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Investigation of MC4 Receptor Polymorphisms and the Effect of Bariatric Surgery on a Selected Group of South African Obese Patients

by

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Submitted in partial fulfilment of the requirements for the degree

Magister Scientae
MSc Immunology

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December 2008

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I would like to dedicate this dissertation to my loving mother Eirene and Jesus Christ my Lord and Saviour, without whom none of this would have been possible.

Acknowledgements

I would like to acknowledge my two Professors, Michael Pepper and Tessa van der Merwe whose help and guidance were crucial to the completion of this dissertation. Jenny Pieters for all the administration and uplifting conversations, you are amazing! Tyren Dodgen for being a massive help during sample collection, much obliged my friend! Y. Sammi from Roche, South Africa for sponsorship of the Pro-BNP kits. To the funding bodies AMPATH, NRF, NHLS and NetCare thank you for your generous contributions to this project.

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List of Symbols and Abbreviations (In order of appearance in text)

BMI	Body Mass Index
WHO	World Health Organization
IHD	Ischemic Heart Disease
HDL	High Density Lipoprotein
NIH	National Institute of Health
NA	Noradrenalin
DA	Dopamine
5-HT	5-hydroxytryptamine
GLP-1	Glucagon-like Peptide 1
BPD	Biliopancreatic Diversion
RYGB	Roux-en-Y Gastric Bypass
LAGB	Laparoscopic Adjustable Gastric Banding
DS	Duodenal Switch
DVT	Deep Venous Thrombosis
PE	Pulmonary Embolism
MI	Myocardial Infarction
SBO	Small Bowel Obstruction
SOS	Swedish Obese Subjects
PYY	Peptide YY
NPY	Neuropeptide Y
GH	Growth Hormone
GHS-R	Growth Hormone Secreatagogue Receptor
α-MSH	α-Melanocyte Stimulating Hormone
GIT	Gastrointestinal Tract
IV	Intravenous
PP	Pancreatic Polypeptide
AGRP	Agouti-Related Protein
IFSO	International Federation for the Surgery of Obesity
DJB	Duodenal-Jejunal Bypass
IRB	Institutional Review Board
BCE	Bariatric Centre of Excellence
DEXA	Dual Energy X-Ray Absorptiometry
DM	Diabetes Mellitus
HT	Hypertension
ASA	Anaesthesiologists Physical Status Score
CAD	Coronary Artery Disease
OSA	Obstructive Sleep Apnoea
ANC	Adjustable Neck Circumference
ESS	Epworth Sleepiness Scale
IM	Intramuscular
PO	per Os or Orally
CI	Confidence Interval
$HbA1_c$	Glycated Haemoglobin
QOL	Quality Of Life
NICE	National Institute for Health and Clinical Excellence
ECG	Electrocardiogram
BNP	B-type Natriuretic Peptide
LV	Left Ventricle

NT-pro-BNP	Amino-Terminal Portion of pro-BNP
MC4R	Melanocortin 4 Receptor
CNS	Central Nervous System
PVH	Paraventricular Nucleus of the Hypothalamus
GPCR	G-protein Coupled Receptor
cAMP	Cyclic Adenosine Monophosphate
ACTH	Adrenocorticotropin
POMC	Pro-opiomelanocortin
MCR	Melanocortin Receptor
G + E	Genotype and Environment
KO	Knock-out
BMD	Bone Mineral Density
BED	Binge Eating Disorder
LEPR	Leptin Receptor
ICV	Intracerebroventricular
MS	Metabolic Syndrome
TM1	Transmembrane Domain 1
TM4	Transmembrane Domain 4
EC_{50}	Median Effective Concentration
NASH	Non-alcoholic Steatohepatitis
LDL	Low Density Lipoprotein
ALT	Alanine Transaminase
AST	Aspartate Transaminase
GGT	Gamma Glutamate Transferase
CRP	C-reactive Protein
SC	Subcutaneous
IGT	Impaired Glucose Tolerance
TG	Triglyceride
CVA	Cardiovascular Accident
BP	Blood Pressure
PCOD	Polycystic Ovarian Syndrome
NAFLD	Non-alcoholic Fatty Liver Disease
SEM	Standard Error of the Mean
EDTA	Ethylene Diamine Tetraacetic Acid
BL	Black African
CA	Caucasian
CL	Mixed Race
IN	Indian
DNA	Deoxyribonucleic Acid
PCR	Polymerase Chain Reaction
EF	External Forward
ER	External Reverse
IF	Internal Forward
IR	Internal Reverse
dNTP	Deoxynucleotide Triphosphate
$MgCl_2$	Magnesium Chloride
UV	Ultraviolet
ABI	Applied Biosystems
SNP	Single Nucleotide Polymorphism
Nt	Nucleotide

AA	Amino Acid
mRNA	Messenger Ribonucleic Acid
NMD	Nonsense Mediated mRNA Decay
PTC	Premature Termination Codon
SAR	Structure Activity Relationship
THIQ	Tetrahydroisoquinoline

List of Conference Contributions

van der Merwe M.T., Logan M., Pieters J.H., Sammi Y., Pepper M.S. Sustained weight loss after an 18 month period of intensive obesity intervention and the successful use of pro-BNP to exclude cardiac disease. 42nd SEMDSA Congress. Bloemfontein, South Africa. 30 June – 2 July, 2007. **J. Endocrinol. Metab. Diabetes South Afr.** 12(1): 46 (2007).

