

Assessment of the Dynamic Insulin Secretion and Sensitivity Test (DISST) in a morbidly obese population pre and post gastric bypass surgery

Thesis Submission for Masters of Medical Science

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Introduction

Type two diabetes mellitus (T2DM) is in epidemic proportions world wide (1, 2) with obesity being the main driver over the last 30-40 years. Obesity increases the risk of T2DM through an increase in insulin resistance. Establishing the best methods of measuring insulin action have occupied researchers for many years, indicating that no single method can be universally adopted or be suitable for all situations. There are limited data about the effect of bariatric surgery on the measurements of insulin resistance. Recently a new test for the assessment of insulin resistance, the Dynamic Insulin Sensitivity and Secretion Test (DISST), has been developed through the bioengineering department at the University of Canterbury as a suitable substitute for the current but complex “gold standard” reference method, the euglycaemic hyperinsulinaemic clamp (EIC). The DISST method has been validated against the clamp in a range of subjects but not after an intervention which is known to fundamentally change glucose homeostasis and insulin sensitivity. The primary aim of this thesis is to test preliminary performance of DISST relative to the EIC in a morbidly obese cohort before and after bariatric surgery. Secondary aims were assessing the performance of other simple fasting tests against the euglycaemic clamp and also assessing weight, blood pressure and lipid changes in the early post surgery phase.

Insulin and its actions

Insulin is a peptide hormone produced in the beta cells of the pancreas. It was discovered in the early 1920s and won its discoverers a Nobel prize for Medicine. It had an immediate effect on the lives of those with Type 1 Diabetes who were usually children and were insulin deficient. Before the advent of use of insulin, Type 1 Diabetes was universally fatal.

Insulin has a number of effects on the body but acts mainly in the hepatocytes, myocytes and adipocytes. It stimulates glycogen synthesis and inhibits glycogenolysis (3, 4). Insulin stimulates lipogenesis and lipid storage, while inhibiting lipolysis (3). Insulin also stimulates protein synthesis and inhibits protein breakdown. Finally insulin stimulates cell growth and differentiation (3). All of these actions are important in energy partitioning and utilisation. However, perhaps the most important action of insulin is in glucose uptake and utilisation.

Insulin is continuously produced and released by the beta cells of the pancreas to maintain background glucose homeostasis. Further release of insulin is mediated by the ingestion of food via various neural and hormonal mechanisms to dispose of glucose and lipids that have been ingested and absorbed into the body, so returning blood glucose concentrations to normal physiological background levels after a meal.

The control and release of insulin is determined by many factors including plasma glucose, free fatty acids, the autonomic nervous system, fat derived hormones and cytokines and gut derived hormones such as Glucagon-like Peptide-1 (GLP-1). GLP-1 stimulates insulin secretion, promotes beta cell mitosis while inhibiting apoptosis, inhibits glucagon secretion and delays gastric emptying.

The actions of insulin are mediated by a cascade of intracellular events which are activated after insulin binds to its cell surface receptor. The scope of these intracellular events is beyond this thesis but are well summarised elsewhere (5).

To perform its function, not only must insulin be produced in sufficient quantity in response to the appropriate signalling for its release from the beta cells, it must also travel via the blood stream, bind to appropriate receptors and the signals produced within the cell must respond appropriately. Impairments of any of these steps produce the phenomenon of insulin resistance.

Insulin Resistance

Insulin resistance is the inability of insulin to produce a normal physiological response to normal physiological concentrations of insulin in the setting of a normal physiological stimulus. Insulin resistance is at times also described in its inverse, “insulin sensitivity”. Hence the more insulin resistant a subject is, the less insulin sensitive they are, and vice versa. The consequence of insulin resistance is an increased secretion of insulin and a higher circulating concentration of insulin required to produce the normal response to maintain normal glucose and lipid homeostasis (6,7).

Insulin resistance is an important entity in health and disease. Insulin resistance is strongly associated with many other illnesses. These include hyperlipidaemia, hypertension, gout, atherosclerosis, polycystic ovary disease and non-alcoholic steatohepatitis (8).

Its most recognised consequence is of the pathophysiological mechanism behind T2DM. Insulin resistance leads to hyperinsulinaemia to maintain normal glucose homeostasis, but when the beta cells of the pancreas are unable to secrete sufficient insulin to maintain normal glucose, T2DM ensues. Some treatments currently available for the management of T2DM primarily act to increase the insulin sensitivity of cells. Examples of this include Metformin, usually the initial treatment in for T2DM (9,10). Metformin acts by altering signalling within the hepatocyte mainly through activation of AMP-activated protein kinase (AMPK) (11). Another example is the “Glitizone” group which enhance insulin sensitivity by modulating the transcription of the insulin sensitive genes via stimulation of the nuclear receptor peroxisome proliferator-activated receptor gamma (PPAR- γ) (12).

The causes of increased insulin resistance may be classified by their relationship with the insulin receptor: Either pre, at or post receptor. Some pre-receptor causes include antibodies produced against insulin or increased insulin degradation. At the receptor level there may be decreased numbers of receptors, reduced binding of insulin to its receptor, insulin receptor mutations or insulin receptor blocking antibodies. Post receptor there may be ineffective intracellular

signal transduction through any of the pathways or mutations in components of the signal pathway (for example the GLUT4 transporter protein). Combinations of the above are also common.

Specific conditions or agents can cause insulin resistance. For example, aging causes insulin resistance through a decreased production of GLUT4. Increased production of insulin antagonists (e.g. Cushing's syndrome, acromegaly) and physiological stress states, such as trauma, surgery, diabetes ketoacidosis, severe infection, uraemia and liver cirrhosis are also causes of insulin resistance. Medications can also cause increased insulin resistance including glucocorticoids, cyclosporine, niacin and protease inhibitors. Anti HIV therapy, protease inhibitor associated lipodystrophy and nucleoside analogues have also been implicated in the development of insulin resistance (13,14). Insulin therapy itself can lead to low titre IgG anti insulin antibodies, though these rarely cause a clinically relevant problem.

Puberty and pregnancy are both normal physiological states that increase insulin resistance and is one mechanism for the accelerated growth that occurs at these times (15,16,17). However, the most common cause world wide is now due to obesity.

Insulin resistance in obesity

The World Health Organisation states that obesity levels as defined by a BMI $\geq 30 \text{ kg/m}^2$ have doubled since 1980. Worldwide 600 million adults (or 13% of world population) are classed as obese. With a further 1.9 billion adults (39% of population) being overweight (18). This has been a feature of developed countries and New Zealand has not been immune from this phenomenon. Data from the 2011/2012 New Zealand Health Survey (19) showed that 28% of our adult population were obese with a further 35% being overweight. There are also particularly important differences between ethnicities in prevalence of obesity. In New Zealand, Maori and Pacific island populations have much higher rates of obesity than people of European decent, with 44% of adult Maori and 62% of Pacific peoples reported as obese.

The worldwide epidemic of T2DM has mirrored the rise of obesity (18,19,20). This is thought to be mainly mediated through the increased insulin resistance caused by obesity. Although most obese subjects are more insulin resistant than normal weight subjects there are many obese individuals who have normal insulin sensitivity.

The increased insulin resistance in obesity is complex and exact mechanisms are still being determined (21,22,23). Obesity leads to a change in a number of hormones, cytokines and adipokines that control glucose and lipid metabolism.

Obesity is defined by an increase of adipose tissue. The last 15 years has seen a greater understanding of adipose tissue biology. It is now recognised that it is more than simply an organ for lipid storage, but that it also releases a number of hormones, adipocytokines and other factors which have an important role in energy balance, lipid and glucose metabolism.

One such example is Tumour Necrosis factor alpha (TNF- α). This was first shown in 1993 by Hotamisligil et al (24) with increased production of TNF- α in the adipose tissue of obese and T2DM mice and that neutralising the TNF- α with

blocking antibodies improved their insulin sensitivity. In humans, a direct correlation between TNF- α production, obesity and insulin resistance has also been shown (25).

In humans TNF- α is not released into the circulation from adipose tissue in any significant concentration, but an increase in production of adipose TNF- α acts in an autocrine/paracrine mechanism. TNF- α represses genes that would normally enhance glucose uptake and increases beta-oxidation (26). These include glucose transporter genes (GLUT-4) (27), the insulin receptor itself and interference in the intracellular signalling from the receptor including interfering with autophosphorylation and activity and Insulin receptor substrate-1 (IRS-1) (26,28,29) and counteracts transcription factors that enhance insulin sensitivity such as peroxisome proliferated activated receptor gamma (PPAR- γ) (26,29,30).

TNF- α also upregulates many genes expressed in adipose tissue that are responsible for inflammation, immune response and energy balance including vascular cell adhesion molecule-1 (VCAM-1), plasminogen activator inhibitor-1 (PAI-1), IL-6, IL-1 β , angiotensinogen, resistin and leptin (26).

TNF- α also down regulates many genes responsible for Free Fatty acid (FFA) uptake and storage and results in enhanced lipolysis and release of FFA and cytokines into the circulation (29,31). FFA then inhibits insulin signalling in insulin responsive tissues, especially muscle. The released FFA, according to the lipid supply hypothesis (Randle hypothesis) (32), act as the predominant substrate in the intermediary metabolism. This results in a switch from glucose to fatty acid metabolism in skeletal muscle which may explain the decreased glucose uptake observed in the insulin resistant obesity state.

Visceral adipose tissue secretes FFA directly into the portal system, thereby exerting its effects directly on the liver. In the liver, high concentrations of FFA provide abundant substrate for Triglycerides and VLDL-synthesis, and for gluconeogenesis. FFA draining from visceral adipose tissue to the portal

circulation and onto the liver decreases hepatic insulin clearance, therefore contributing to the hyperinsulinaemia (33). There is also added contribution from cytokines and other inflammatory markers such as IL-6. Excess triglycerides accumulate in the liver further increasing hepatic insulin resistance.

In muscle high FFA concentrations favour beta oxidation, which diminishes glucose uptake (34). Beta-oxidation doesn't adequately clear FFA from the circulation, and even more so in the absence of physical activity (35) so entering a vicious circle of on going negative effect on glucose uptake and utilisation. In addition excess FFA are stored as triglyceride droplets in muscles, further increasing muscle insulin resistance (36,37).

Glycogen synthesis in muscle is also inhibited and as muscle is the main site of glucose disposal (80-90%), diminished uptake contributes greatly to hyperglycaemia (31,38). In Adipose tissue, FFA inhibits lipoprotein lipase activity, which is otherwise stimulated by insulin, and thereby reduces clearance of FFA from circulation (39) so further exacerbating the problem.

In the Beta cells of the pancreas prolonged exposure to high FFA concentrations impairs insulin secretion. This is an additive effect on individuals already genetically predisposed to altered insulin sensitivity of beta cell signal molecules e.g. protein kinases (40). This is the main mechanism of pre-receptor insulin resistance in obesity.

TNF- α has been shown to down regulate the genes for adiponectin. Adiponectin is exclusively produced and excreted by adipocytes and is strongly inversely related to insulin resistance in obesity (41). Both insulin and insulin-like growth factor (IGF-1) stimulate adiponectin synthesis. Adiponectin improves insulin sensitivity by various methods. In the liver it induces fatty acid oxidation, decreases lipid synthesis, decreases uptake of FFA and represses gluconeogenesis by enzyme down regulation (42,43). In muscle, adiponectin favours glucose and FFA oxidation. These effects are partly due to activation of

AMP-kinase (41). Thereby adiponectin decreases plasma FFA and glucose levels (42,43).

Adiponectin exerts effects on gene transcription also through inhibition of nuclear transcription factor kappaB (44). Additionally adiponectin suppresses secretion of TNF-alpha (45) in a typical feedback loop. Obesity is associated with reduced adiponectin concentration proportional to the degree of increased fat mass. The infusion of Adiponectin has been shown to improve insulin sensitivity (46).

Leptin, another hormone produced by adipose tissue and secreted in proportion to fat mass, is primarily a signal of energy stores to the hypothalamus where it is one of the factors influencing appetite and energy intake. It also has a direct role in insulin sensitivity (47). Leptin deficiency causes an increase in insulin resistance. As weight increases leptin levels rise but this may be a relatively leptin resistant state as levels get high. The rise in leptin should result in CNS/hypothalamic signals to down regulate appetite and to increase metabolic use of stored energy. There is somehow a "leptin resistance" so higher levels of leptin that are actually required.

Measuring Insulin Sensitivity

There are many ways of measuring insulin sensitivity which suggests that no one way is ideal in all conditions.

There are multiple factors that can influence insulin sensitivity. These include production and release of insulin, glucose sensing by the pancreas and the response to this, glucose absorption, factors in the liver increasing and decreasing gluconeogenesis or glycolysis, peripheral handling of glucose in muscle and other organs and tissues, as well as the interplay of other endocrine and paracrine hormones such as glucagon, GLP-1, cortisol and growth hormone.

The current reference method for measuring insulin sensitivity is the Euglycaemic Hyperinsulinaemic Clamp (EIC). All other indices of insulin resistance are measured against their ability to correlate to the EIC. The EIC was first described in 1957 by Andres et al (48) but modified to its current form by DeFronzo in 1979 (49). The basic technique is an infusion of high dose of insulin given to maintain a high predetermined hyperinsulinaemic concentration. This has the effect of shutting down hepatic glucose production and release. At the same time a variable rate glucose infusion is given to avoid hypoglycaemia. The glucose infusion rate is altered according to frequently sampled blood glucose concentrations, measured every few minutes from arterialized blood to maintain a steady glucose concentration usually around 5mmol/L. The principal of the high dose insulin infusion is that it shuts down hepatic glucose output so that the only source of circulating glucose is from the glucose infusion. By maintaining a euglycaemic level below the renal glucose threshold this also eliminates urinary glucose losses. An index of insulin sensitivity can then be calculated from the average insulin level and the rate of glucose infusion over the last part of the clamp, which equals the rate of glucose uptake into cells.

However the euglycaemic clamp technique is a complicated test involving multiple intravenous cannulae, multiple blood samples and a considerable length of time to perform. It is not practical for large studies or population studies but remains useful as a research technique. Because it shuts down hepatic production of glucose it really is a measure of peripheral insulin resistance.

Because of the very high doses of insulin and glucose used the clamp is also not being done under physiological conditions. The very high doses of insulin and glucose may be causing effects that we are not aware of. Although it is considered the reference method, the euglycaemic clamp still has a coefficient of variation of around 10% (49). Furthermore, in individuals who have high degrees of insulin resistance it cannot be assumed that the standard insulin infusion rate is adequate to fully suppress hepatic glucose output, which may introduce additional error. In those with morbid obesity there may also be marked differences in distribution of the glucose and insulin infusions compared to lean subjects.

Other methods of estimating insulin sensitivity include the intravenous glucose tolerance test with minimal modelling which uses a bolus of intravenous glucose and then frequent measures of insulin and glucose concentrations. Glucose disposal is modelled using simplified mathematical representations of the glucose insulin relationships (50). Using 2 equations, firstly the glucose kinetics with assuming single compartment models for glucose distribution, secondly the insulin effect. The sensitivity index represents the link between insulin levels in the effect compartment and the glucose disappearance from the glucose department. Again the difficulty of this test is the time involved (up to 4 hours) and the need for multiple blood tests. A major limitation of this method for estimating insulin sensitivity in those with established diabetes is the need for adequate insulin secretion by the pancreas. The minimal model again largely measures peripheral insulin resistance (51).

The Botnia clamp (52) uses a combination of both the intravenous glucose tolerance test and the euglycaemic clamp. This is so an estimate of both insulin secretion and insulin sensitivity could be achieved during the one test. The intravenous tolerance test gives an estimate of first phase insulin secretion and the euglycaemic clamp was begun one hour after the glucose bolus. The M-value correlated very well with the standard euglycaemic clamp ($r=0.953$, $p<0.005$). The same issues with the standard euglycaemic clamp are still present.

There have been numerous attempts to derive a simple fasting blood test to measure insulin resistance. One off fasting tests include fasting plasma insulin, fasting glucose, Homeostasis Model Assessment (HOMA) (53,54,55), 1/LogHOMA, Quantitative Insulin Sensitivity Check Index (QUICKI)(56), Revised QUICKI(57,58), Glucose/insulin ratio, insulin/glucose ratio, Fasting Insulin Resistance Index (FIRI)(59), Belfiore GLY (60), Belfiore FFA(60), and McAuley Index (61). All of these are very similar in derivation. All have insulin as part of their formula and all are products of insulin with glucose and/or triglycerides or free fatty acids, or are the inverse or log transformations of these. Multiple small validation studies have been conducted to compare these methods of estimating insulin sensitivity with the euglycaemic clamp or the minimal model (62).

The HOMA test has been the most widely used and published of the simple tests. HOMA was developed in 1985 (53) and relies on the simultaneous measurement of fasting insulin and glucose. The simple formula is:

$$\text{HOMA}(\text{equation}) = (\text{Glucose}(\text{mmol/L}) \times \text{Insulin}(\text{pmol/L}))/22.5.$$

It is easy to calculate and relies on a simple single fasting blood test which makes it attractive for epidemiological or large studies where more prolonged and complicated measures are impracticable or impossible. The results rely on a steady state of insulin and glucose and as it is measured in the fasting state it more likely reflects hepatic glucose homeostasis so therefore is more likely to be a marker of hepatic insulin resistance. The correlation with clamp studies range from $r=0.45$ to $r=0.89$ (62) with the best correlation reported by the original authors (53). In the original publication the glucose and insulin concentrations are taken as the average of three measurements five minutes apart, to account for the pulsatility of insulin release. However, in most studies utilising HOMA only one sample is taken, which increases error of the estimate. The authors have modified the equation of HOMA in a more recent publication (54) and now have a website for calculating HOMA (55). The two HOMA values however are not the same as the HOMA calculator value is calibrated against a young insulin sensitive population. Coefficient of variation is measured at 30% which is mainly due to biological variation (53).

