad mellan grupperna när det gäller välbefinnande, och komplikationer efter höftprotesoperation.

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## 13 APPENDIX

### KOMPLIKATIONER

*Gradera som 0-1-2-3 (svårighetsgrad) och beskriv kortfattat!*

1. Ventilationshjälp (syrgas, ventilator m.m.) \_\_\_\_\_
2. Feber och antibiotika utan känt fokus \_\_\_\_\_
3. Smärta (opioider behövs) \_\_\_\_\_
4. Illamående och kräkningar \_\_\_\_\_
5. Högt/lågt blodtryck \_\_\_\_\_
6. Hjärtarytmier \_\_\_\_\_
7. Tolererar inte fast föda \_\_\_\_\_
8. Neurologiska problem (förvirring etc.) \_\_\_\_\_
9. Sårkomplikation \_\_\_\_\_
10. Hemtologisk komplikation (blodtransfusion etc.) \_\_\_\_\_
11. Njurproblem \_\_\_\_\_
12. Postspinal huvudvärk \_\_\_\_\_
13. Blodförgiftning (sepsis) \_\_\_\_\_
14. Lunginflammation \_\_\_\_\_
15. Lungödem \_\_\_\_\_
16. Hjärtinfarkt \_\_\_\_\_
17. Stroke \_\_\_\_\_
18. Inflammation i urinblåsan \_\_\_\_\_

Annat (beskriv) \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

## W-BQ12

	<u>Hela tiden</u>			<u>Inte alls</u>
1. Jag gråter ibland eller känner mig gråtfärdig.	3	2	1	0
2. Jag känner mig nedstämd och tungsint.				
3. Jag känner rädsla utan någon som helst orsak.				
4. Jag blir lätt upprörd eller skärrad.				
5. Jag känner mig energisk, aktiv och pigg.				
6. Jag känner mig slö och trög.				
7. Jag känner mig trött, utsliten eller slutkörd.				
8. Jag känner mig fräsch och utvilad när jag vaknar.				
9. Jag har varit lycklig, nöjd och tillfreds med mitt personliga liv.				
10. Jag har levt det liv som jag har önskat.				
11. Jag har känt mig ivrig att ta itu med dagliga göromål eller att fatta nya beslut.				
12. Jag har känt att jag lätt kunnat handskas med eller klara av allvarliga problem eller större förändringar i mitt liv.				

## HÄLSOINDEX

### 1. Hur är det med Din ork?

Orkar ingenting	orkar ganska lite	orkar ganska mycket	orkar nästan hur mycket som helst
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

---

### Hur är det med Ditt humör?

Är så ledsen	är ganska ledsen	är ganska glad	är så glad man kan bli
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

---

### 2. Hur trött känner Du dig?

Mycket trött	ganska trött	inte särskilt trött	inte alls trött
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

---

### 3. Känner Du Dig ensam och isolerad?

Mycket ensam	ganska ensam	inte särskilt ensam	inte alls ensam
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

---

### 5. Hur är din sömn?

Sover mycket dåligt	sover ganska dåligt	sover ganska bra	sover mycket bra
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

---

### 6. Har du yrsel?

Har ständig yrsel	har ofta yrsel	har sällan yrsel	har aldrig yrsel
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

---

-

### 7. Hur tycker Du att Din mage fungerar?

Har mycket besvär med magen	har ganska mycket besvär med magen	har lite besvär med magen	har inget besvär alls med magen
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

---

8. Besväras Du av värk eller smärta?

Har ständig värk	har ofta värk	har sällan värk	har aldrig värk
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

---

9. Har Du svårt att röra dig?

Har mycket svårt att röra mig	har ganska svårt att röra mig	har lite svårt att röra mig	är inte alls hindrad
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

---

10. Hur har Din hälsa varit i stort den senaste veckan?

Mycket dåligt	ganska dåligt	ganska bra	mycket bra
<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

---

## FQ

### Fysiska symtom

1. Har du problem med trötthet?
2. Skulle du vilja vila med (oftare)?
3. Känner du dig trött eller slö?
4. Har du problem med att komma igång med saker?
5. Kommer du igång med saker utan svårighet men blir svagare med tiden?
6. Känner du brist på energi?
7. Har du för lite styrka i dina muskler?
8. Känner du dig svag?