The second most commonly cited fasting test is QUICKI (Quantitative Insulin Sensitivity Check Index) first published in 2000 (56). Further validation tests performed in 2005 (63). The basic formula is:

$$1 / ((\log(\text{fasting insulin } \mu\text{U/mL}) + \log(\text{fasting glucose mg/dL}))$$

To convert to our units of fasting glucose in mmol/L divided by 18. The initial study was also performed on a cross section of subjects with 28 nonobese, 13 obese and 15 type 2 diabetes subjects (56) with a very good correlation with the euglycaemic clamp ($r=0.78$). A further validation study by same group used 116 subjects ranging from nonobese, obese, type 2 diabetic and hypertensive (63). Again correlation was very good ($r=0.75$). In a recent meta-analysis (62) compared to the euglycaemic clamp this has a correlation of $r= 0.61$. QUICKI has also been modified with the addition of a free fatty acid component (revised QUICKI). Subsequent analysis (62) shows that this is the best of the fasting tests compared to the EIC with $r=0.67$ but this involved only 7 studies. This however compares very favourably with the best OGTT based tests and is within their confidence intervals.

Another way of estimating insulin sensitivity is to use a more dynamic test integrating glucose disposal and insulin concentrations from an oral rather than intravenous glucose load. Several indices have been derived using a 75g oral glucose tolerance test measurements of glucose and insulin from various subsequent time points. These include the Matsuda index (64), AUC insulin, Stumvoll metabolic clearance rate (65), Stumvoll insulin sensitivity index (65), Gutt (66), Belfiore(60) and Cederholm (67).

A recent meta-analysis (62) of measures of insulin sensitivity versus the hyperinsulinaemic-euglycaemic clamp looked at all articles from 1979 (when DeFronzo published his Clamp paper) (49) up until 2012 which reported bivariate correlations between the euglycaemic clamp and the surrogate measure for insulin sensitivity. They performed random effects meta-analysis for each surrogate measure to integrate the correlation coefficients of the different studies. The pooled correlations (r) were for the fasting surrogate markers computer generated HOMA $r=0.57$, logHOMA-IR $r=-0.60$ QUICKI $r=0.61$ and

revised QUICKI $r=0.68$. For the OGTT based tests Stumvoll metabolic clearance rate $r=0.70$, oral glucose insulin sensitivity $r=0.70$, Matsuda index $r=0.67$, Stumvoll insulin sensitivity index 0.67 and the Gutt index $r=0.65$. In this meta-analysis there were no studies with mean BMI in the morbidly obese range and most studies had average BMI in the mildly overweight.

Some methods of estimating insulin sensitivity also enable estimates of insulin secretion. Again these can be simple EG HOMA-beta%, or complex such as the Hyperglycaemic euinsulinaemic clamp also described by DeFronzo et al (49) but unable to be performed at the same time.

Differentiating between the different sites of insulin resistance is also difficult. There have been a number of EIC studies done using radioisotope glucose or other similar agents to differentiate hepatic versus peripheral insulin resistance. These add a further complexity to an already difficult and expensive study.

In validation studies for various tests most of the individuals included are not obese and do not have diabetes. When used in obese subjects or those with T2DM the methods all have limitations due to tissue specific effects of increasing insulin resistance or altered beta cell function, such that the relationship between insulin and glucose cannot be assumed to be the same, and therefore the correlation between simple methods and the more complex reference methods may not remain as robust.

The intra-individual variation of insulin is predominantly determined by biological variation on a day to day basis. The insulin test and retest variation on separate days was 60 % (68), whereas glucose was 15% (68) and triglycerides and Free fatty acids were similar (69). The analytical variation made only a minor contribution (68). Biological variation will always be present whereas analytical variability will lessen over time as better techniques evolve. Comparing between different results is also difficult as there are also interlab variations up to 3 fold (70).

Other problems with the simple tests of insulin resistance are the reliance on insulin secretion as part of test. If secretion is impaired this will alter results and this can be more an issue with dynamic tests.

Hyperglycaemia can affect some results of insulin sensitivity. Hyperglycaemia above the renal threshold will cause glucose loss in the urine so affecting results. Furthermore, hyperglycaemia itself can cause insulin resistance.

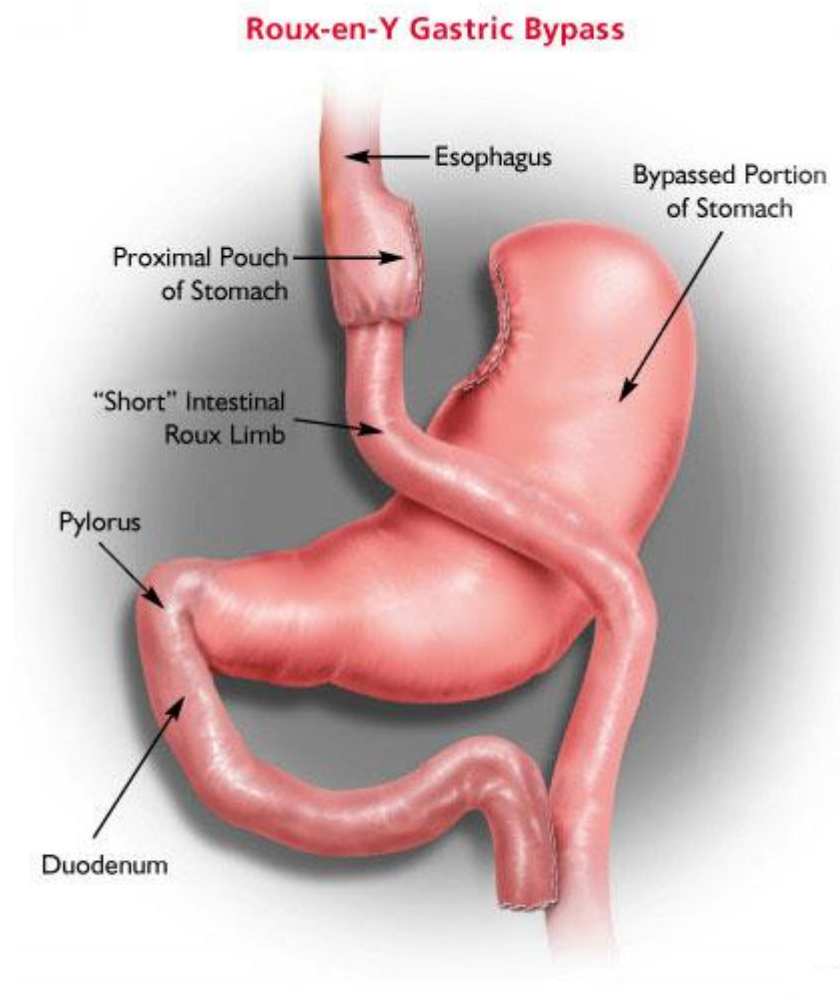
There is also the problem of glucose uptake by non insulin dependent cells or organs, for example the brain.

Changes in insulin resistance post Roux-en-y gastric bypass

Roux-en-y Gastric bypass (RYGB) remains the most common bariatric surgical procedure performed today and is considered the gold standard (71).

There are two parts to the Roux-en-y gastric bypass. First, a small stomach pouch is made by dividing the top of the stomach from the rest of the stomach. Next, the first part of the small intestine is divided, and the distal end is brought up and connected to the newly formed small stomach pouch. The proximal end of the divided small intestine is then attached to the small intestine further down. See figure 1 (72).

Figure 1. Roux-en-y gastric bypass (72).



One of the features of RYGB is the resolution of T2DM within days of the operation, before any significant weight loss has been achieved (73,74). This is

not seen in another common bariatric surgical procedure the gastric band. This suggests the operation itself has had an effect on glucose homeostasis. The gastric bypass is both a malabsorptive as well as a dietary restrictive procedure and it changes the anatomy and physiology of the gut. The gastric band is purely a restrictive procedure lessening food intake.

Following Roux-en-y gastric bypass there is rapid improvement within days of markers of glucose metabolism and before any substantial weight loss. There are 2 common theories proposed for the quick improvement in type 2 diabetes mellitus. One is the gut hormone theory where the changes in the anatomy and the way the food is presented to different parts of the intestine from the stomach produce alteration in neural signals and gut hormone release. Commonly associated hormones include PYY from the distal intestine which markedly increases post prandially after gastric bypass (75) and GLP-1 (76) which also markedly increases post prandially after gastric bypass. Both these cause an increase in insulin output, and they may also have an effect on the target organs for insulin including muscle cells and hepatic cells. There may also be yet unknown gut-derived factors that may be being released that are yet to be discovered. The operation itself may also interfere with signalling via the vagus nerve and its direct control over satiety and appetite from the hypothalamus (77). There are falls in both fasting glucose and fasting insulin and changes in other hormones associated with glucose metabolism including glucagon, GLP-1, free fatty acids and PYY (74). These have a direct effect on the markers of insulin resistance that use fasting insulin and glucose to estimate insulin resistance. Indices such as HOMA-IR and others based on fasting glucose and insulin and lipids all rapidly improve within days of RYGB surgery. This suggests that these are predominantly estimates of hepatic resistance as it has been shown that hepatic fat rapidly clears post gastric bypass surgery (73,74,78,79,80,81).

A similar picture of rapid improvement in insulin sensitivity without loss of fat mass can be found with short term severe caloric restriction with the same pattern of lower fasting glucose and lower fasting insulin. The second common theory for the quick improvement in diabetes and insulin resistance post

bariatric surgery is that of caloric restriction. This has been shown in the use of Very Low calorie diets (VLCD) (78,82,83,84,85). Anecdotally I have seen this also in the hospital setting where patients with known T2DM are admitted to hospital and kept nil by mouth, their blood glucose concentrations improve markedly. By dietary restriction and malabsorption there is the decreased absorption of free fatty acids and carbohydrate into the portal system. Therefore the liver is less exposed to elements known to increase hepatic insulin resistance. There are also less free fatty acids and lipids in the systemic circulation, so less uptake and accumulation in the myocytes. This enables lipid stored in hepatocytes and myocytes to be cleared relatively quickly, allowing normal switching from lipid to glucose oxidation and this improving the insulin sensitivity of these cells. There is also clearance of lipids from the pancreatic beta cells which enables increased insulin secretion (82).

The actual mechanism is probably a combination of all these as no one individual cause is shown to work as well.

Isbell et al (78) compared 9 subjects' pre and post RYGB with 9 subjects who had not undergone surgery but were on the same post procedure diet. They took fasting bloods and performed a mixed meal test at baseline and average 4 days post procedure. HOMA improved in both groups, insulin response blunted in both groups but no change in glucose response. GLP1 response to the meal test increased in the RYGB group but stayed same in diet only group. The authors concluded that this suggested the improved insulin sensitivity was due to caloric restriction.

Euglycaemic hyperinsulinaemic clamp studies do not show this rapid improvement in insulin sensitivity. Paradoxically the opposite occurs with using the hyperinsulinaemic euglycaemic clamp where this is more of a measure of peripheral insulin resistance. Clamp studies done at 2-3 weeks show no change in the degree of insulin resistance (86,87), and some studies done at 1 -2 weeks post surgery show worsening of insulin resistance, which may be the result of surgical stress (88,89). Studies done at 4 weeks to 3 months show variable

results (90) where as studies done 6 months or more post surgery show improvements in insulin resistance directly correlated to the amount of weight that has been lost (86,87,91).

The Dynamic insulin sensitivity and secretion test

The Dynamic Insulin sensitivity and secretion test (DISST) was designed to mimic the euglycaemic clamp so therefore being a true measure compared to the current reference method and more accurate than other markers of insulin resistance which use surrogate comparisons. It was also designed to be much simpler and shorter to perform, requiring a small number of blood tests and being performed over less than 1 hour. It involves the measurement of glucose, insulin and C-peptide at a number of time points with a low dose intravenous glucose (10g) infusion and low dose intravenous insulin (1 unit) bolus as stimulus for glucose disposal. The DISST test also has the advantage of being both a measure of insulin resistance and of insulin secretion.

The DISST test was developed using data from a series of euglycaemic clamps originating from a diet and exercise intervention study (92). The clamps were performed pre trial and at 16 weeks post trial. The study group consisted of 73 subjects (so 146 clamps) who were mainly mildly obese. The mean BMI pre intervention was 34.4 (SD 4.9) kg/m² with a range of 24.5-45.2kg/m² and post intervention mean BMI of 33.2 (SD 5.0) kg/m² and a range of 23.6-44.8 kg/m². These clamp data and pharmacodynamic modelling derived from Bergman (93) and others (94,95) was used to form a simulation cohort and the resulting DISST model (96).

A number of assumptions are made for the model. These included assuming insulin and c-peptide had similar plasma and interstitial volume distribution, renal clearance and diffusion constant. This was assumed as insulin and c-peptide have a similar molecular weight and similar passive properties. As the subjects were fasted the equilibrium glucose was set at 0. With low dose injection, saturation by insulin and glucose of available receptors and channels was unlikely. Other parameters were identified using a fitting model (97).

Using the above assumptions and the known error in accuracy of assays, timing, insulin and glucose dilution errors and an unmodelled suppression of

endogenous glucose production, a Monte Carlo analysis (98,99) was performed. The metabolic model was then fitted to the stimulated test profiles (glucose, insulin, C-Peptide) resulting in an insulin sensitivity index. The clamp fitted DISST insulin sensitivity index and the measured clamp ISI correlated very well ($r=0.93$).

The validation study (100) was performed on a group of 123 subjects of which 50 had DISST, Matsuda index and euglycaemic clamp performed. Of the 50 subjects having all 3 tests performed 10 were lean ($BMI < 25 \text{ kg/m}^2$), 20 were over weight ($BMI > 25$ but $< 30 \text{ kg/m}^2$) and 20 were obese ($BMI > 30 \text{ kg/m}^2$). Correlation between the euglycaemic clamp and the DISST was very good at $r=0.82$. This is much better than the other OGTT and fasting measures (62).

However, the DISST test has not been performed on a very obese group of subjects or on a group who have had an intervention which fundamentally changes glucose metabolism such as bariatric surgery. Therefore the aim of this thesis was to examine the performance of the DISST test compared with a euglycaemic clamp in an obese group of subjects and before and after gastric bypass surgery.

Methods

Participants:

All subjects were recruited through Wakefield Obesity Clinic from patients about to undergo Roux-en-y gastric bypass with Fobi pouch for weight loss by Professor Richard Stubbs. All potential participants were initially approached by Prof Stubbs. If patients expressed interest, I then made contact and provided an information sheet and then followed up with a telephone call. All subjects were obese (BMI greater than 35 kg/m²) and. All participants as part of their preoperative assessment underwent an oral glucose tolerance test (OGTT) unless they were known to have T2DM. Their glucose status ranged from normal glucose control (as per normal OGTT) through to known T2DM. There were no specific additional inclusion or exclusion criteria. The project was discussed by myself by telephone with each subject after they had adequate time to review the information sheet. Written informed consent was then obtained. The study was approved by the Central Regional Ethics Committee study number CEN/10/05/017.

Study Procedures:

Subjects underwent 3 procedures on 3 separate occasions prior to surgery and three to five weeks post surgery.

Prior to the initial test, demographic data were obtained as well as the collection of anthropometric and clinical data such as weight, height, waist circumference, hip circumference and blood pressure. From these Body Mass Index (BMI), waist to hip ratio and body surface area (BSA) were calculated by the Dubois method (101).

The tests involved were; Dynamic Insulin Secretion and Sensitivity test (DISST), a Euglycaemic Hyperinsulinaemic clamp and a standard meal test. The standard meal test was part of another project not included in this thesis but enabled us to obtain a further set of fasting glucose and insulin levels.

All subjects continued with their normal diet pre-operatively. They attended in the morning after a 12 hour overnight fast for their investigations. Postoperatively all subjects were on low calorie diets of between 600 and 1000 calories and attended for their investigations after a 12 hour overnight fast. Patients with T2DM on medication had individualised plans in regards to their medication management.

DISST Study:

Each subject arrived at the research centre after a 10 hour overnight fast, and was instructed not to take any of their regular medications. An intravenous cannula was placed in the arm and blood samples taken for glucose, insulin, C-peptide, lipids and a full blood count (time zero). Samples were centrifuged immediately and aliquot of serum and plasma were frozen for later analysis. After the fasting sample was taken, 10g of glucose (20ml 50% glucose) was then given intravenously at 5 minutes. At 10 and 15 minutes further bloods were taken for glucose, insulin and C-peptide. Immediately after the 15 minute bloods were taken 1 unit of Actrapid insulin was given intravenously. To prevent binding of insulin to plastic tubing 10 units of insulin were placed with 0.5ml patients own blood and made up to 10ml with normal saline. Then 1mL of this solution was then administered. Further bloods were then taken at t25 and t35 minutes for glucose, insulin and C-peptide.

Euglycaemic Hyperinsulinaemic Clamp Study

The Euglycaemic hyperinsulinaemic clamps were performed using standard technique as per DeFronzo (49). The subject was cannulated in the antecubital fossa of one arm and in the dorsum of the other hand. The cannulated hand was placed into a heated hand box to arterialise the venous blood (102). The temperature of the upper part of the hand box was set at 50 degrees centigrade giving an approximate setting of 42-43 degrees around hand. Baseline blood samples were taken 10 minutes after hand had been in hand warmer. An infusion of insulin of 25% glucose was set up at the antecubital fossa via infusion pumps. The established insulin dose for the euglycaemic insulin clamp is

40mU/min/m². The amount to be added to the 50ml syringe is calculated as: BSA (m²) x 40 mU/min/m² x 60min ÷ 15mL (infused volume per hour) x 50 mL (syringe volume) = mU of insulin to add to syringe. This can be simplified to BSA x 8 = Units of insulin to add to syringe. The insulin infusion was made up from Actrapid insulin in units equivalent to 8x BSA added to 3ml of subjects own blood, to prevent binding of insulin to plastic tubing(103), and 47 ml Normal saline to give total volume of 50ml. This was attached to a Y-non return valve extension to the antecubital fossa cannula and initial rate of infusion was 60ml/hour for 4 minutes, then 30 ml/hr for 3 minutes then a continuous infusion at 15 ml/hr after that (so rapidly decreasing hepatic glucose production). On the other leg of the Y non-return valve a bag of 1000ml 25% glucose was attached. This infusion started at weight in kg as ml/hour (so giving an infusion rate of 1mg/kg/min) at 4 minutes (unless blood glucose level was elevated above 5. If blood glucose level was above 5 the start of the infusion was delayed until blood glucose level was below 5). A blood sample for glucose was taken at 0, 4, 7 and 10 minutes then every 5 minutes after that. The blood glucose level was used to alter the infusion of 25% glucose with aim to maintain steady state of glucose between 4.0 and 5.0 mmol/L. Once stable, further measures of insulin were taken from 60 minutes and every 20 minutes for 3 occasions. If levels were not stable, the blood specimen for insulin was delayed until stable. After the third sample was taken the insulin infusion was stopped but the glucose infusion continued for a further 20 minutes to ensure subject did not become hypoglycaemic. The subject was then also provided a meal. An index of Insulin resistance was then calculated using the standard formula:

M = glucose disposal (consumption) during the steady state time
 = glucose infusion rate (mg/kg/min) – urinary loses – space correction
 Urinary loses of glucose when euglycaemic are assumed to be stable and negligible. The space correction should also be assumed to be zero if steady state is reached.

Standard Meal Test

The Standard meal test was Fortisip® 200ml. Subjects arrived at the research centre at 7am after a 10 hour overnight fast. An intravenous cannula was

inserted into the arm. Baseline blood samples were taken for glucose, insulin, C-peptide and incretins. When the test was performed prior to surgery, subjects then drank the Fortisip® over less than 1 minute. Postoperatively, subjects were asked to drink it over 30 minutes, as the rapid gastric emptying seen post gastric bypass surgery precluded them from consuming it quickly as dumping syndrome occurs as the meal immediately moves into small intestine. Subjects with normal anatomy have meal absorption half-times of about 60 minutes (104). Repeat bloods were then taken at 15, 30, 45, 60, 90 and 120 minutes. The baseline fasting samples were used in the derivation of fasting indices of insulin sensitivity for this thesis. The remainder of the test is beyond the scope of this thesis.

Due to practical time factors, subjects underwent these three tests preoperatively simply as they were able to attend. As there was no reason to believe that the effects of one test would influence the outcome of another, this was not randomised and was usually in the order DISST, then euglycaemic clamp then meal. There was a minimum washout time of two days between tests.

All subjects then underwent an open Roux-en-y gastric bypass with Fobi pouch. The Fobi Pouch gastric bypass has the same type of pouch construction as the Roux-en-Y gastric bypass. However rather than staples, it uses a silastic ring around distal end of the pouch to simulate the pyloric valve and prevent stretching of the opening between the pouch and the section of small bowel.

Post operatively all 3 tests were repeated. The DISST test was always performed first at 3 weeks post surgery, followed by the clamp test usually within 2 days (but not the following day) of the DISST test (mean, median, range) and finally by the meal test.

Subjects were required to be fasting for all investigations and all tests were performed between 0730 and 0900 with each subject keeping to specific time. All tests were performed at Wakefield hospital in Wellington.

Sample Analysis

All glucose levels were measured on an YSI 2300 Stat Plus, (YSI Life Sciences, Yellow Springs, Ohio, USA) which uses the glucose oxidase method. This gives accurate glucose results within a minute with a resolution of 0.1mmol/L and a precision of +/- 2% or 0.2 mmol/L (which ever is larger) (105).

All blood samples for insulin and C-peptide were immediately centrifuged at 4 degrees centigrade, and the plasma was then stored at -80 degrees for subsequent batch analysis.

Insulin and C-peptide concentrations were all performed at Department of Nutrition laboratory, University of Otago, Dunedin. Insulin and C-Peptide were both measured by a Cobas electrochemiluminescence immunoassay. Insulin levels were reported in both pmol/L and microU/mL. For insulin CV% for lab was 3.4%. For C-peptide the %CV for lab was 5.44%

Lipids and full blood count were all processed at the local community laboratory, Aotea pathology, Wellington.

For the DISST test, the results were modelled by Dr Paul Docherty, University of Canterbury, who was part of the team who have developed the test, using computer modelling and an index of insulin resistance (ISI-DISST) and an estimation of first phase and second phase insulin secretion was obtained.

For the euglycaemic clamp test a measure of glucose disposal (M) at steady state was obtained from the formula:

$M = \text{glucose infusion rate (mg/kg/min)} - \text{urinary losses} - \text{space correction}.$

With a glucose level below the renal threshold the urinary losses were estimated to be zero. With the glucose being at steady state the space correction was also estimated to be zero. An index of insulin sensitivity was then obtained using the formula:

$\text{ISI-Clamp} = M / I \times 100$ (where I is the mean of the measured steady state insulin values).

For all subjects there were a number of fasting insulin and glucose pairings from each of the dynamic tests available (up to three both preoperative and three post operative). HOMA (equation) was obtained for each available samples using:

$$\text{HOMA}(\text{equation}) = (\text{Glucose (mmol/L)} \times \text{Insulin (pmol/L)}) / 22.5 \quad (52).$$

The mean of available HOMA(equation) values for each subject pre and postoperatively was used as their actual value.

As the modified and available online calculator will not obtain values with insulin above certain values (57.6µU/mL or 400 pmol/L) those that were able to be done were calculated and again a mean of the values was obtained to give a HOMA(calculator) value (54,55).

Of the matched pairs where both a HOMA(equation) value and a HOMA(calculator) value were available these were analysed to ensure adequate correlation between them to assess whether just HOMA(equation) values could be used so as not to exclude the most insulin resistant subjects.

1/logHOMA(equation) was also calculated for each available test. Again the mean value was used as actual value.

QUICKI (56) was calculated using the standard formula:

$$\text{QUICKI} = 1 / (\log \text{Insulin (microU/ml)} + \log \text{Glucose (mmol/L} \times 18))$$

Again the mean value of available tests for each subject pre and post operative was used as actual value.

Statistical Analysis:

Values were compared for significance by standard student T test of values of ISI-clamp, ISI-DISST, HOMA(equation), 1/logHOMA(equation), QUICKI, weight, BMI, blood pressure and lipids. Pearson correlation_coefficient calculations were performed to test correlation of ISI-Clamp with ISI-DISST, HOMA(equation), 1/logHOMA(equation) and QUICKI as well as comparing HOMA(equation) with HOMA(calculator). Bland-Altman plot analysis was performed on the ISI-DISST versus ISI-Clamp. As the ISI-DISST and the ISI-Clamp have different units a conversion factor is required. This was converted using the formula:

$$\text{ISI DISST} = 18000 \times \text{Glucose baseline} \times \text{distribution volume of glucose (0.19} \times \text{weight)} \times \text{steady state ratio between plasma and interstitial fluid (0.5)} / \text{weight. (100)}.$$

As this was a pilot study to assess usability of the DISST test in a morbid obese population and assess its potential use in the early post bariatric surgery setting and we were uncertain on how many potential recruits we would be able to obtain no actual target number of participants or power calculations were performed.

Results

A total of 11 subjects were recruited for the study with 11 completing preoperative and 10 completing postoperative DISST testing, 10 completing preoperative euglycaemic clamp and 8 completing postoperative euglycaemic clamp and 11 completing preoperative mixed-meal test and 9 completing postoperative mixed-meal testing. Baseline characteristics of these subjects are shown in Table 1. Of the 11 participants, nine were female and two male. Their mean age was 51.2yrs (SD 12.1yrs), with a mean preoperative BMI of 48.7 (SD 9.5). Of the 11 subjects, 5 had normal glucose tolerance, 2 had impaired glucose metabolism, and 4 had T2DM (2 known and 2 diagnosed from preoperative OGTT). Only 1 (subject 6) of the T2DM subjects was on any glucose lowering medication (Gliclazide and metformin). This was withheld the evening before and the morning of each test preoperatively. The Subjects were on no diabetes medication at the time of the postoperative tests. The mean Blood pressure was 137.8/78.4 mmHg (SD 21.9/9.9). The mean Total Cholesterol was 5.2 mmol/L (SD 0.5), mean triglycerides were 1.8 mmol/L (SD 0.7) and mean HDL was 1.29 mmol/L (SD 0.24).

Table 1: Baseline preoperative individual characteristics of the 11 subjects.

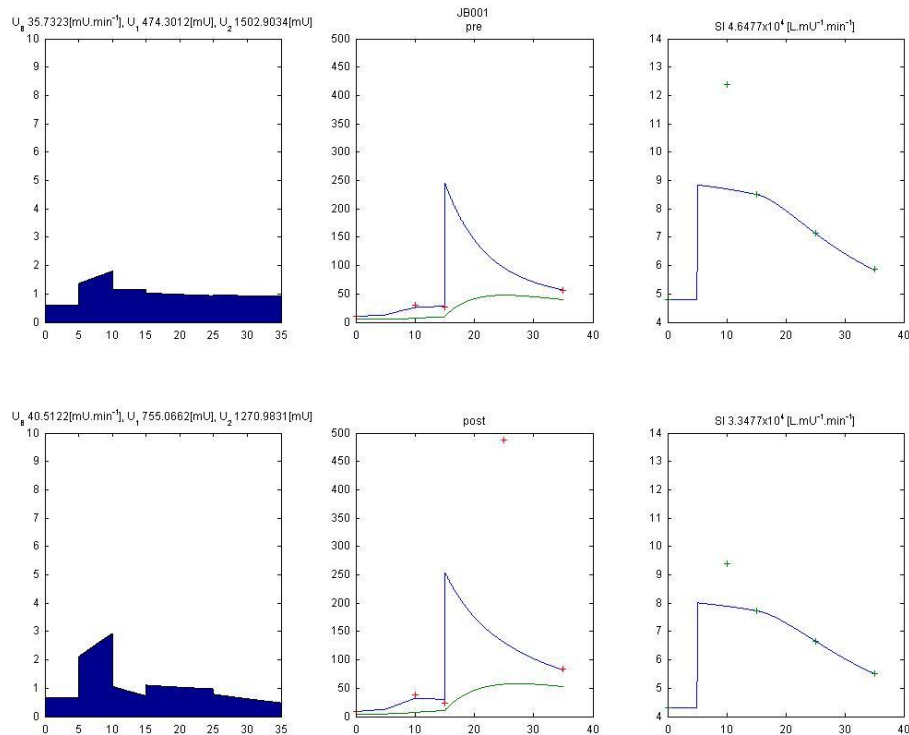
	Age	Sex	Weight (kg)	BMI (Kg/m ²)	Glucose status	BP (mmHg)	Total chol (mmol/L)	Tg (mmol/L)	HDL (mmol/L)
1	59	F	114.2	41.3	Normal	192/100	5.5	2.8	1.15
2	63	F	116.5	44.1	IGT	140/74	5.9	3.4	1.18
3	49	F	117.4	51.4	Normal	130/70	5.5	1.1	1.52
4	48	F	117.5	45.0	Normal	128/76	5.7	1.5	1.40
5	24	F	215.6	75.5	Normal	112/66			
6	61	F	121.2	41.8	Known T2DM	140/70	4.3	1.7	1.86
7	42	F	143.6	49.8	New T2DM		5.6	1.7	1.06
8	68	M	119.6	39.5	New T2DM	130/80	5.1	2.2	1.08
9	41	F	166.0	53.3	Normal	126/80	4.6	0.8	1.56
10	60	M	120.0	44.1	IFG	160/92	5.6	1.0	1.27
11	48	F	120.4	49.5	Known T2DM	120/76	4.1	2.0	0.83
mean	51.2		133.8	48.7		137.8/78.4	5.2	1.8	1.29
SD	12.1		29.8	9.5		21.9/9.9	0.5	0.7	0.24

Abbreviations: F=female, M=male, IGT=impaired glucose tolerance, IFG=Impaired fasting glucose, SD=standard deviation, BMI=Body mass index, BP=blood pressure, chol=cholesterol, Tg=triglycerides, HDL=high density lipoprotein

DISST results

A computer modeled Insulin Sensitivity Index (ISI-DISST) was calculated on 11 subjects preoperatively and 10 subjects postoperatively. An example of the modeling of subject 1 is given in figure 2. The mean ISI-DISST reduced from $3.07 \times 10^{-4} \text{L} \cdot \text{pmol}^{-1} \cdot \text{min}^{-1}$ (SD 2.18) preoperatively to $2.36 \times 10^{-4} \text{L} \cdot \text{pmol}^{-1} \cdot \text{min}^{-1}$ (SD 0.78) postoperatively, though this reduction was not statistically significant ($p=0.37$).

Figure 2. Sample of DISST analysis for subject one



Euglycaemic Clamp results:

The insulin sensitivity index for the euglycaemic clamp (ISI-Clamp) was calculated on 10 subjects preoperatively and 8 subjects post operatively. Preoperatively Clamp ISI was $2.14 \times 10^{-2} \text{ mg.L.kg}^{-1} \text{ min}^{-1} \text{ pmol}^{-1}$ (SD 1.80) and post operatively was $2.00 \times 10^{-2} \text{ mg.L.kg}^{-1} \text{ min}^{-1} \text{ pmol}^{-1}$ (SD 0.76) again this was not a significant change ($p=0.86$). Actual values for DISST ISI and Clamp ISI can be seen in table 2.

Correlation between the entire matched ISI-DISST and ISI-Clamp was strong at $r=0.76$ (95% CI 0.45-0.90) (figure 5). Sub analysis of the correlation between ISI-DISST and ISI-Clamp preoperatively was strong at $r=0.81$ (95% CI 0.37-0.95) (figure 3). However the correlation in the post surgical group was weaker $r=0.47$ (95% CI 0-0.88) (figure 4).

Bland-Altman plot analysis (Figure 6) shows the bias between the 2 tests, where the DISST underestimated the clamp by $0.96 \times 10^{-2} \text{ mg.L.kg}^{-1} \text{ min}^{-1} \text{ pmol}^{-1}$ (95%

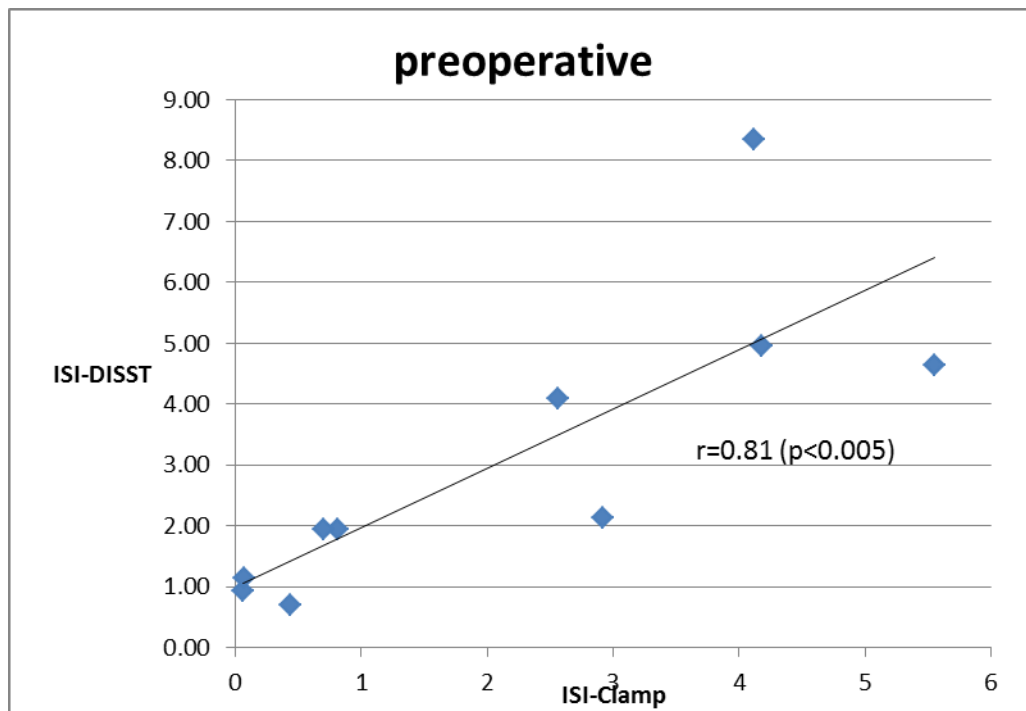
confidence intervals -2.24 to 0.32). Just analysing the pre surgery group (figure 7), DISST again under estimates the clamp by $1.16 \times 10^{-2} \text{mg.L.kg}^{-1}.\text{pmol}^{-1}$ (95% confidence intervals -2.65 to 0.33) and in the post surgery group (figure 8) DISST underestimated the clamp by $0.71 \times 10^{-2} \text{mg.L.kg}^{-1}.\text{pmol}^{-1}$ (95% confidence intervals -1.61 to 0.19)