### Mentala symtom

1. Har du svårt att koncentrera dig?
2. Har du svårt att tänka klart?
3. Har tungan svårt att formulera orden när du talar?
4. Finner du ibland svårt att hitta rätt ord?
5. Hur är ditt minne
6. Har du förlorat intresse för saker som brukade roa dig förr?



EQ- VAS

**Ditt  
nuvarande  
hälsotillstånd**

Bästa  
tänkbara  
tillstånd

100

9 0

8 0

7 0

6 0

5 0

4 0

3 0

2 0

1 0

0

Sämsta  
tänkbara  
tillstånd

## 14 REFERENCES

- Abdul-Ghani, M. A., C. P. Jenkinson, D. K. Richardson, D. Tripathy and R. A. DeFronzo (2006). "Insulin secretion and action in subjects with impaired fasting glucose and impaired glucose tolerance: results from the Veterans Administration Genetic Epidemiology Study." Diabetes **55**(5): 1430-1435.
- Ahren, B. and G. Pacini (2004). "Importance of quantifying insulin secretion in relation to insulin sensitivity to accurately assess beta cell function in clinical studies." Eur J Endocrinol **150**(2): 97-104.
- Altman, D. G. (1980). "Statistics and ethics in medical research: III How large a sample?" Br Med J **281**(6251): 1336-1338.
- Aronsson, A., N. A. Al-Ani, K. Brismar and M. Hedstrom (2009). "A carbohydrate-rich drink shortly before surgery affected IGF-I bioavailability after a total hip replacement. A double-blind placebo controlled study on 29 patients." Aging Clin Exp Res **21**(2): 97-101.
- Ashby, E., M. P. Grocott and F. S. Haddad (2008). "Outcome measures for orthopaedic interventions on the hip." J Bone Joint Surg Br **90**(5): 545-549.
- Awad, S., K. K. Varadhan, O. Ljungqvist and D. N. Lobo (2013). "A meta-analysis of randomised controlled trials on preoperative oral carbohydrate treatment in elective surgery." Clin Nutr **32**(1): 34-44.
- Bacha, F., S. Lee, N. Gungor and S. A. Arslanian (2010). "From pre-diabetes to type 2 diabetes in obese youth: pathophysiological characteristics along the spectrum of glucose dysregulation." Diabetes Care **33**(10): 2225-2231.
- Barthelsson, C., B. Anderberg, S. Ramel, C. Bjorvell, K. Giesecke and G. Nordstrom (2008). "Outpatient versus inpatient laparoscopic cholecystectomy: a prospective randomized study of symptom occurrence, symptom distress and general state of health during the first post-operative week." J Eval Clin Pract **14**(4): 577-584.
- Beard, J. C., R. N. Bergman, W. K. Ward and D. Porte, Jr. (1986). "The insulin sensitivity index in nondiabetic man. Correlation between clamp-derived and IVGTT-derived values." Diabetes **35**(3): 362-369.
- Bennett-Guerrero, E., I. Welsby, T. J. Dunn, L. R. Young, T. A. Wahl, T. L. Diers, B. G. Phillips-Bute, M. F. Newman and M. G. Mythen (1999). "The use of a postoperative morbidity survey to evaluate patients with prolonged hospitalization after routine, moderate-risk, elective surgery." Anesth Analg **89**(2): 514-519.
- Bergman, R. N. (1989). "Lilly lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach." Diabetes **38**(12): 1512-1527.
- Bergman, R. N., Y. Z. Ider, C. R. Bowden and C. Cobelli (1979). "Quantitative estimation of insulin sensitivity." Am J Physiol **236**(6): E667-677.
- Bergman, R. N., R. Prager, A. Volund and J. M. Olefsky (1987). "Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp." J Clin Invest **79**(3): 790-800.

- Berndtson, D., J. Olsson and R. G. Hahn (2008). "Hypovolaemia after glucose/insulin infusions in volunteers." Clin Sci (Lond) **115**(12): 371-378.
- Bisgaard, T., V. B. Kristiansen, N. C. Hjortso, L. S. Jacobsen, J. Rosenberg and H. Kehlet (2004). "Randomized clinical trial comparing an oral carbohydrate beverage with placebo before laparoscopic cholecystectomy." Br J Surg **91**(2): 151-158.
- Borai, A., C. Livingstone and G. A. Ferns (2007). "The biochemical assessment of insulin resistance." Ann Clin Biochem **44**(Pt 4): 324-342.
- Borai, A., C. Livingstone, I. Kaddam and G. Ferns (2011). "Selection of the appropriate method for the assessment of insulin resistance." BMC Med Res Methodol **11**: 158.
- Brandi, L. S., M. Frediani, M. Oleggini, F. Mosca, M. Cerri, C. Boni, N. Pecori, G. Buzzigoli and E. Ferrannini (1990). "Insulin resistance after surgery: normalization by insulin treatment." Clin Sci (Lond) **79**(5): 443-450.
- Brandstrup, B., H. Tonnesen, R. Beier-Holgersen, E. Hjortso, H. Ording, K. Lindorff-Larsen, M. S. Rasmussen, C. Lanng, L. Wallin, L. H. Iversen, C. S. Gramkow, M. Okholm, T. Blemmer, P. E. Svendsen, H. H. Rottensten, B. Thage, J. Riis, I. S. Jeppesen, D. Teilum, A. M. Christensen, B. Graungaard and F. Pott (2003). "Effects of intravenous fluid restriction on postoperative complications: comparison of two perioperative fluid regimens: a randomized assessor-blinded multicenter trial." Ann Surg **238**(5): 641-648.
- Chalder, T., G. Berelowitz, T. Pawlikowska, L. Watts, S. Wessely, D. Wright and E. P. Wallace (1993). "Development of a fatigue scale." J Psychosom Res **37**(2): 147-153.
- Cobelli, C., G. M. Toffolo, C. Dalla Man, M. Campioni, P. Denti, A. Caumo, P. Butler and R. Rizza (2007). "Assessment of beta-cell function in humans, simultaneously with insulin sensitivity and hepatic extraction, from intravenous and oral glucose tests." Am J Physiol Endocrinol Metab **293**(1): E1-E15.
- Copeland, K. C., F. A. Kenney and K. S. Nair (1992). "Heated dorsal hand vein sampling for metabolic studies: a reappraisal." Am J Physiol **263**(5 Pt 1): E1010-1014.
- Dalla Man, C., F. Piccinini, R. Basu, A. Basu, R. A. Rizza and C. Cobelli (2013). "Modeling hepatic insulin sensitivity during a meal: validation against the euglycemic hyperinsulinemic clamp." Am J Physiol Endocrinol Metab **304**(8): E819-825.
- De Geest, T., P. Vansintjan and G. De Loore (2013). "Direct anterior total hip arthroplasty: complications and early outcome in a series of 300 cases." Acta Orthop Belg **79**(2): 166-173.
- De Lorenzo, A., A. Andreoli, J. Matthie and P. Withers (1997). "Predicting body cell mass with bioimpedance by using theoretical methods: a technological review." J Appl Physiol **82**(5): 1542-1558.
- DeFronzo, R. A. (2009). "Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus." Diabetes **58**(4): 773-795.
- DeFronzo, R. A., J. D. Tobin and R. Andres (1979). "Glucose clamp technique: a method for quantifying insulin secretion and resistance." Am J Physiol **237**(3): E214-223.
- Elia, M., A. Carter, S. Bacon, C. G. Winearls and R. Smith (1981). "Clinical usefulness of urinary 3-methylhistidine excretion in indicating muscle protein breakdown." Br Med J (Clin Res Ed) **282**(6261): 351-354.
- Faerch, K., C. Brons, A. C. Alibegovic and A. Vaag (2010). "The disposition index: adjustment for peripheral vs. hepatic insulin sensitivity?" J Physiol **588**(Pt 5): 759-764.