Table 2. ISI indices pre and postoperative

	Preoperative		Postoperative	
subject	ISI-DISST	ISI-clamp	ISI-DISST	ISI-clamp
1	4.65	5.55	3.35	3.10
2	2.99	xx	2.25	xx
3	4.95	4.18	xx	xx
4	4.08	2.57	2.05	2.92
5	0.94	0.06	1.64	xx
6	0.69	0.44	1.36	1.86
7	1.13	0.07	1.05	0.32
8	1.94	0.82	2.75	1.76
9	8.35	4.12	2.47	2.43
10	2.14	2.92	3.09	1.98
11	1.93	0.70	3.64	1.66
mean	3.07	2.14	2.36	2.00
SD	2.18	1.80	0.78	0.76

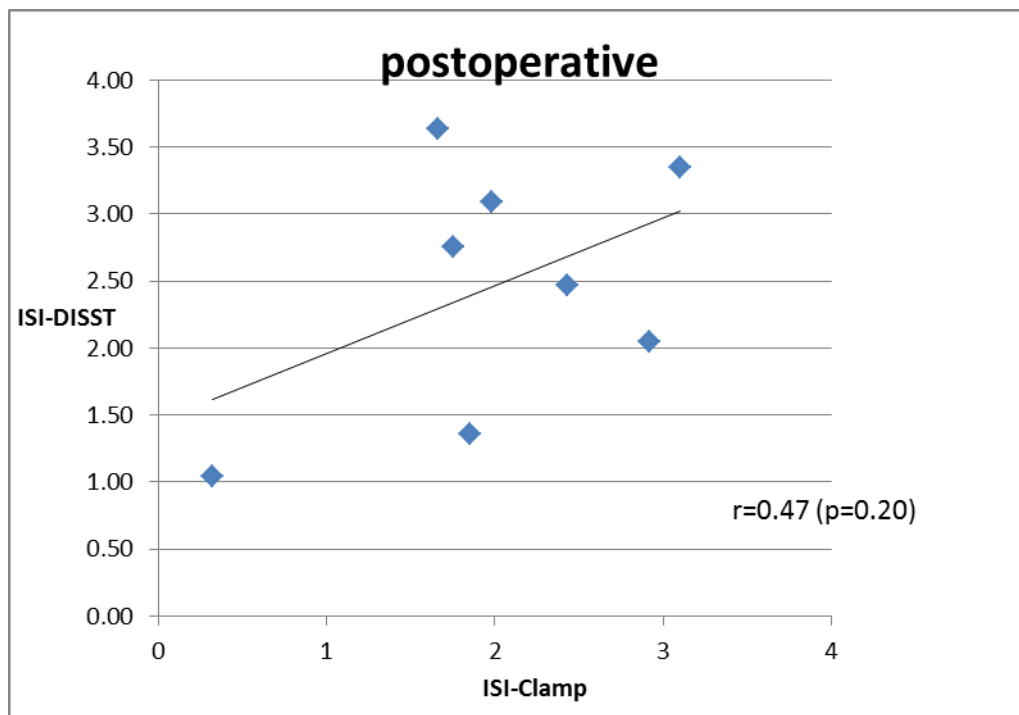
Units: ISI-DISST ($\times 10^{-4} \text{L.pmol}^{-1}.\text{min}^{-1}$), ISI-Clamp ($\times 10^{-2} \text{mg.L.kg}^{-1}.\text{min}^{-1}.\text{pmol}^{-1}$)

Figure 3. ISI-Clamp versus ISI-DISST preoperative



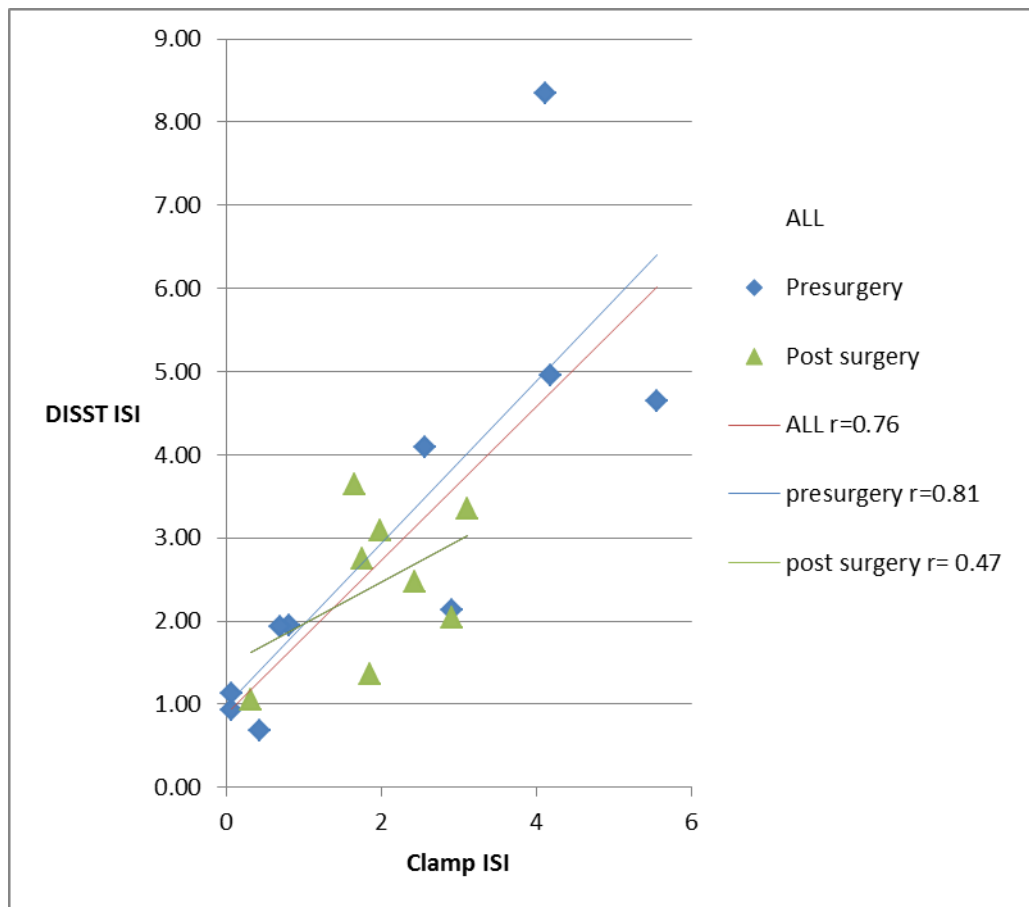
Units: ISI-DISST ($\times 10^{-4}$ L.pmol $^{-1}$.min $^{-1}$), ISI-Clamp ($\times 10^{-2}$.mg.L.kg $^{-1}$.min $^{-1}$.pmol $^{-1}$)

Figure 4. ISI-Clamp versus ISI-DISST postoperative



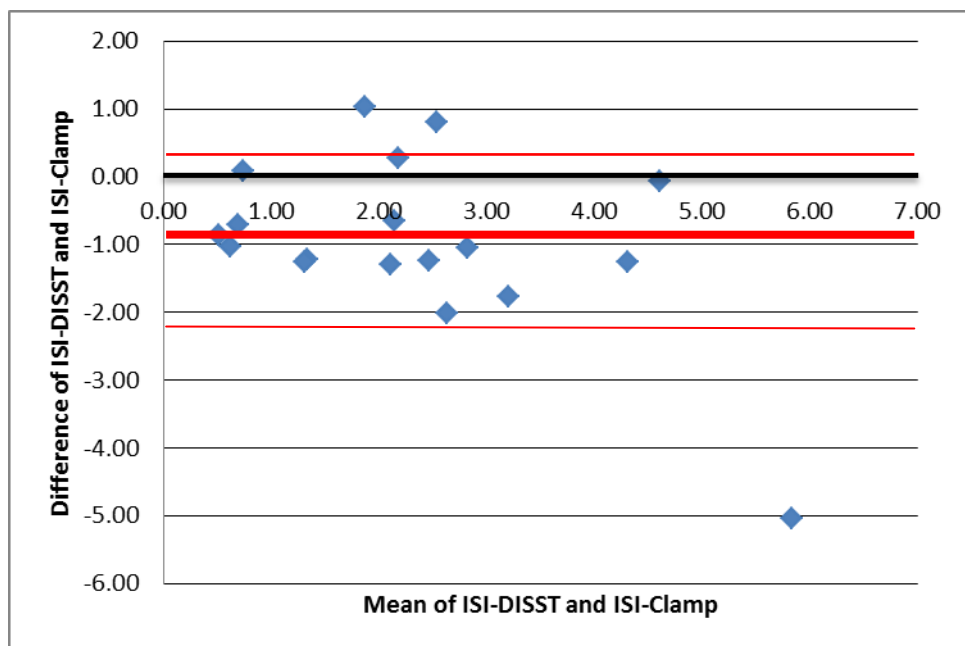
Units: ISI-DISST ($\times 10^{-4}$ L.pmol $^{-1}$.min $^{-1}$), ISI-Clamp ($\times 10^{-2}$.mg.L.kg $^{-1}$.min $^{-1}$.pmol $^{-1}$)

Figure 5. Combined ISI-Clamp versus ISI-DSST pre and postoperative



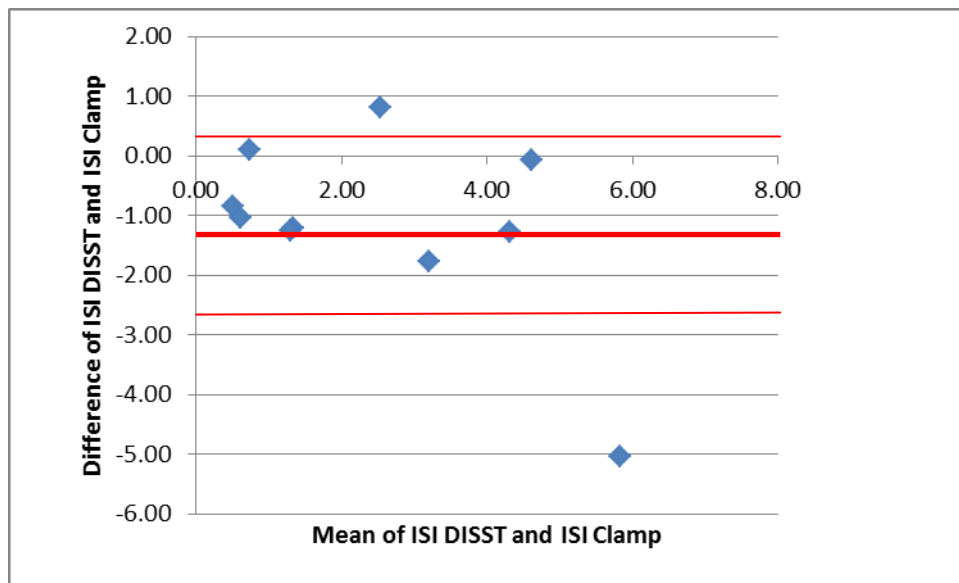
Units: ISI-DISST ($\times 10^{-4} \text{L.pmol}^{-1}.\text{min}^{-1}$), ISI-Clamp ($\times 10^{-2}.\text{mg.L.kg}^{-1}.\text{min}^{-1}.\text{pmol}^{-1}$)

Figure 6. Bland-Altman plot for ISI-DISST versus ISI-Clamp (Combination of pre surgery and post surgery)



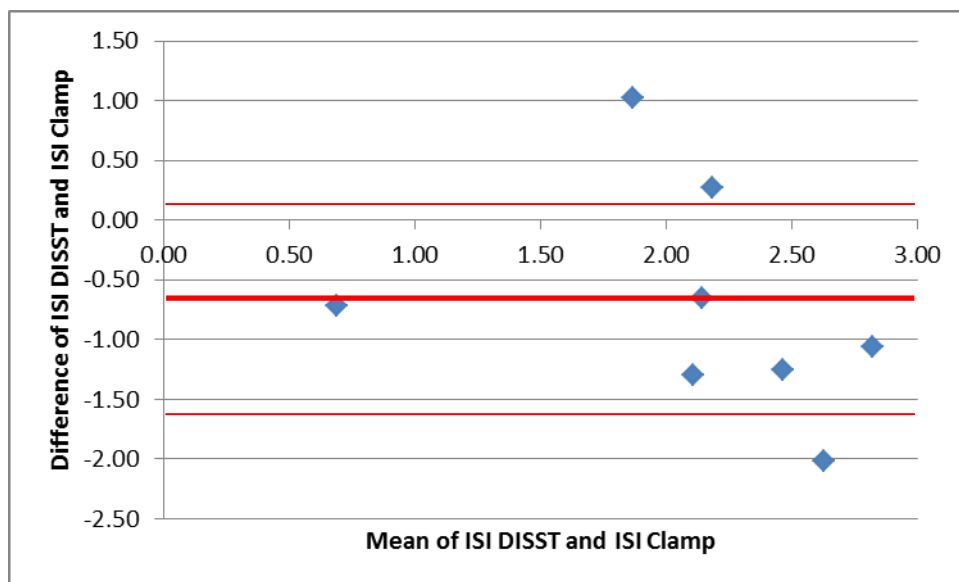
Units: ($\times 10^{-2}.\text{mg.L.kg}^{-1}.\text{min}^{-1}.\text{pmol}^{-1}$)

Figure 7. Bland-Altman plot for ISI DISST versus ISI Clamp (pre surgery)



Units: ($\times 10^{-2} \cdot \text{mg} \cdot \text{L} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \text{pmol}^{-1}$)

Figure 8. Bland-Altman plot for ISI DISST versus ISI Clamp (post surgery)



Units: ($\times 10^{-2} \cdot \text{mg} \cdot \text{L} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \text{pmol}^{-1}$)

Insulin Sensitivity Pre and Post Surgically assessed with Simple Fasting Indices

A HOMA(equation), HOMA(calculator), 1/logHOMA(equation) and QUICKI were obtained from the fasting sample data for each dynamic test performed. These were averaged to give one estimate of insulin sensitivity preoperatively and one postoperatively. Individual data for HOMA using the standard formula and the online HOMA calculator are shown in Table 3. Summary data for individuals and the group as a whole are shown in Table 4.

Table 3: Individual data for the 11 subjects for HOMA(equation) calculated by standard formula or HOMA(calculator) by online calculator for fasting data from each of the three dynamic tests.

Subject	HOMA(equation)				HOMA(calculator)			
	DISST	Clamp	Meal	Average	DISST	Clamp	Meal	Average
1 pre	2.43	2.62	2.70	2.59	1.26	1.3	1.52	1.36
1 post	1.93	1.63	1.95	1.84	1.08	0.99	1.07	1.05
2 pre	5.75	NT	5.40	5.58	2.7	NT	2.62	2.66
2 post	4.39	NT	3.28	3.84	2.23	NT	1.84	2.04
3 pre	3.93	2.75	1.96	2.88	1.92	1.48	1.01	1.47
3 post	NT	NT	NT	NT	NT	NT	NT	NT
4 pre	4.02	3.39	4.93	4.11	1.93	1.66	2.19	1.93
4 post	2.71	3.09	5.61	3.80	1.44	1.62	2.6	1.89
5 pre	31.21	39.83	NT	35.52	UTC	UTC	NT	UTC
5 post	13.17	NT	NT	13.17	5.71	NT	NT	5.71
6 pre	6.64	10.03	5.02	7.23	1.85	2.79	1.5	2.05
6 post	5.37	3.91	3.54	4.27	1.96	1.47	1.3	1.58
7 pre	90.76	72.73	86.31	83.27	UTC	UTC	UTC	UTC
7 post	17.09	28.87	26.31	24.09	6.94	UTC	UTC	6.94
8 pre	10.55	11.80	24.85	15.73	4.85	5.32	UTC	5.09
8 post	6.07	3.43	2.69	4.06	3.04	1.94	1.53	2.17
9 pre	3.95	2.93	4.75	3.88	2.05	1.64	2.34	2.01
9 post	2.38	1.81	4.63	2.94	1.31	1.03	2.41	1.58
10 pre	9.43	7.25	6.85	7.84	4.03	3.02	3.09	3.38
10 post	4.26	3.02	3.33	3.54	2	1.52	1.66	1.73
11 pre	6.28	5.48	NT	5.88	2.79	2.48	NT	2.64
11 post	7.52	5.58	6.77	6.62	3.32	2.79	2.95	3.02

*Abbreviations : NT=Not tested, UTC=unable to calculate

For the available complete data both pre and post operatively combined, comparing HOMA(equation) versus HOMA(calculator) the correlation was excellent (figure 9) with $r = 0.96$ (95% CI 0.91-0.99) . If the 3 outlying (most insulin resistant) individuals were removed the correlation value was $r = 0.88$ (95% CI 0.70-0.95). Looking at differences preoperative alone or postoperative alone and again with or without the most insulin resistant outliers all gave similar results with r values ranging from $r = 0.84$ to $r = 0.97$. Therefore subsequent analysis using HOMA data the HOMA(equation) value was used so as not to exclude samples obtained from the most insulin resistant subjects.