- Fellander, G., J. Nordenstrom, I. Tjader, J. Bolinder and P. Arner (1994). "Lipolysis during abdominal surgery." J Clin Endocrinol Metab **78**(1): 150-155.
- Ferrannini, E. and A. Mari (1998). "How to measure insulin sensitivity." J Hypertens **16**(7): 895-906.
- Gibson, A. (1950). "Posterior exposure of the hip joint." J Bone Joint Surg Br **32-B**(2): 183-186.
- Group, T. E. (1990). "EuroQol--a new facility for the measurement of health-related quality of life. The EuroQol Group." Health Policy **16**(3): 199-208.
- Gunerhan, Y., N. Koksall, U. Y. Sahin, M. A. Uzun and E. Eksioglu-Demiralp (2009). "Effect of preoperative immunonutrition and other nutrition models on cellular immune parameters." World J Gastroenterol **15**(4): 467-472.
- Hahn, R. G., S. Ljunggren, F. Larsen and T. Nystrom (2011). "A simple intravenous glucose tolerance test for assessment of insulin sensitivity." Theor Biol Med Model **8**: 12.
- Hailer, N. P., G. Garellick and J. Karrholm (2010). "Uncemented and cemented primary total hip arthroplasty in the Swedish Hip Arthroplasty Register." Acta Orthop **81**(1): 34-41.
- Hardinge, K. (1982). "The direct lateral approach to the hip." J Bone Joint Surg Br **64**(1): 17-19.
- Hausel, J., J. Nygren, A. Thorell, M. Lagerkranser and O. Ljungqvist (2005). "Randomized clinical trial of the effects of oral preoperative carbohydrates on postoperative nausea and vomiting after laparoscopic cholecystectomy." Br J Surg **92**(4): 415-421.
- Hedstrom, M., O. Ljungqvist and T. Cederholm (2006). "Metabolism and catabolism in hip fracture patients: nutritional and anabolic intervention--a review." Acta Orthop **77**(5): 741-747.
- Henriksen, M. G., I. Hesselov, F. Dela, H. V. Hansen, V. Haraldsted and S. A. Rodt (2003). "Effects of preoperative oral carbohydrates and peptides on postoperative endocrine response, mobilization, nutrition and muscle function in abdominal surgery." Acta Anaesthesiol Scand **47**(2): 191-199.
- Hope, S. V., A. G. Jones, E. Goodchild, M. Shepherd, R. E. Besser, B. Shields, T. McDonald, B. A. Knight and A. Hattersley (2013). "Urinary C-peptide creatinine ratio detects absolute insulin deficiency in Type 2 diabetes." Diabet Med.
- Hunter, S. J. and W. T. Garvey (1998). "Insulin action and insulin resistance: diseases involving defects in insulin receptors, signal transduction, and the glucose transport effector system." Am J Med **105**(4): 331-345.
- Häussinger D (1995). "Regulation of metabolism by changes in cellular hydration." Clin Nutr **1995**; **14**: 4-12.
- Jaffrin, M. Y. and H. Morel (2008). "Body fluid volumes measurements by impedance: A review of bioimpedance spectroscopy (BIS) and bioimpedance analysis (BIA) methods." Med Eng Phys **30**(10): 1257-1269.
- Johnson, H. L., S. P. Virk, P. Mayclin and T. Barbieri (1992). "Predicting total body water and extracellular fluid volumes from bioelectrical measurements of the human body." J Am Coll Nutr **11**(5): 539-547.

Kahn, R., J. Buse, E. Ferrannini and M. Stern (2005). "The metabolic syndrome: time for a critical appraisal: joint statement from the American Diabetes Association and the European Association for the Study of Diabetes." Diabetes Care **28**(9): 2289-2304.

Kahn, S. E. (2003). "The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes." Diabetologia **46**(1): 3-19.

Kahn, S. E., R. L. Prigeon, D. K. McCulloch, E. J. Boyko, R. N. Bergman, M. W. Schwartz, J. L. Neifing, W. K. Ward, J. C. Beard, J. P. Palmer and et al. (1993). "Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function." Diabetes **42**(11): 1663-1672.