Figure 9. HOMA(equation) versus HOMA(calculator)

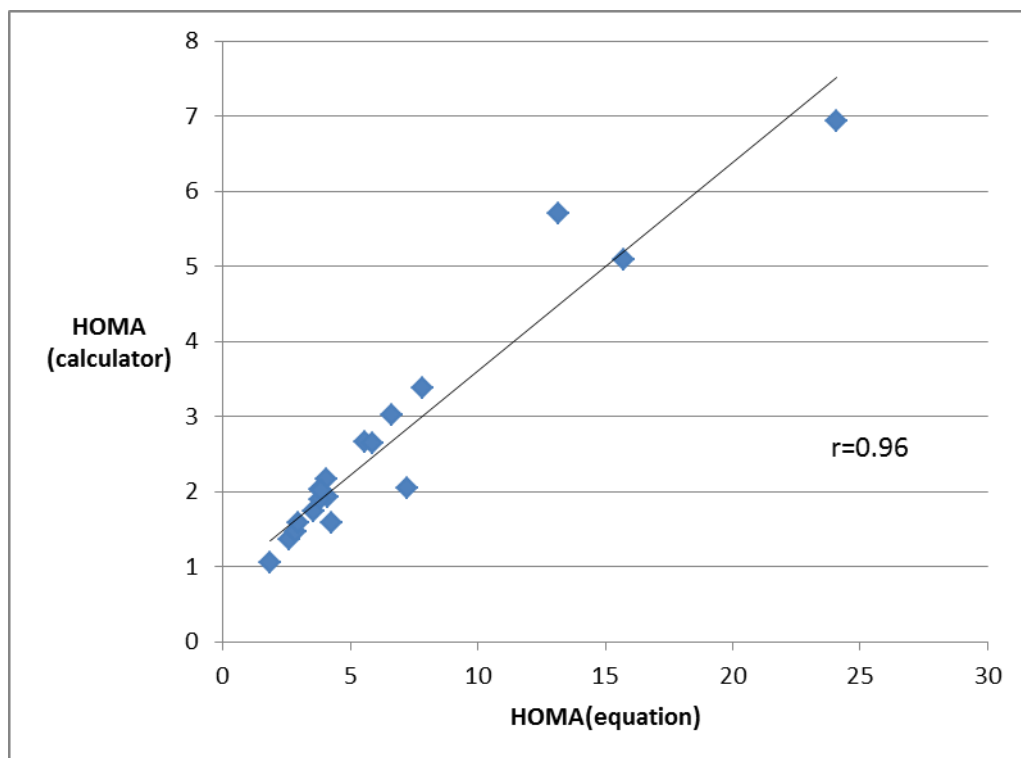


Table 4. Mean values for HOMA(equation), 1/logHOMA(equation) and QUICKI

	preoperative			postoperative		
subject	HOMA (equat)	1/log (HOMA)	QUICKI	HOMA (equat)	1/log (HOMA)	QUICKI
1	2.59	2.42	0.34	1.84	3.79	0.36
2	5.58	1.34	0.3	3.84	1.71	0.32
3	2.88	2.18	0.34	ND	ND	ND
4	4.11	1.63	0.32	3.8	1.72	0.32
5	35.52	0.64	0.24	13.17	0.89	0.27
6	7.23	1.16	0.3	4.27	1.59	0.32
7	83.27	0.52	0.22	24.09	0.72	0.26
8	15.73	0.84	0.27	4.06	1.64	0.32
9	3.88	1.7	0.32	2.94	2.13	0.34
10	7.84	1.12	0.29	3.54	1.82	0.32
11	5.88	1.3	0.3	6.62	1.22	0.3
mean	15.86	1.35	0.29	6.82	1.72	0.31
SD	23.16	0.57	0.03	6.49	0.8	0.03

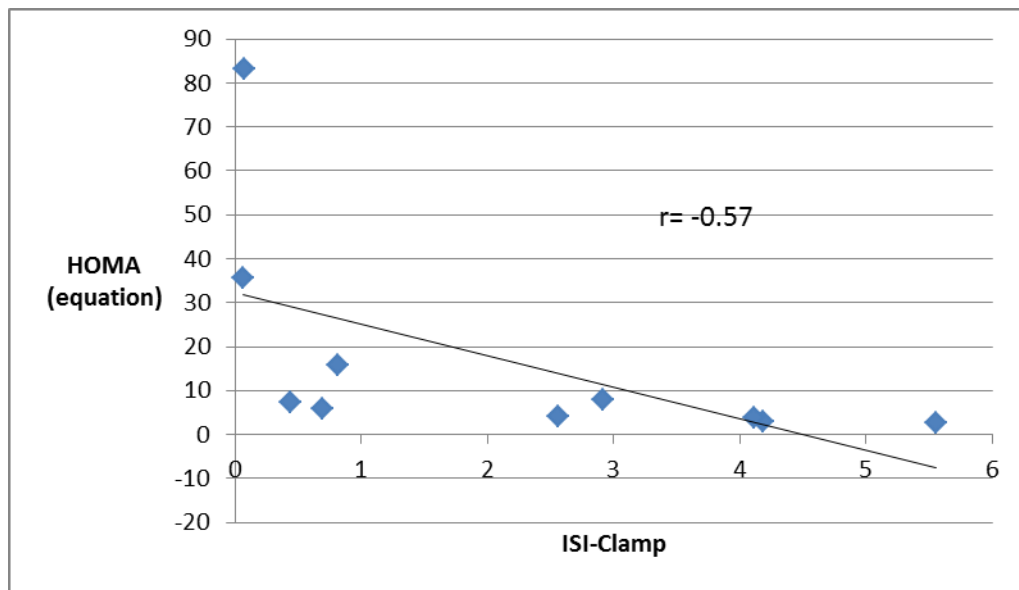
HOMA(equation)

Mean values of HOMA(equation) preoperative were 15.86 (SD 23.16) and postoperative were 6.82 (SD 6.49) (See Table 4). Although all but one value fell there was no statistical difference between preoperative and postoperative HOMA(equation) ($p=0.14$)

HOMA(equation) versus ISI clamp

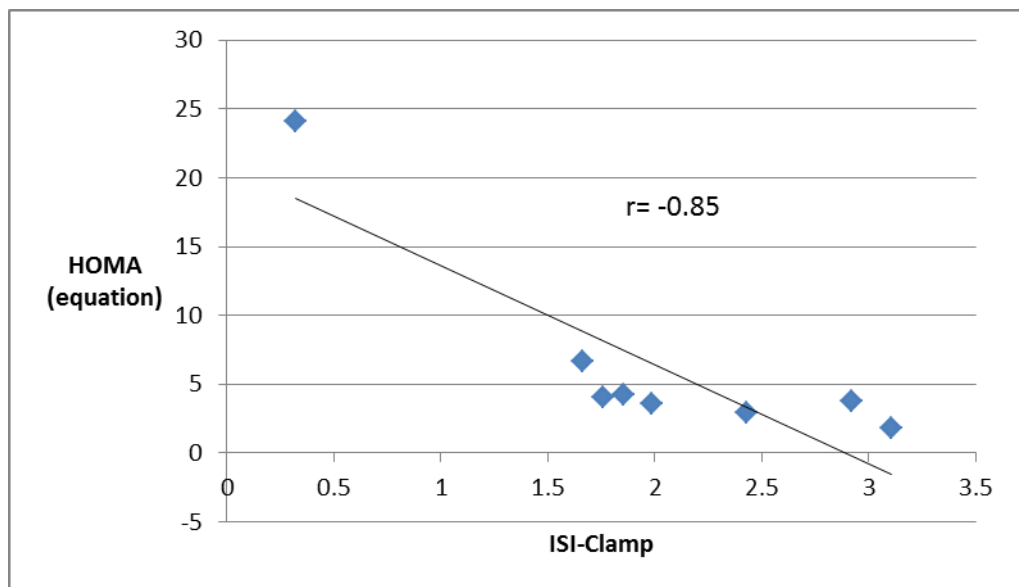
The correlation of HOMA(equation) versus Clamp ISI preoperative is consistent with previously reported studies with $r=-0.57$ (95% CI 0.0-0.87)(figure 10). Postoperative the correlation improves with $r= -0.85$ (95% CI 0.49-0.97) (figure 11). When comparing the regression lines between the pre and postoperative correlations (figure 12), the slope is shifted to the left postoperatively. Therefore for the same apparent insulin sensitivity by euglycaemic clamp, the HOMA index indicates improved insulin sensitivity postoperatively. This suggests that the two methods are not estimating the same physiological entity.

Figure 10. ISI-Clamp versus HOMA(equation) preoperative



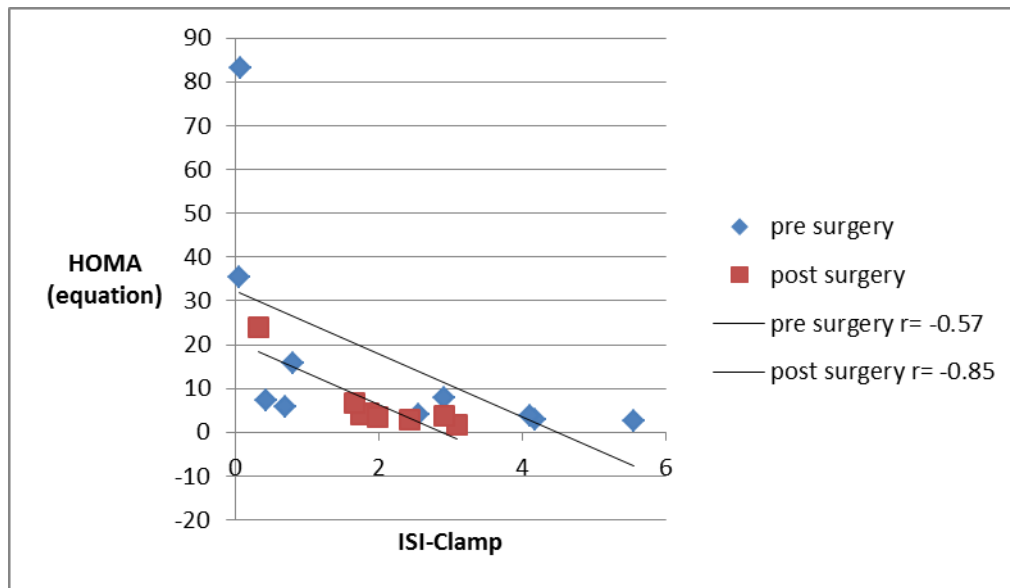
Units: ISI-Clamp ($\times 10^{-2} \cdot \text{mg} \cdot \text{L} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \text{pmol}^{-1}$)

Figure 11. ISI-Clamp versus HOMA(equation) postoperative



Units: ISI-Clamp ($\times 10^{-2} \cdot \text{mg} \cdot \text{L} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \text{pmol}^{-1}$)

Figure 12. ISI-Clamp versus HOMA(equation) combined

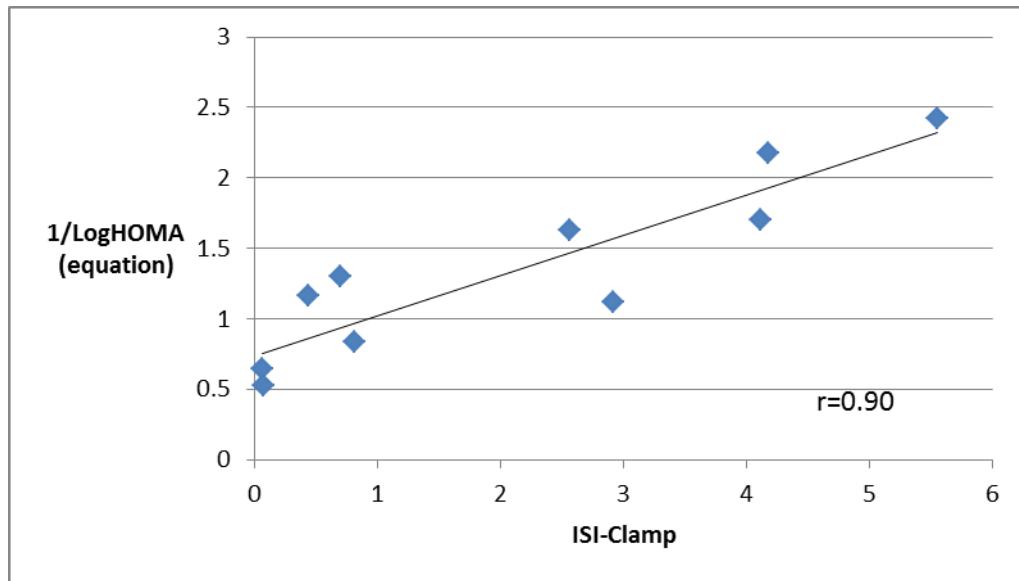


Units: ISI-Clamp ($\times 10^{-2} \cdot \text{mg} \cdot \text{L} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \text{pmol}^{-1}$)

1/logHOMA(equation)

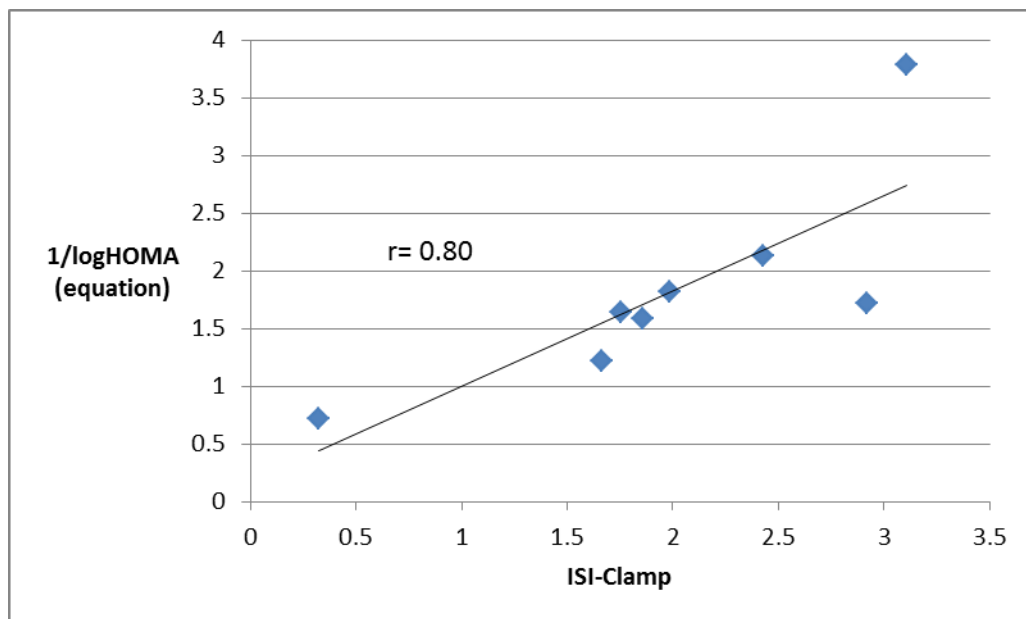
1/logHOMA values preoperative were mean 1.35 (SD 0.57) compared with 1.72 (SD 0.80) postoperatively. Again this difference was not significantly different ($p=0.13$). Using 1/logHOMA preoperative correlation with the ISI-clamp were $r=0.90$ (95% CI 0.67-0.98) (figure 13) and postoperative $r= 0.80$ (95% CI 0.29-0.95) (figure 14). The combined correlation lines can be seen in figure 15.

Figure 13. ISI-Clamp versus 1/logHOMA(equation) preoperative



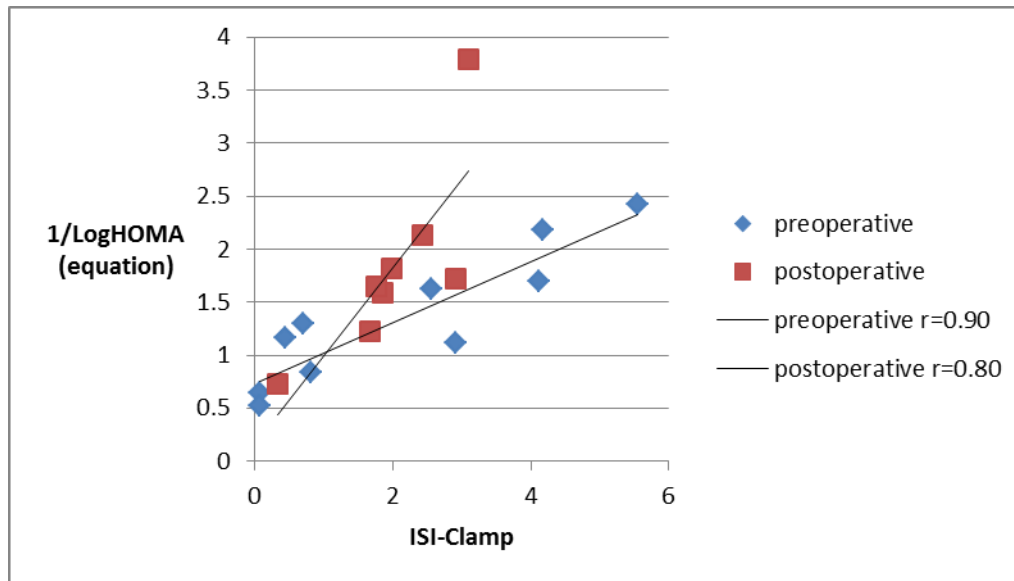
Units: ISI-Clamp ($\times 10^{-2} \cdot \text{mg} \cdot \text{L} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \text{pmol}^{-1}$)

Figure 14. ISI-Clamp versus 1/logHOMA(equation) postoperative



Units: ISI-Clamp ($\times 10^{-2} \cdot \text{mg} \cdot \text{L} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \text{pmol}^{-1}$)