Katz, A., S. S. Nambi, K. Mather, A. D. Baron, D. A. Follmann, G. Sullivan and M. J. Quon (2000). "Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans." J Clin Endocrinol Metab **85**(7): 2402-2410.

Kocalevent, R. D., A. Hinz, E. Brahler and B. F. Klapp (2011). "Determinants of fatigue and stress." BMC Res Notes **4**: 238.

Kratzing, C. (2011). "Pre-operative nutrition and carbohydrate loading." Proc Nutr Soc **70**(3): 311-315.

Laakso, M. (1999). "Hyperglycemia and cardiovascular disease in type 2 diabetes." Diabetes **48**(5): 937-942.

Laakso, M., J. Zilinskaite, T. Hansen, T. W. Boesgaard, M. Vanttinen, A. Stancakova, P. A. Jansson, F. Pellme, J. J. Holst, T. Kuulasmaa, M. L. Hribal, G. Sesti, N. Stefan, A. Fritsche, H. Haring, O. Pedersen and U. Smith (2008). "Insulin sensitivity, insulin release and glucagon-like peptide-1 levels in persons with impaired fasting glucose and/or impaired glucose tolerance in the EUGENE2 study." Diabetologia **51**(3): 502-511.

Lauwick, S. M., A. Kaba, S. Mawaja, E. E. Hamoir and J. L. Joris (2009). "Effects of oral preoperative carbohydrate on early postoperative outcome after thyroidectomy." Acta Anaesthesiol Belg **60**(2): 67-73.

Li, L., Z. Wang, X. Ying, J. Tian, T. Sun, K. Yi, P. Zhang, Z. Jing and K. Yang (2012). "Preoperative carbohydrate loading for elective surgery: a systematic review and meta-analysis." Surg Today **42**(7): 613-624.

Lin, J. D., Y. L. Chen, C. H. Hsu, C. Z. Wu, A. T. Hsieh, C. H. Hsieh, J. B. Chang, Y. J. Liang and D. Pei (2013). "Beta-cell function and insulin sensitivity at various degrees of glucose tolerance in Chinese subjects." Diabetes Res Clin Pract **100**(3): 391-397.

Ljungqvist, O. (2010). "Insulin resistance and outcomes in surgery." J Clin Endocrinol Metab **95**(9): 4217-4219.

Ljungqvist, O. and A. Alibegovic (1994). "Hyperglycaemia and survival after haemorrhage." Eur J Surg **160**(9): 465-469.

Ljungqvist, O., J. Nygren and A. Thorell (2000). "Insulin resistance and elective surgery." Surgery **128**(5): 757-760.

Ljungqvist, O., E. Sandberg, G. Nylander and J. Ware (1989). "Glucose kinetics in haemorrhagic hyperglycemia." Circ Shock **28**(4): 347-356.

Ljungqvist, O., M. Soop and M. Hedstrom (2007). "Why metabolism matters in elective orthopedic surgery: a review." Acta Orthop **78**(5): 610-615.

Ljungqvist, O., A. Thorell, M. Gutniak, T. Haggmark and S. Efendic (1994). "Glucose infusion instead of preoperative fasting reduces postoperative insulin resistance." J Am Coll Surg **178**(4): 329-336.

Matsuda, M. and R. A. DeFronzo (1999). "Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp." Diabetes Care **22**(9): 1462-1470.

Matthews, D. R., J. P. Hosker, A. S. Rudenski, B. A. Naylor, D. F. Treacher and R. C. Turner (1985). "Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man." Diabetologia **28**(7): 412-419.

Messier, S. P., R. F. Loeser, M. N. Mitchell, G. Valle, T. P. Morgan, W. J. Rejeski and W. H. Ettinger (2000). "Exercise and weight loss in obese older adults with knee osteoarthritis: a preliminary study." J Am Geriatr Soc **48**(9): 1062-1072.