Figure 15. ISI-Clamp versus 1/logHOMA(equation) combined

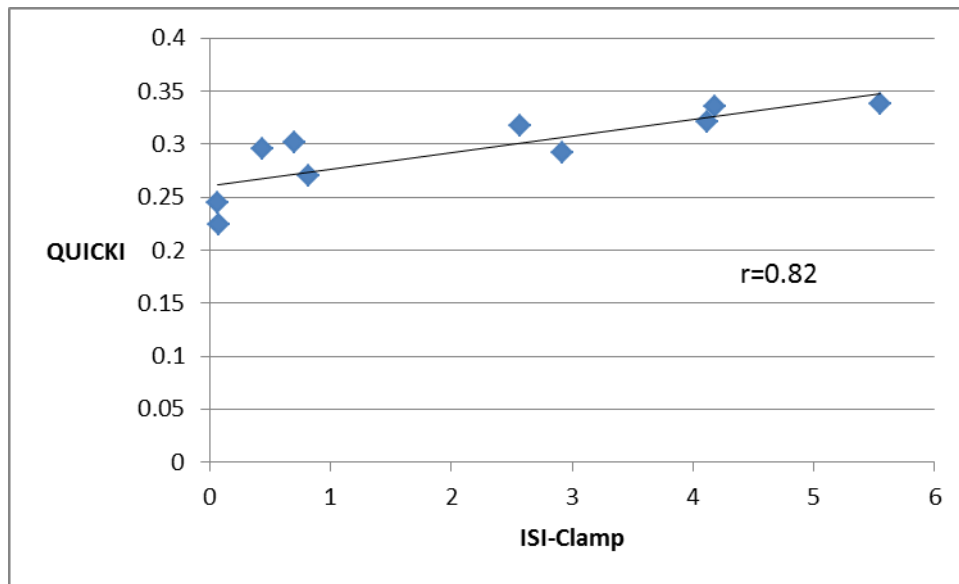


Units: ISI-Clamp ($\times 10^{-2} \cdot \text{mg} \cdot \text{L} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \text{pmol}^{-1}$)

QUICKI

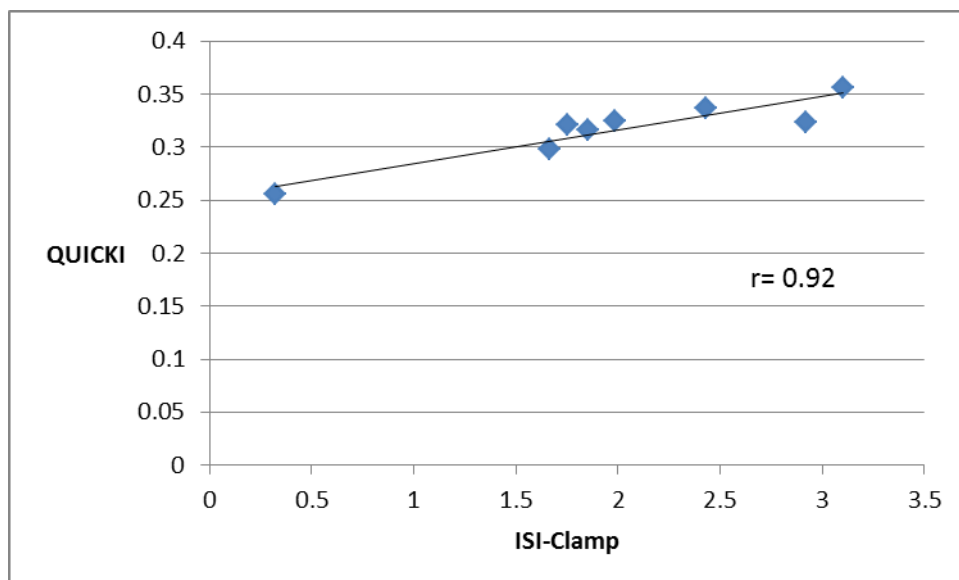
Calculating QUICKI gave a mean preoperative value of 0.29 (SD 0.03) and mean postoperative value of 0.31 (SD 0.03). Again this was not statistically different ($p=0.12$). Correlation with the clamp was excellent preoperative with $r= 0.82$ (95% CI 0.44-0.95)(figure 16) and was even better postoperative at $r= 0.92$ (95% CI 0.70-0.98) (figure 17). However there was a difference in the slope of the curves with separation of curves as Clamp ISI increased so again unlikely to be directly comparable (figure 18).

Figure 16. ISI-Clamp versus QUICKI preoperative



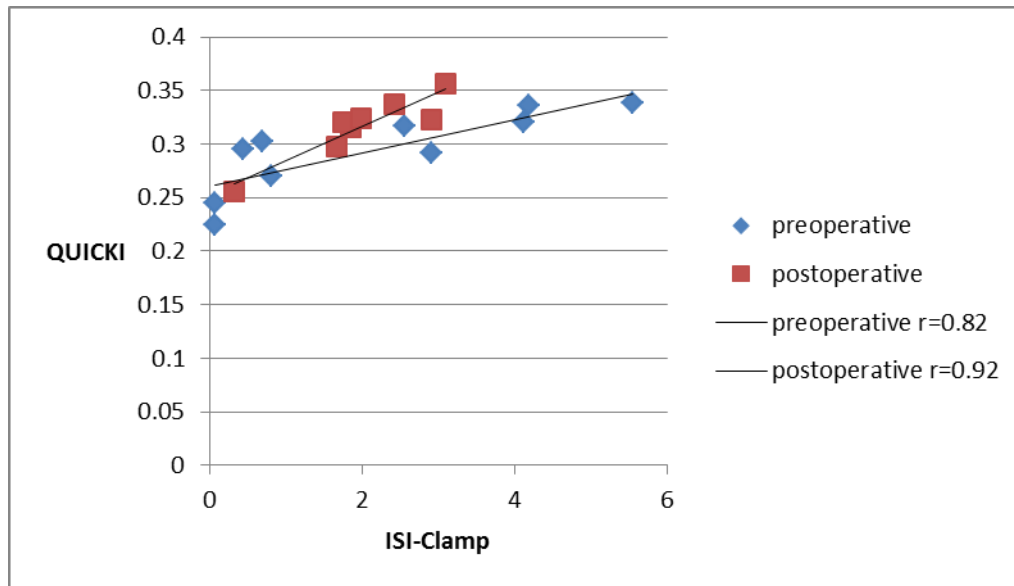
Units: ISI-Clamp ($\times 10^{-2} \cdot \text{mg} \cdot \text{L} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \text{pmol}^{-1}$)

Figure 17. ISI-Clamp versus QUICKI postoperative



Units: ISI-Clamp ($\times 10^{-2} \cdot \text{mg} \cdot \text{L} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \text{pmol}^{-1}$)

Figure 18. ISI-Clamp versus QUICKI combined



Units: ISI-Clamp ($\times 10^{-2} \cdot \text{mg} \cdot \text{L} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \cdot \text{pmol}^{-1}$)

Insulin secretion

The DISST test was able to give estimates of insulin secretion in both first phase of insulin secretion and in the second phase of insulin secretion. Although there was a trend upwards in the secretion of insulin in the first phase of insulin secretion from a mean of 823.5 mU (SD 862.3 mU) to 1091.6 mU (SD 765.8 mU) this was not significant with $p=0.28$ (figure 19). There was no change in the second phase of insulin secretion. 2129.4 mU (SD 862.3 mU) to 2217.3mU (SD 765.8 mU) ($p=0.82$) (figure 20). There was no correlation between either estimated first phase insulin release or second phase insulin release before surgery or after surgery with the clamp.

Figure 19. First phase insulin preoperative versus post operative

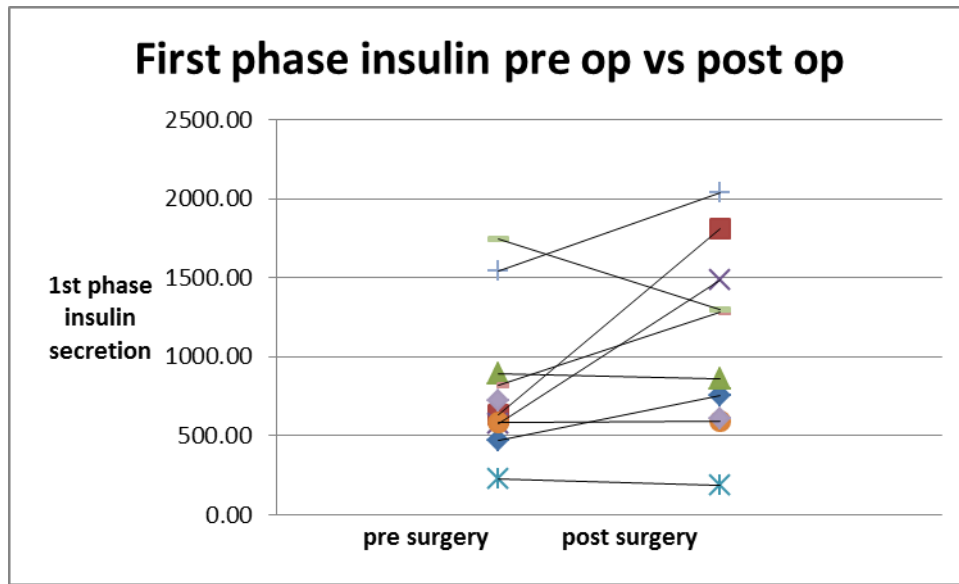
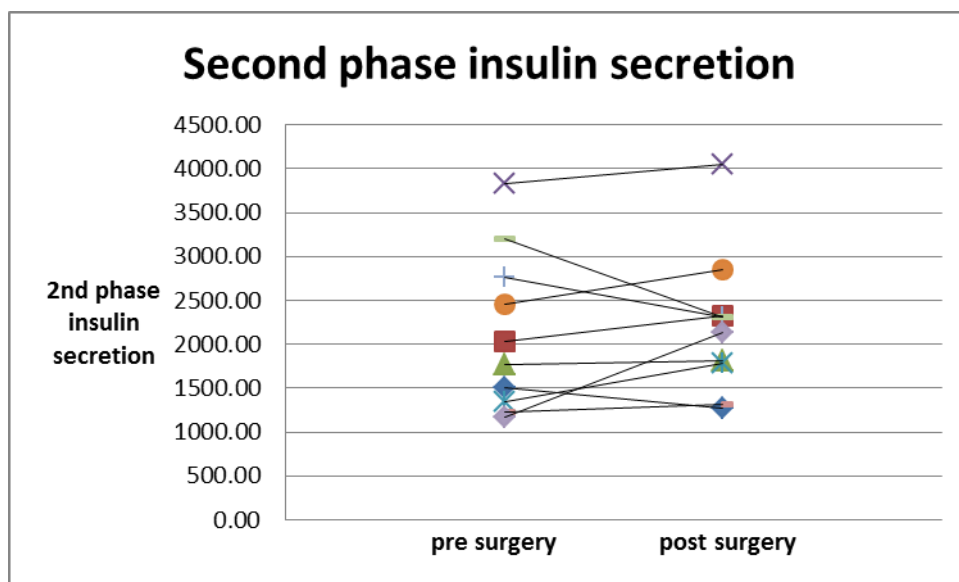


Figure 20. Second phase insulin secretion preoperative versus post operative



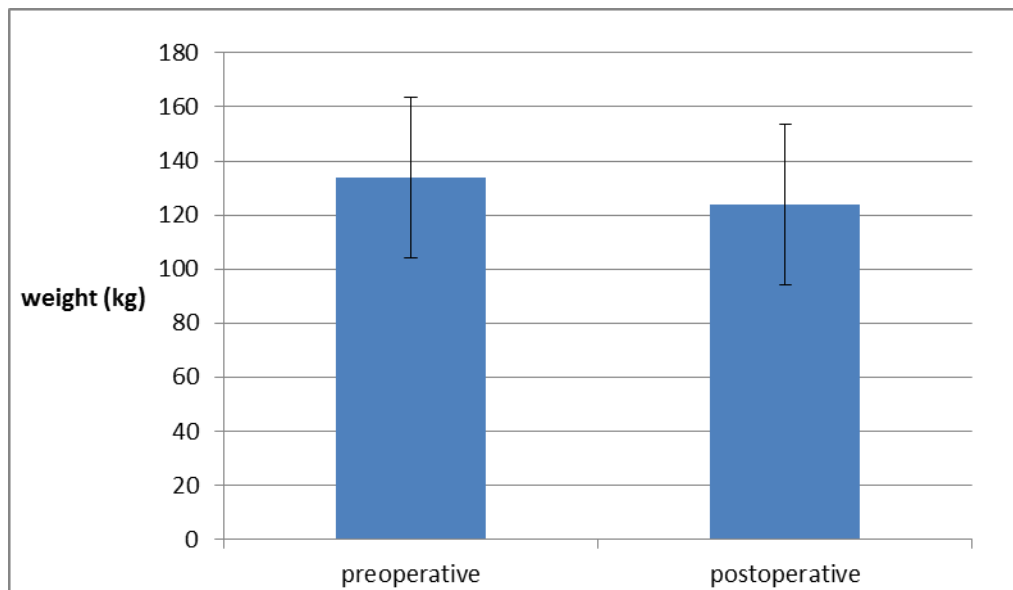
Weight

For the 11 subjects mean initial weight was 133.8kg (SD 29.8, range 114.2-215.6). If only the 10 subjects with a postoperative weight then mean 135.5kg, (SD 30.8, with the same range). For the 10 subjects with post surgery weight taken an average of 23 days after surgery mean weight 123.8kg (SD 28.9, range 102.9-198.4). BMI in the preoperative group was 48.7 kg/m² (SD 9.5, range 39.5-75.5) and in the post operative group was 44.2 kg/m² (SD 9.3, range 35.5-69.5). There was no statistical difference between preoperative weight (figure 21) or BMI. However if the largest patient was excluded, BMI loss then became significant (p=0.02) but weight remained non significant.

Table 5. Weight based changes preoperative versus postoperative

	Preoperative		Postoperative			
Subject	Weight (kg)	BMI (kg/m ²)	Weight (kg)	Weight loss (kg)	BMI (kg/m ²)	% of original weight
1	114.2	41.3	102.9	11.3	37.2	90.1
2	116.5	44.1	109.4	7.1	41.6	93.9
3	117.4	51.4				
4	117.5	45	108.6	8.9	41.6	92.4
5	215.6	75.5	198.4	17.2	69.5	92
6	121.2	41.8	109.7	11.5	37.8	90.5
7	143.6	49.8	130.1	13.5	45.1	90.6
8	119.6	39.5	107.5	12.1	35.5	89.9
9	166	53.3	154.8	11.2	49.7	93.3
10	120	44.1	107.3	12.7	39.4	89.4
11	120.4	49.5	109.6	10.8	45	91
Mean	133.8	48.7	123.8	11.6	44.2	91.3
SD	29.8	9.5	28.9	2.5	9.3	1.4

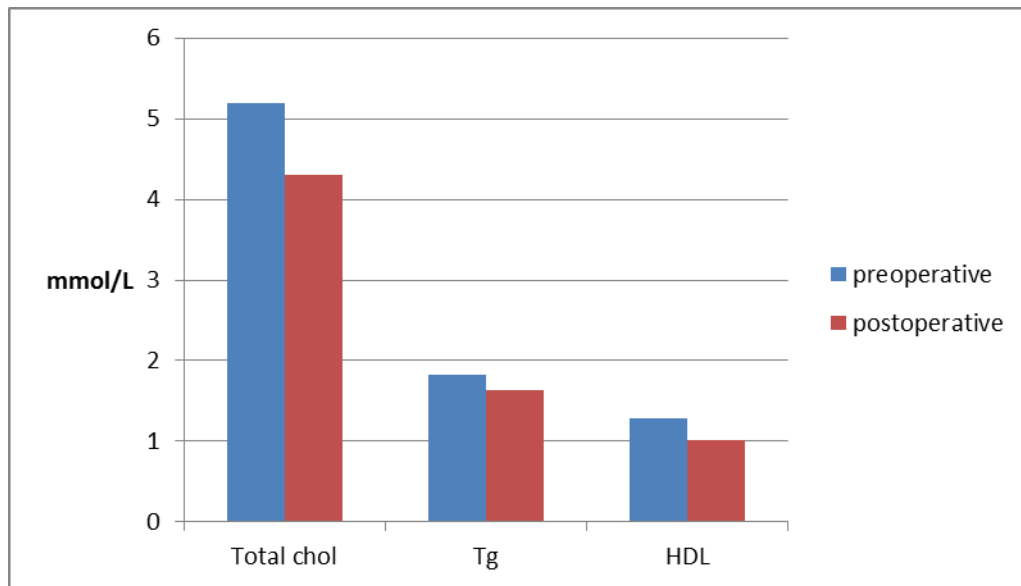
Figure 21. Weight change



Lipids

Preoperative Total Cholesterol mean was 5.2mmol/L (SD 0.6) and postoperative mean was 4.3 mmol/l (SD 0.7). Of the nine subjects with both preoperative and postoperative samples the mean fall in Total cholesterol was 0.9mmol/L (SD 1.15). This just reached statistical significance at $p=0.048$. Triglycerides preoperative mean was 1.82mmol/L (SD 0.77) and postoperative 1.63 mmol/L (SD 0.51). Of the nine subjects with both preoperative and postoperative samples the mean fall in Triglycerides was 0.4 (SD 0.64). This did not reach statistical significance. The preoperative HDL mean was 1.29mmol/L (SD 0.28) and postoperative mean was 1.01 mmol/L (SD 0.23). Again of the nine subjects with both preoperative and postoperative samples the mean fall in HDL was 0.23mmol/L (SD 0.12). This is significant at $p<0.001$ (figure 22).

Figure 22. Lipid values



Blood Pressure

10 of 11 subjects had Blood pressure (BP) preoperative (subject 7 missing) and 10 BP were available post surgery (subject 3 missing). Mean systolic BP preoperative was 137.8 mmHg, (SD 21.8, range 112-192). Mean systolic BP postoperative was 123.8 mmHg, (SD 10.4, range 110-142). The mean fall in BP postoperative was 13.3 mmHg (SD 24.1). Mean diastolic BP preoperative was 78.4 mmHg, (SD 9.9, range 66-100) and mean postoperative diastolic BP was 76.3 mmHg, (SD 10.5, range 60-94). There was no statistical difference with any of these values.

Discussion

Two of the modern world's most common chronic medical conditions are obesity and T2DM with a common relationship between the two being the obesity related increase in insulin resistance, thought to contribute causally to the increasing incidence of T2DM.

For a number of years there has been increasing research interest in establishing a easy, repeatable and accurate measure of insulin resistance that correlates well with the current reference standard of the hyperinsulinaemic euglycaemic clamp as described by DeFronzo (49) in 1979.

Multiple indices derived from fasting insulin and glucose, and some with addition of free fatty acids or triglycerides, have only moderate correlation with the euglycaemic clamp. The best of these (revised QUICKI) still only having a pooled correlation coefficient of $r=0.69$ (62).

There are nearly as many indices derived from the dynamic changes in glucose and insulin during an OGTT (oral glucose tolerance test), which have slightly stronger r values, with the best of these having a pooled correlation coefficient of $r=0.70$ (62). However these are still within the confidence intervals of most of the fasting tests (62). Therefore we are still in search of a simple method that can be utilized in large studies or in clinical practice.

The DISST test was developed to attempt to fill this gap, with a better correlation than the simple fasting tests to the hyperinsulinaemic euglycaemic clamp, to reflect more the measure of insulin sensitivity of the clamp and be easier to administer and cheaper, as well shorter and more palatable to the research subject than the OGTT based tests. The whole test is designed to take less than an hour with about the same number of blood tests. It is modeled on data derived from hyperinsulinaemic euglycaemic clamps. The validation studies described in chapter 1.g support this.

The study reported in this Masters thesis was designed to test the preliminary performance of the DISST test in a group of individuals who were more obese than those included in the validation studies and therefore may have different physiological characteristics determining insulin sensitivity. Furthermore, DISST had not previously been assessed after an intervention which is known to fundamentally change glucose metabolism such as bariatric surgery.

This study demonstrates a strong correlation between DISST and the euglycaemic clamp preoperatively ($r = 0.81$) and matches nicely with the correlation values from the validation studies. However the relationship was not as strong postoperatively ($r=0.47$). This would suggest that the factors determining insulin sensitivity when measured by the euglycaemic clamp may change in a different way than those determining the DISST index. This suggests that the DISST test may be more affected by changes in hepatic insulin resistance which happen early in the postoperative gastric bypass state which do not affect the results of the EIC which mainly reflects peripheral insulin sensitivity.

However, with very small numbers in our study (only 10 preoperative DISST versus clamp pairings and only 8 postoperative pairings), we must be very cautious in generalizing from this. The confidence intervals for the correlation between the preoperative and postoperative ISI-DISST and the ISI-clamp were wide and overlapping so we cannot say there is any difference at all.

Bland-Altman plot analysis also showed some underestimation of ISI-DISST versus the ISI-Clamp but did cross zero with its wide confidence intervals again likely due to the small numbers involved (figure 6). When split into pre surgery and post surgery there was still an under estimation with the post surgery group performing better but again with even wider confidence intervals (figures 7 and 8).

With the data available measures for HOMA, $1/\log\text{HOMA}$ and QUICKI were able to be calculated.

As described in the methods chapter, HOMA was calculated using both the standard equation (53) to give HOMA(equation) and also using the downloadable HOMA calculator (55) to give the HOMA(calculator) value. These values were not directly interchangeable as the computer model recalibrated the base line to young fit and healthy insulin sensitive subjects. However, because the online calculator only accepts insulin concentrations between to a certain concentration, in many very insulin resistant subjects HOMA(calculator) was not able to calculated for this method. When the two HOMA values were compared for available individuals, the correlation was very strong at $r > 0.95$ with narrow confidence intervals. For this reason HOMA(equation) was used for further evaluation so as to include those who were the most insulin resistant where a computer generated HOMA(calculator) could not be obtained. However this correlation may not be valid for the very insulin resistant subjects.

The correlation between the euglycaemic clamp and HOMA in the preoperative studies was $r = -0.57$ which is similar to published literature. A recent meta-analysis (62) has HOMA(calculator) pooled correlation coefficient as $r = -0.57$. Notably the strength of the relationship increased in the post operative studies ($r = -0.85$) but with the numbers in the study being so small the confidence intervals are wide and overlap.

The slope of the regression line for the preoperative and postoperative correlations was similar (Figure 12). However, the postoperative comparison was shifted to the left, indicating a lower HOMA and therefore better insulin sensitivity for the matched comparative clamp ISI estimate. This matches with previous data that shows a rapid improvement in HOMA without improvement in Clamp ISI (78,106,107,108) in the early postoperative stage. It also highlights the potential differences what each of these methods is measuring in fundamental terms. It may be speculated that because the key physiological determinants of fasting glucose and insulin, the parameters used in HOMA, are beta cell function and hepatic insulin sensitivity, that RYGB surgery is having its predominant effect on these parameters within the first few weeks. This would

be in keeping with the observation that such improvements in glucose metabolism occur prior to any loss of fat mass.

Certainly there is data to suggest that differing changes occur. Foo et al (109) performed a study where 8 morbidly obese subjects undertook a very low calorie diet for 6 days and subsequently had gastric bypass surgery a few weeks later. Both had significant falls in HOMA at 6 days after each intervention although the post gastric bypass group changes were larger. A short intravenous insulin tolerance test was also performed which showed a worsening of glucose disposal suggesting worsening of peripheral insulin resistance. Martinussen et al (110) using HOMA and an IVGTT showed rapid improvement in HOMA at 1 week but peripheral resistance was not improved until 3 months as shown in the ivGTT.

The other simple methods assessed for estimating insulin resistance from fasting blood samples performed even better than HOMA itself. For example, $1/\log\text{HOMA}(\text{equation})$ gave preoperative correlation value of $r=0.90$ and postoperative value of $r=0.80$. The recent meta-analysis (62) gave a pooled correlation value of $r=0.60$. QUICKI also performed very well with preoperative correlation value of $r=0.82$ and postoperative value of $r=0.92$. The recent meta-analysis (62) had a pooled correlation value of $r=0.61$. With these methods, the slope of the regression lines differed between pre and postoperative studies. This would suggest the possibility that individuals with different degrees of insulin sensitivity preoperatively respond to surgery in different ways, and in different magnitude, though the same hypothesis that hepatic insulin sensitivity is the early determinant still holds true (See figures 15 and 18). Again though with the small numbers involved in the study confidence intervals for each of the reported correlation figures are large. Unfortunately we were unable to calculate revised QUICKI, the best performing fasting test in the recent meta-analysis (62).

Previous studies have shown weak correlation between HOMA, QUICKI and the EIC in the early postoperative period. There is rapid improvement in the fasting

indices whereas no improvement in clamp is seen until a significant amount of weight is lost. In our study there was not a statistically significant weight or BMI loss although we would expect these to be clinically significant. Dietary and lifestyle studies show improved glucose control from as little as 5% weight loss (111,112). The lack of statistical significance is likely due to the small numbers involved and also the effect of an outlier, the largest patient, which potentially has skewed the data. When this outlier was excluded there was still no significant change in weight however the BMI loss was now significant at $p=0.02$.

It is postulated that one of the reasons that whole body measures of insulin sensitivity such as the euglycaemic clamp may not improve immediately postoperatively because of ongoing systemic factors related to the surgery. Being only 3-4 weeks post operative there may still also be ongoing healing and inflammatory markers so influencing effects on insulin resistance. Currently the most commonly performed roux-en-y gastric bypass procedures are usually done by laparoscopic methods so having faster healing times. However, the operation performed during this study was always an open procedure so having larger wounds and slower healing times. Certainly at least a couple of subjects had delay in healing of their abdominal wounds which has the potential to affect results with increased inflammatory markers having a direct effect on insulin resistance.

If we ignore the effect of insulin resistance and focus on glucose outcomes per se there are marked improvements in diabetes control with glucose levels falling throughout. This is seen in both fasting glucose levels and also peak glucose levels seen in meal study and DISST tests (data not presented). Multiple studies have shown that type 2 diabetes mellitus can rapidly improve following gastric bypass surgery (before any appreciable change in ISI-clamp) (73,74).

There are multiple mechanisms involved for this improvement. These include calorie restriction, changes in appetite regulating hormones and changes in insulin secretion.

Calorie restriction is an important component of the gastric bypass operation as with the small stomach pouch subjects cannot eat large amount. With the small stomach pouch the subject postoperatively cannot have a large meal as there is no room for it in the stomach. Large meals also lead to the dumping phenomenon where there is rapid transit of meal from the stomach to the small intestine. This is more common with higher carbohydrate meals. Common symptoms include abdominal pains, nausea and vomiting, flushing, dizziness, lightheadedness and palpitations and most subjects find it particularly unpleasant (113). There are a number of studies that do show rapid improvement in glucose control just by restricting food intake (109,110,114). These show rapid improvement in the fasting measures of insulin resistance such as HOMA and can be equal to that of gastric bypass.

Other mechanisms include a change in gut hormone release with changes occurring almost immediately in important appetite regulating hormones such as GLP-1, PYY and Ghrelin (76,107,108,115) so not having the desire or need to eat. These do not occur in very low calorie diets suggesting that it is the operation and the change in anatomy that causes this alteration.

The DISST test also models insulin secretion. Results suggest possible improved first phase insulin secretion although this does not reach statistical significance with our small numbers. Multiple studies largely using oral glucose tests show an early and exaggerated rise in insulin secretion post gastric bypass. There is then a fall of insulin so the area under curve for total secretion remains the same (107). The reasons for this could be multi factorial but the two likely causes are firstly decreased fat/lipid accumulation in the pancreas allowing beta cells to perform better and secrete more insulin (82,110). The second possibility is the effect of the operation itself. There is a marked increase in Glucagon-like peptide 1 (GLP-1) secretion in the post prandial setting (76,116) which directly increases insulin production, as well as affecting the vagal impact so both increasing pancreatic output (110,117,118). This also has effects on central mediated satiety so decreased oral intake (118). Increased concentration of postprandial Peptide YY₃₋₃₆ in the gastric bypass subject also has alters vagal stimulation (75).

A third possibility could be the decreased calorie load required from having only a small stomach remnant and so the pancreas does not have to produce as much insulin. This same phenomenon is seen in those on very low calorie diets (82,110). Certainly this increased first phase insulin release has been demonstrated elsewhere (110) following gastric bypass surgery.

Interestingly there is little change in the measured second phase insulin secretion. There is limited data to show change in this second phase secretion. Available data suggests a decrease in this second phase over the first few months (106,119). This may be because of the exaggerated first phase reaction so down regulation of the second phase.

There is also sustained control of T2DM in the medium term of 1-2 years (120,121,122) post gastric bypass surgery. This is likely to be a combination of the early initial changes in hepatic insulin sensitivity and gut hormone release and then the addition of increased peripheral insulin sensitivity as the subjects weight falls. The peripheral insulin sensitivity as measured by the clamp improves in direct correlation to the weight lost (86,87,91). Sustained weight loss by lifestyle measures can also markedly improve glucose metabolism. This is seen in two major diabetes prevention studies, the Finnish Prevention Study (111) and the Diabetes Prevention Program (112) with prevention of T2DM of between 43 - 58% on as little as 5% weight loss, much less than the weight loss achieved usually by gastric bypass surgery.

Changes in lipid profile were less predictable. There was a significant improvement in total cholesterol but also a significant drop in HDL cholesterol. Surprisingly there was no change seen in Triglyceride concentrations. In the Buchwald meta-analysis hyperlipidaemia improved in 70% (121) but again this was over a longer follow up period. Only one subject in our study was on lipid lowering medication which was stopped at the time of surgery and remained off at the postoperative assessment.

There were no changes seen in blood pressure in the current study. However the effect of medication confounds this analysis. Five of the subjects were on blood pressure medication prior to surgery and all remained off the medication at the time of postoperative assessment. This same issue is also described by Schauer et al (122). The first major metaanalysis (121) of effects of bariatric surgery showed that 78.5% of subjects had improved blood pressure. This though was over prolonged follow up and not in the immediate postoperative phase.

Limitations of the research:

There were a number of limitations to this research. These can be simplified into recruitment, technical and analytical issues.

The major limiting factor of this study was of course the small numbers of participants. The total number of participants was limited by the number of consenting subjects who underwent surgery during the time available for this Masters. Recruitment was not as good as was hoped with a total of only eleven subjects recruited. Based on previous throughput it was expected that we would have enrolled twenty five subjects in the same timeframe. A number of factors limited the ability to recruit more. All subjects for practical reasons had to be from the greater Wellington region as they were asked to come into the research centre on six occasions (three pre surgery visits and three post surgery visits). Even with this limitation two of the research subjects were based in the Kapiti Coast with round trips of almost 100km and both very happy to do this. As greater than 50% of the patients undergoing surgery were from other regions (Canterbury, Hawkes Bay and Manawatu being the bulk of these) this immediately restricted the number of potential subjects. Of those who met the geographical criteria there were still a number of factors that limited ability to take part in the study. Some understandably were just not interested in taking part in the study, others had work commitments that they could not get around, and others had not enough time to be able to do the pre surgery studies given their surgery scheduling. Furthermore there was a significant reduction in the

numbers of people coming forward for privately funded surgery over the time of this study due to the world financial crisis.

This lack of numbers means making any strong conclusions in regards to the use of DISST in the morbidly obese or in a postoperative bariatric surgery population impossible to make.

Technical issues with each of the individual tests were common. For the Euglycaemic hyperinsulinaemic clamp a number of difficulties were encountered. Cannulation of obese individuals can be very difficult. All subjects required 2 intravenous cannulae; one in the dorsum of the hand and another placed in the antecubital fossa. The antecubital fossa cannula was for the infusion of the glucose and insulin solutions and no problems were encountered during any of clamps once was in place. The cannula in the dorsum of the hand though was used for obtaining blood specimens for glucose assessment every 5 minutes and on a number of occasions this failed usually only once though often in the middle of the clamp study. This required a rapid recannulation knowing that you continued to need regular blood samples.

The protocol used for the clamp worked very well for the majority of the patients. The two exceptions were the two patients with marked insulin resistance. Both required almost no glucose infusion (1 and 3 ml/hour of 25% glucose whereas the protocol started them both of at about 100 ml/hr). This prolonged the clamp study significantly before steady state was reached.

Analysis issues include the suitability of the euglycaemic clamp in the obese and the very insulin resistant subjects. In obesity the dispersion of both insulin and glucose differs from lean subjects and this requires differing rates of infusion. For the very insulin resistant subjects it is debated whether the infused insulin doses may not be sufficient to switch off all hepatic glucose output, thus create a measurement error in determining insulin sensitivity by exogenous glucose infusion rates.

The DISST test was technically straight forward. The two main issues were again IV cannulation, though only one was required with only one failure during all DISST tests performed. The most challenging aspect of test was to give each subject one unit of insulin intravenously. This is such a small dose that 10 units were mixed with some solution of patients blood and Normal saline made up to 10 ml and 1 ml infused. The results suggested that this was effective with a clear increase in the insulin concentration measured at the first post bolus blood sample.

With the meal test the main issue was with nausea and vomiting in the postoperative test. This is likely to be a combination of volume (200ml into a small remnant stomach pouch) and dumping as gastric emptying would still be faster than what would be a normal rate of about 60 minutes. Subject one drank their meal over 10 minutes and had severe nausea coming on at about 30 minutes and vomited twice at 50 and 60 minutes. All other subjects subsequently had their meal test drink over 30 minutes. There was no further vomiting but all had marked nausea between 40 and 60 minutes. There was not funding to further explore some of these factors although samples continue to be stored for future use.

There were a number of analytical issues. With the hyperinsulinaemic clamp the last part of the clamp was used to determine insulin sensitivity once steady state was achieved. However in the two most insulin resistant subjects both were very slow to reach steady state so potentially introducing more variability.

As discussed above, the computer generated HOMA insulin values could not be derived at all in two subjects and in the preoperative value for a further one subject. This excluded results from our most insulin resistant pair and one of the results from a third. This may have had adverse effects on using HOMA as a comparator to the clamp.

All fasting measures were done on single point insulin and glucose measures. The release of insulin is pulsatile so ideally 3 samples over 10minutes would give

better mean basal concentrations. This was somewhat mitigated by having a number of differing tests on different days but due to chance each of these may have been done at a peak or a trough of an insulin pulse so affecting overall average (53).

The fasting tests are also measures of basal insulin resistance whereas any dynamic test (Clamp, DISST, OGTT based) is more a measure of stimulated insulin resistance.

There was also some missing data as not all tests were able to be completed in all subjects.

There were also a number of strengths to this study. The DISST test was easy to perform and well tolerated. There were no problems reported with the glucose infusion in comparison to the OGTT which can be poorly tolerated both with the difficulty drinking 300ml of a very sweet drink and some nausea that can occur. There were also no problems associated with the insulin bolus. Being only 1 unit makes hypoglycaemia very unlikely and given that the dose is intravenous any issue would occur within the time of the test. With a simple and easy to follow protocol the DISST test was quick with the whole test taking about 40-45 minutes including preparation time. The ISI-DISST also at least in the preoperative group performed as expected compared to previous studies.