Muniyappa, R., S. Lee, H. Chen and M. J. Quon (2008). "Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage." Am J Physiol Endocrinol Metab **294**(1): E15-26.

Nadon, G. W., J. A. Little, W. E. Hall and M. O. O'Sullivan (1964). "A COMPARISON OF THE ORAL AND INTRAVENOUS GLUCOSE TOLERANCE TESTS IN NON-DIABETIC, POSSIBLE DIABETIC AND DIABETIC SUBJECTS." Can Med Assoc J **91**: 1350-1353.

Nittala, A., S. Ghosh, D. Stefanovski, R. Bergman and X. Wang (2006). "Dimensional analysis of MINMOD leads to definition of the disposition index of glucose regulation and improved simulation algorithm." Biomed Eng Online **5**: 44.

Nordstrom, G., C. R. Nyman and T. Theorell (1992). "Psychosocial adjustment and general state of health in patients with ileal conduit urinary diversion." Scand J Urol Nephrol **26**(2): 139-147.

Nygren, J., M. Soop, A. Thorell, S. Efendic, K. S. Nair and O. Ljungqvist (1998). "Preoperative oral carbohydrate administration reduces postoperative insulin resistance." Clin Nutr **17**(2): 65-71.

Nygren, J., M. Soop, A. Thorell, K. Sree Nair and O. Ljungqvist (1999). "Preoperative oral carbohydrates and postoperative insulin resistance." Clin Nutr **18**(2): 117-120.

Nygren, J., A. Thorell, S. Efendic, K. S. Nair and O. Ljungqvist (1997). "Site of insulin resistance after surgery: the contribution of hypocaloric nutrition and bed rest." Clin Sci (Lond) **93**(2): 137-146.

Nygren, J., A. Thorell and O. Ljungqvist (2001). "Preoperative oral carbohydrate nutrition: an update." Curr Opin Clin Nutr Metab Care **4**(4): 255-259.

Nygren, J. O., A. Thorell, M. Soop, S. Efendic, K. Brismar, F. Karpe, K. S. Nair and O. Ljungqvist (1998). "Perioperative insulin and glucose infusion maintains normal insulin sensitivity after surgery." Am J Physiol **275**(1 Pt 1): E140-148.

Ostendorf, M., H. F. van Stel, E. Buskens, A. J. Schrijvers, L. N. Marting, A. J. Verbout and W. J. Dhert (2004). "Patient-reported outcome in total hip replacement. A comparison of five instruments of health status." J Bone Joint Surg Br **86**(6): 801-808.

Pacini, G. and R. N. Bergman (1986). "MINMOD: a computer program to calculate insulin sensitivity and pancreatic responsivity from the frequently sampled intravenous glucose tolerance test." Comput Methods Programs Biomed **23**(2): 113-122.

Pacini, G. and A. Mari (2003). "Methods for clinical assessment of insulin sensitivity and beta-cell function." Best Pract Res Clin Endocrinol Metab **17**(3): 305-322.

Pietropaolo, M. (2013). "Persistent C-peptide: what does it mean?" Curr Opin Endocrinol Diabetes Obes **20**(4): 279-284.

Pouwer, F., F. J. Snoek, H. M. van der Ploeg, H. J. Ader and R. J. Heine (2000). "The well-being questionnaire: evidence for a three-factor structure with 12 items (W-BQ12)." Psychol Med **30**(2): 455-462.

Reaven, G. M. (1997). "Banting Lecture 1988. Role of insulin resistance in human disease. 1988." Nutrition **13**(1): 65; discussion 64, 66.

Riediger, W., S. Doering and M. Krismer (2010). "Depression and somatisation influence the outcome of total hip replacement." Int Orthop **34**(1): 13-18.

Robinson, L. E. and M. H. van Soeren (2004). "Insulin resistance and hyperglycemia in critical illness: role of insulin in glycemic control." AACN Clin Issues **15**(1): 45-62.

Saad, M. F., G. M. Steil, M. Riad-Gabriel, A. Khan, A. Sharma, R. Boyadjian, S. D. Jinagouda and R. N. Bergman (1997). "Method of insulin administration has no effect on insulin sensitivity estimates from the insulin-modified minimal model protocol." Diabetes **46**(12): 2044-2048.