Conclusion

This study was designed to compare the performance of the newly developed DISST method for measuring insulin sensitivity with other methods in a group of morbidly obese individuals before and after bariatric surgery. Overall the DISST test strongly correlated with the reference method, the euglycaemic clamp in the preoperative tests. DISST had a stronger correlation with the clamp than did HOMA, the most commonly used simple surrogate measure of insulin resistance. Notably in this study both $1/\log\text{HOMA}$ and QUICKI also had very strong correlation with the clamp. However as the study numbers were small the confidence intervals were all wide and overlapped.

After surgery, when it is known that there are major changes in glucose homeostasis, DISST had a much weaker correlation with the clamp. The nature of the relationship between the different measurements postoperatively was also altered, suggesting that the fundamental physiological determinants of insulin sensitivity being measured by each change in different ways with RYGB surgery. Gaining a better understanding of this with further studies in larger numbers may help to tease out the mechanistic causes of insulin resistance associated with obesity. For example euglycaemic clamp studies with glucose tracers enabling better compartmentalization of the glucose kinetics with respect to liver output and whole body uptake would be extremely valuable. However this study has demonstrated that the DISST method is a method which can be used to estimate insulin sensitivity in morbidly obese individuals.

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Appendices

Information Sheet

Comparison of DIST versus euglycaemic hyperinsulinaemic clamp before and after Roux-en-Y Gastric Bypass for the measurement of insulin resistance

Principal investigator: Professor Richard Stubbs, Director, Wakefield Biomedical Research Unit, Wakefield Clinic, Wakefield Hospital, Wellington

Co-investigators: Dr John Wilson, Wakefield Clinic, Wakefield Hospital, Wellington, Dr Mark Hayes, Wakefield Biomedical Research Unit, Wakefield Clinic, Wakefield Hospital, Wellington, Dr Jeremy Krebs, Clinical Leader Diabetes and Endocrinology, Wellington Hospital, Wellington

Introduction:

You are invited to take part in a study assessing the methods of measuring insulin resistance in a group of subjects before gastric bypass surgery and after gastric bypass surgery. Please take as long as you need to consider whether or not you wish to take part. You have the right not to take part.

Participation:

Your participation is entirely voluntary (your choice). You do not have to take part in this study, and if you choose not to take part you will receive the standard treatment/care available. This will not affect any future care or treatment.

If you do agree to take part in the study, you are free to withdraw from the study at any time, without having to give a reason, and this will in no way affect your future or continuing health care.

Participation in this study will be stopped should any harmful effects appear or if the doctor feels it is not in your best interests to continue.

About the study

The main aims of the study are to analyse the use of a new test (DIST) that measures insulin resistance and compare that test with the current gold standard for insulin resistance (Euglycaemic hyperinsulinaemic clamp) in a group of people before gastric bypass and after gastric bypass. As part of this study we would also wish to analyse gut hormone response and insulin response to a mixed meal (combination of carbohydrate, fat, and protein) before and after surgery, to see how this relates to insulin resistance.

Subjects for the study will be selected from those about to undergo gastric bypass surgery at Wakefield Hospital. The investigators involved in the study will be involved in the selection.

We aim to assess at least 24 subjects.

The study will be run at Wakefield Hospital and all investigations will be performed in the clinical investigation room within the Wakefield Clinic.

The recruiting period will be over a 12-month period. The initial investigations will be done at 1-2 weeks prior to bypass surgery and 2-4 weeks after surgery and 6-12 months after surgery.

What will happen during the study?

You will be asked for written consent for the study. We will go through the information sheet and any outstanding questions will be answered. You will then sign a consent form agreeing to your willing participation. You will then be asked about some demographic details as well as medical and family history and medication use. You will have your weight taken as well as your height as these are needed for certain calculations for investigations. These will be updated on the visits after surgery.

During the pre bypass study week you will be asked to come into the research facility on 3 separate days having fasted overnight.

On one of these days you will have a test called a euglycaemic hyperinsulinaemic clamp. This is the current best test we have for measuring insulin resistance. This test involves the insertion of 2 intravenous lines in one arm, usually one in the elbow region and one in the hand. One of these lines is for the infusion of insulin and glucose at a set rate. The second line will be to take blood samples from. This hand will be placed in a warming box to increase the blood flow. A small blood sample will be taken every 5 minutes from the line to ensure your glucose levels remain constant. The test itself will take up to 2.5 hours but with setup and post test observation the time involved will be about 4 hours in total.

The second test (DIST) involves putting another intravenous line in. A blood sample is then taken just prior to the commencement of the test. At the beginning of the test you are given an intravenous dose of glucose (10g). Blood samples are then done at 5 minutes and 10 minutes through the line. After the 10-minute blood sample you are given a dose of intravenous insulin (1unit fast acting insulin) and further blood samples are taken at 20,25 and 35 minutes. The test is then completed but you are observed for a further half hour. The total time with us should be approximately 1.5 hours.

The third test involves a mixed meal test where you are given a set volume of a liquid food containing a known amount of calories made up of carbohydrate, fat and protein.

You will have an intravenous cannula placed to enable simpler blood sampling to take place. You will have some blood samples done at baseline (before the mixed meal) then further samples at 15, 30, 45, 60, 90 and 120 minutes. The total time with us should be about 3 hours.

The above tests will then be repeated at 2-4 weeks after your gastric bypass and again between 6-12 months after gastric bypass.

Benefits, risks and safety

The benefits of the study

There will be no direct benefits to you for participating in the study. The study is being performed to establish easier and perhaps better ways of assessing insulin resistance, which is a key factor in the problems associated with severe obesity. Such assessments are likely to contribute importantly in the future to the development of better treatments for obesity and its related problems.

The study is a non-therapeutic study. This means we will not be giving you any medication or other treatment that will give you any tangible benefit. The data we gain may however be useful in deciding in the future who may benefit most from gastric bypass surgery.

The risks and/or inconveniences of the study

The inconveniences of the study include the time involved. Each clamp study will involve a time commitment of 4 hours in the morning (3 separate times) and each DIST test will involve 1.5 hours in the mornings (3 separate occasions). The Mixed meal test will take about 3 hours in the morning. (3 separate occasions). IV line sites have risks of bruising, inflammation and infection although this is minimized by removal of lines at completion of the observation period after testing. The lines occasionally stop working during the testing process so additional lines may be necessary during the test.

During the test and at the completion of the test the other main side effect will be hypoglycaemia (low blood sugar). During the clamp study you will have blood sugars measured at 5-minute intervals to ensure this does not happen. After both tests are completed there is an observation period mainly to ensure that you do not become hypoglycaemic following the procedure. For the clamp study the main risk of hypoglycaemia is during the study and we expect about 5% of subjects will become hypoglycaemic during the study. This will be treated immediately with glucose and altering of insulin infusion. We expect the episodes of hypoglycaemia to be only minor in the majority. For the DIST test we are not expecting any abnormalities with hypoglycaemia as the insulin dose is so small but we will be observing for this as a safety measure.

Occasionally people feel nauseous with glucose or insulin infusions and with a mixed meal test. The mixed meal test also has a taste that some people find unpleasant.

Inclusion and exclusion criteria

Inclusion criteria

those awaiting gastric bypass and willing to participate in study.

Exclusion criteria

Age under 18 years

Individuals with poor venous access

Significant known cardiac / renal impairment which may increase any risk arising from hypoglycaemia.

Costs, Payments and Reimbursements

There will be no additional costs for taking part in the study and no payments required. There will be no reimbursements of costs. Parking is available at Wakefield Hospital at no cost.

What happens if there are any ill effects from the trial? What compensation will be available?

We do not expect any major side effects from the trial. Any untoward event will be treated as medically appropriate. Each test will be done under the direct supervision of a trained medical professional.

In the unlikely event of a physical injury as a result of your participation in this study, you may be covered by ACC under the Injury Prevention and Compensation ACT. ACC cover is not automatic and your case will need to be assessed by ACC according to provisions of the 2002 Injury Prevention and Compensation ACT. If your claim is accepted by ACC, you still might not get any compensation. This depends on a number of factors such as whether you are an earner or non-earner. ACC usually provides only partial reimbursement of costs and expenses and there may be no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue the investigators. For more details, refer to <http://www.acc.co.nz>

General

Will my GP be told I am in the study?

Your GP will be informed you are participating in this study unless you specifically wish this not to be done.

What will happen at the end of the study?

At the end of the study all data will be collated together with others who have been involved in the study and the data will be analysed. There will be no direct follow up of the tests that you have performed but you will continue to be followed through the usual way following your bypass surgery.

Where can I get more information about the study?

You can contact the investigators and we would be happy to talk further about the study.

If I need an interpreter, can one be provided?

A qualified interpreter will be available if required.

Additional support

You may have a friend, family or whānau support to help you understand the risks and/or benefits of this study and any other explanation you may require.

If you have any queries or concerns regarding your rights as a participant in this study, you may wish to contact an independent health and disability advocate:

Free phone: 0800 555 050

Free fax: 0800 2 SUPPORT (0800 2787 7678)

Email: advocacy@hdc.org.nz

Confidentiality

No material that could personally identify you will be used in any reports on this study.

Each subject will be assigned a unique code and this will be used to identify subjects with specimen results. Some of the investigations performed however will be part of the current surgical workup and will as such be identifiable to others.

Records of the personal details and other history and examination findings as well as results of investigations will be kept in a secure and confidential manner. This data will only be available to the investigators.

Results

The data collected will be analysed in a scientific manner using appropriate statistical methods. The aim is publication of the results in appropriate scientific journals and presentation of findings at appropriate medical meetings. There is always a delay following data collection and subsequent analysis and presentation of findings. A copy of the final results will be available to any subjects if they wish.

Statement of approval

This study has received ethical approval from the Central Regional Ethics Committee; ethics reference number (insert ethics reference number).

Please feel free to contact the researcher if you have any questions about this study.

Study protocol

Comparison of DIST versus euglycaemic hyperinsulinaemic clamp before and after Roux-en-Y Gastric Bypass for the measurement of insulin resistance

Principal investigator: Professor Richard Stubbs, Director, Wakefield Biomedical Research Unit, Wakefield Clinic, Wakefield Hospital, Wellington

Co-investigators: Dr John Wilson, Wakefield Clinic, Wakefield Hospital, Wellington, Dr Mark Hayes, Wakefield Biomedical Research Unit, Wakefield Clinic, Wakefield Hospital, Wellington, Dr Jeremy Krebs, Clinical Leader Diabetes and Endocrinology, Wellington Hospital, Wellington

Insulin Resistance is a marker for metabolic disease and predicts the development of type 2 diabetes and cardiovascular disease (1). The current gold standard for testing whole body insulin sensitivity is the euglycaemic hyperinsulinaemic clamp technique (2). However this is a time consuming and intensive investigation for both investigator and subject. An alternative is the intravenous glucose tolerance test with minimal modelling as developed by Bergman (2,3,4). However this is also invasive, time consuming and expensive. Other surrogate measures for insulin resistance based on fasting blood samples of insulin and glucose (5), the most commonly used being HOMA (6), or indices using the oral glucose tolerance test (Matsuda index) (7) have been developed. Although much simpler, less invasive and less expensive, these measures have variable correlation with the euglycaemic hyperinsulinaemic clamp, particularly in individuals with established diabetes.

A new technique called the DIST (Dynamic Insulin Sensitivity Test) has been developed and validated against the euglycaemic hyperinsulinaemic clamp in subjects of various weights and non-diabetic and diabetic ($r=0.97$, $n=60$). (8) Recent further validation has been performed on a different population group. ($r=0.98$, $n=146$, unpublished).

Additionally, because DIST does not require supra-physiological levels of insulin, it may offer a more appropriate measure of insulin sensitivity than a clamp study. DIST is less time consuming and less expensive and therefore appears well suited for the measurement of whole body insulin resistance in place of the euglycaemic insulin clamp.

In the obese there is an increased preponderance of metabolic syndrome and diabetes. This is thought to be related to a reduction in insulin sensitivity with increased fat mass. However not all obese and super-obese subjects have increased insulin resistance.

Conducting euglycaemic hyperinsulinaemic clamps is more difficult in obese and super-obese subjects, mainly through the technical aspects of venous access. A simpler method of assessing insulin resistance would make subsequent investigation of this group of subjects simpler and easier both for the subjects and for the investigators.

Although DIST has been validated in obese subjects, it has not been assessed before and after an intervention known to alter insulin sensitivity. Gastric bypass surgery is known to profoundly improve insulin sensitivity. Therefore it offers an ideal model to assess how DIST performs in this setting. The mechanism for improved insulin sensitivity following gastric bypass surgery remains unclear, but one hypothesis is that there are changes in the entero-insular axis. Known putative hormones include GLP-1, GIP, PYY and Ghrelin.

In this study we plan to assess the new DIST technique in comparison with the euglycaemic hyperinsulinaemic clamp technique in the obese and super obese both pre op Roux-en-y gastric bypass, within 2-4 weeks post gastric bypass and at 6-12 months post gastric bypass.

As part of this project we will also assess changes in gut hormones and insulin in response to a mixed meal.

Methods

Participants

We will recruit 24 participants for this study. The subjects will be privately funding their gastric bypass through Prof Richard Stubbs at Wakefield Clinic. They will undergo the usual bypass surgery assessments. Prof Stubbs, or other investigators will approach the subjects, regarding their potential participation in the study. Their participation will be entirely voluntary and declining participation will have no effect on any subsequent surgery or other treatment.

Written consent will be obtained from all participants.

The study will consist of 3 separate tests over 3 separate days in the same week at 3 time points, preoperatively, 2-4 weeks post operatively and 6-12 months post-operatively.

The 3 tests will be:

- euglycaemic hyperinsulaemic clamp
- DIST
- Mixed meal test

The euglycaemic hyperinsulinaemic clamp

The euglycaemic clamp consists of the participant arriving at 8am having fasted overnight. A retrograde cannula is inserted in the dorsum of hand or wrist and

connected to a saline primed microbore 30cm extension tubing and a three way tap. The hand is placed in a box heated to 60°C to allow maximal vasodilation to “arterialise” the venous blood. This line is used for sampling. A second cannula is inserted in the antecubital fossa of the same arm and is connected, via a dual adapter with a non-return valve, to insulin and glucose infusions.

Body Surface Area (BSA) is calculated from measured height and weight using the DuBois and DuBois formula.

Baseline insulin and glucose values are measured.

Insulin infusion is set up after the patient has been cannulated. 47 mL of saline and 3 mL of the participant’s blood are mixed in a 50 mL syringe. The blood is needed to reduce insulin binding to the infusion tubing. The established insulin dose for euglycaemic hyperinsulinaemic clamp is 40mU/min/m². The amount to be added to the 50ml syringe is calculated $(BSA (m^2) \times 40 \text{ mU/min/m}^2 \times 60\text{min} \div 15\text{mL (infused volume per hour)} \times 50 \text{ mL (syringe volume)}) = \text{mU of insulin to add to syringe}$. This can be simplified to $BSA \times 8 = \text{Units of insulin to add to syringe}$.

The clamp then commences with a loading dose of insulin of 60mL/hr for 4 minutes, then 30mL/hr for 3 minutes then 15mL/hr as the ongoing infusion rate for the rest of the test. This rapid loading of insulin allows for rapid declines in hepatic glucose production over the first approximately 20minutes of the procedure.

The glucose infusion (Glucose solution 25% = 250mg/ml) is started 4 minutes after the insulin. However if the glucose value is above 5.5mmol/L at baseline the glucose infusion is started once the glucose level is 4.5mmol/L.

Dose of glucose infusion is calculated by $\text{Weight} \times 60 \text{ min} \div 250 \text{ mg/ml (G25\% solution)} = \text{ml/hr}$ (Simplified $\text{Weight} \times 0.24$). This delivers 1mg/kg/min. Start rate of glucose is the same as weight (kg) in ml/hr, which is just above 4 mg/kg/min. The glucose rate is then adjusted over the remainder of the test to maintain euglycaemia.

The set point of the test is 4.5mmol/L of glucose unless the baseline value is between 4-5mmol/L. Then the baseline value is the set value. Steady state is deemed to be when Glucose level and Glucose infusion rate are stable $\pm 10\%$.

At 60 minutes, 80 minutes, 100 minutes and 120 minutes Insulin levels are measured.

The data is then analysed using standard formula to obtain an insulin sensitivity index.

The participant will need about 4 hours of time for the test. During the test the participant’s glucose is measured every 5 minutes using a rapid glucose analyser.

DIST

The DIST technique requires participants to arrive fasted at 0800. They then have a baseline insulin/c-peptide /glucose measured. They then have an iv glucose injection (10g). A repeat insulin/c-peptide/glucose is done at +5 and +10 minutes. An insulin

dose (1 unit) is given at (+10 minute) and repeat insulin/glucose/C-peptide performed at +20 minutes and +30minutes.

These results are then used to give an insulin sensitivity index using a standard formula. The results will then be compared via statistical analysis to verify whether the technique is satisfactory for use as investigative procedure in Gastric bypass population.

Mixed meal test

A mixed meal test using a liquid meal of known volume, calories, fat, carbohydrate and protein to assess insulin, GLP-1 and GIP profiles before and after surgery. The participant will arrive fasted at 0800. Baseline blood samples of glucose, insulin, GLP-1 and GIP will be measured. The participant will then drink the mixed meal (Ensure plus). Repeat Glucose, insulin, GLP-1 and GIP will be done at 15, 30, 45, 60, 90, and 120 minutes.

These will be done in the same week as the other tests but on separate days.

The data will be analysed using appropriate statistical analysis and to determine the value of using the DIST test in post-gastric bypass subjects.

The Mixed meal data will be analysed separately and compared against insulin resistance and may form part of a subsequent pilot study.

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