Saltiel, A. R. (2000). "Series introduction: the molecular and physiological basis of insulin resistance: emerging implications for metabolic and cardiovascular diseases." J Clin Invest **106**(2): 163-164.

Saltiel, A. R. and C. R. Kahn (2001). "Insulin signalling and the regulation of glucose and lipid metabolism." Nature **414**(6865): 799-806.

Sandberg, A. A., M. Woodruff, H. Rosenthal, S. Nienhouse and W. R. Slaunwhite, Jr. (1964). "TRANSCORTIN: A CORTICOSTEROID-BINDING PROTEIN OF PLASMA. VII. HALF-LIFE IN NORMAL AND ESTROGEN-TREATED SUBJECTS." J Clin Invest **43**: 461-466.

Sato, H., G. Carvalho, T. Sato, R. Lattermann, T. Matsukawa and T. Schricker (2010). "The association of preoperative glycemic control, intraoperative insulin sensitivity, and outcomes after cardiac surgery." J Clin Endocrinol Metab **95**(9): 4338-4344.

Shulman, G. I. (2000). "Cellular mechanisms of insulin resistance." J Clin Invest **106**(2): 171-176.

Sjolin, J., G. Hjort, G. Friman and L. Hambræus (1987). "Urinary excretion of 1-methylhistidine: a qualitative indicator of exogenous 3-methylhistidine and intake of meats from various sources." Metabolism **36**(12): 1175-1184.

Sjostrand, F., L. Edsberg and R. G. Hahn (2001). "Volume kinetics of glucose solutions given by intravenous infusion." Br J Anaesth **87**(6): 834-843.

Sjostrand, F. and R. G. Hahn (2003). "Validation of volume kinetic analysis of glucose 2.5% solution given by intravenous infusion." Br J Anaesth **90**(5): 600-607.

- Soop, M., J. Nygren, K. Brismar, A. Thorell and O. Ljungqvist (2000). "The hyperinsulinaemic-euglycaemic glucose clamp: reproducibility and metabolic effects of prolonged insulin infusion in healthy subjects." Clin Sci (Lond) **98**(4): 367-374.
- Soop, M., J. Nygren, P. Myrenfors, A. Thorell and O. Ljungqvist (2001). "Preoperative oral carbohydrate treatment attenuates immediate postoperative insulin resistance." Am J Physiol Endocrinol Metab **280**(4): E576-583.
- Soop, M., J. Nygren, A. Thorell, L. Weidenhielm, M. Lundberg, F. Hammarqvist and O. Ljungqvist (2004). "Preoperative oral carbohydrate treatment attenuates endogenous glucose release 3 days after surgery." Clin Nutr **23**(4): 733-741.
- Strandberg, P. and R. G. Hahn (2005). "Volume kinetics of glucose 2.5% solution and insulin resistance after abdominal hysterectomy." Br J Anaesth **94**(1): 30-38.
- Svanfeldt, M., A. Thorell, J. Hausel, M. Soop, J. Nygren and O. Ljungqvist (2005). "Effect of "preoperative" oral carbohydrate treatment on insulin action--a randomised cross-over unblinded study in healthy subjects." Clin Nutr **24**(5): 815-821.
- Svensen, C. H., J. Olsson and R. G. Hahn (2006). "Intravascular fluid administration and hemodynamic performance during open abdominal surgery." Anesth Analg **103**(3): 671-676.
- Thorell, A., S. Efendic, M. Gutniak, T. Haggmark and O. Ljungqvist (1993). "Development of postoperative insulin resistance is associated with the magnitude of operation." Eur J Surg **159**(11-12): 593-599.
- Thorell, A., S. Efendic, M. Gutniak, T. Haggmark and O. Ljungqvist (1994). "Insulin resistance after abdominal surgery." Br J Surg **81**(1): 59-63.
- Thorell, A., A. Loftenius, B. Andersson and O. Ljungqvist (1996). "Postoperative insulin resistance and circulating concentrations of stress hormones and cytokines." Clin Nutr **15**(2): 75-79.
- Thorell, A., J. Nygren and O. Ljungqvist (1999). "Insulin resistance: a marker of surgical stress." Curr Opin Clin Nutr Metab Care **2**(1): 69-78.
- Tripathy, D., Y. Wessman, M. Gullstrom, T. Tuomi and L. Groop (2003). "Importance of obtaining independent measures of insulin secretion and insulin sensitivity during the same test: results with the Botnia clamp." Diabetes Care **26**(5): 1395-1401.
- Tura, A., S. Sbrignadello, E. Succurro, L. Groop, G. Sesti and G. Pacini (2010). "An empirical index of insulin sensitivity from short IVGTT: validation against the minimal model and glucose clamp indices in patients with different clinical characteristics." Diabetologia **53**(1): 144-152.
- Turpeinen, U. and U. H. Stenman (2003). "Determination of urinary free cortisol by liquid chromatography-tandem mass spectrometry." Scand J Clin Lab Invest **63**(2): 143-150.
- Uchida, I., T. Asoh, C. Shirasaka and H. Tsuji (1988). "Effect of epidural analgesia on postoperative insulin resistance as evaluated by insulin clamp technique." Br J Surg **75**(6): 557-562.
- Wallace, T. M., J. C. Levy and D. R. Matthews (2004). "Use and abuse of HOMA modeling." Diabetes Care **27**(6): 1487-1495.
- Wallberg-Henriksson, H., J. Rincon and J. R. Zierath (1998). "Exercise in the management of non-insulin-dependent diabetes mellitus." Sports Med **25**(1): 25-35.



- Wang, Z. G., Q. Wang, W. J. Wang and H. L. Qin (2010). "Randomized clinical trial to compare the effects of preoperative oral carbohydrate versus placebo on insulin resistance after colorectal surgery." Br J Surg **97**(3): 317-327.
- Weaver, J. K. (1975). "Total hip replacement: a comparison between the transtrochanteric and posterior surgical approaches." Clin Orthop Relat Res(112): 201-207.
- Weeden, S. H., W. G. Paprosky and J. W. Bowling (2003). "The early dislocation rate in primary total hip arthroplasty following the posterior approach with posterior soft-tissue repair." J Arthroplasty **18**(6): 709-713.
- Westacott, D. J., J. McArthur, R. J. King and P. Foguet (2013). "Assessment of cup orientation in hip resurfacing: a comparison of TraumaCad and computed tomography." J Orthop Surg Res **8**: 8.
- Vigano, J., E. Cereda, R. Caccialanza, R. Carini, B. Cameletti, M. Spampinato and P. Dionigi (2012). "Effects of preoperative oral carbohydrate supplementation on postoperative metabolic stress response of patients undergoing elective abdominal surgery." World J Surg **36**(8): 1738-1743.
- Wiklund, I. and B. Romanus (1991). "A comparison of quality of life before and after arthroplasty in patients who had arthrosis of the hip joint." J Bone Joint Surg Am **73**(5): 765-769.
- Vogeser, M. (2003). "Liquid chromatography-tandem mass spectrometry--application in the clinical laboratory." Clin Chem Lab Med **41**(2): 117-126.
- Vogeser, M. and K. G. Parhofer (2007). "Liquid chromatography tandem-mass spectrometry (LC-MS/MS)--technique and applications in endocrinology." Exp Clin Endocrinol Diabetes **115**(9): 559-570.
- Wredling, R., J. Stalhammar, U. Adamson, C. Berne, Y. Larsson and J. Ostman (1995). "Well-being and treatment satisfaction in adults with diabetes: a Swedish population-based study." Qual Life Res **4**(6): 515-522.
- Yamada, H., Y. Yoshihara, O. Henmi, M. Morita, Y. Shiromoto, T. Kawano, A. Kanaji, K. Ando, M. Nakagawa, N. Kosaki and E. Fukaya (2009). "Cementless total hip replacement: past, present, and future." J Orthop Sci **14**(2): 228-241.