Logan M.G., Pepper M.S., van der Merwe M.T. and Fetter G.K. Early changes following bariatric surgery in South Africa. SASOM Conference. Unitas Hospital, Pretoria, South Africa. 14 January, 2008.

Logan M.G., Pepper M.S., van der Merwe M.T. and Fetter G.K. Early changes following bariatric surgery in South Africa. 43rd SEMDSA Congress. Cape Town, South Africa. 12-15 April, 2008. **J. Endocrinol. Metab. Diabetes South Afr.** 13 (1): 35 (2008).

Logan M., Pepper M.S. and van der Merwe M.T. Early changes following bariatric surgery in South Africa. 16th European Congress on Obesity ECO. Geneva, Switzerland. 14-17 May, 2008. **Int. J. Obesity** 32 (suppl 1): S5 (2008).

Logan M.G., Pepper M.S., van der Merwe M.T. and Fetter G.K. Early changes following bariatric surgery in South Africa. Faculty Day, University of Pretoria, Pretoria, South Africa. 20 August, 2008.

Summary:

A) Bariatric surgery for the treatment of obesity has shown much promise. The Roux-en-Y gastric bypass is a procedure that combines both restrictive and malabsorptive elements. Post-operative weight loss and co-morbidity improvements previously achieved are over and above those which are seen during life style modification and drug therapy. 330 patients (2005-2007) with a mean BMI of 45.87 ± 0.63 were characterised pre-operatively with regard to clinical, anthropometric and DEXA scan measurements. 130 were matched for the same parameters post-operatively over a 9-12 month observation period. The data was analysed statistically using paired t-tests and regression analyses. Significant post-operative improvements were observed with regard to patients' weight loss and co-morbidity improvement. Positive and significant correlations of anthropometric measures to biochemical parameters ensued. Risk factor scoring methodology produced an average total score of 17 points / 36. Average post-op weight loss at 9-12 months follow-up was 20% of initial pre-op weight. Co-morbid diseases and anthropometric measurements illustrated significant changes following surgery. Risk factor scoring is a valuable pre-op tool for assessing eligibility for medical aid re-imbursement for surgery.

B) Obesity is a global epidemic and is increasing the worlds' mortality rate. Genetic predisposition to obesity is recognized as being significant. Polymorphisms within the Melanocortin 4 Receptor (MC4R) gene, which encodes a G-protein coupled receptor responsible for post-prandial satiety signalling, have been associated with monogenic obesity. Obesity prevalence in South Africa is drastically increasing, however there has been no causative investigation done. Thus we sought to perform an initial assessment of the prevalence of MC4R polymorphisms within a South African representative group. Blood was drawn from a mixed Body Mass Index (BMI) cohort of 259 adult individuals and their DNA was extracted. The MC4R gene was PCR amplified from the DNA, the amplicon sequenced and the sequence data was analyzed for polymorphisms. A polymorphism prevalence of 13.51% was found within the patients across a BMI range that spanned from underweight

(19.6) to super-obese (126.0). In addition to MC4R polymorphisms that had been identified previously, two new polymorphisms namely R7H and S36T were observed. Four haplotypes were also identified. MC4R mutation frequency was observed to be ethnically dependant; however the hypothesis of differing ethnic backgrounds illustrating varying mutational penetrance was not confirmed. The expected trend regarding MC4R polymorphism functional effect and associated pathogenicity was not followed in light of our results. The question of whether or not MC4R polymorphisms contribute to the development of obesity is indisputable; however the current accepted trend regarding their precise role may be incorrect and must be challenged.

Samevatting (Afrikaans):

A) Bariatriese chirurgie vir die behandeling van vetsug, toon baie hoop. Die Roux-en-Y gastrieseverlegging is ‘n prosedure wat beide beperkende- asook malabsorpsie-elemente kombineer. Post-operatiewe gewigsverlies en mede-siektetoestand-verbeterings wat vroeër behaal is, is bo en behalwe dit wat gesien word tydens lewensstyl veranderings en middel-terapie. 330 pasiënte (2005-2007) met ‘n gemiddelde LMI van 45.87 ± 0.63 is pre-operatief gekarakteriseer met betrekking tot kliniese, antropometriese en DEXA skanderings-mates. 130 pasiënte was gepaar vir dieselfde parameters post-operatief oor ‘n 9-12 maande waarnemings-periode. Die data was statisties geanaliseer deur van ‘n gepaarde t-toets en regressie analise gebruik te maak. Positiewe en betekenisvolle korrelasies tussen antropometriese metings en biochemiese parameters het gevolg. Risikofaktor metings metodologie het ‘n gemiddelde totale telling van 17 / 36 punte gegenereer. Gemiddelde post-operatiewe gewigsverlies by die 9-12 maande opvolg was 20% van die oorspronklike pre-operatiewe gewig. Mede-siektetoestande en antropometriese metings het betekenisvolle veranderings na chirurgie geïllustreer. Risikofaktor meting is ‘n waardevolle pre-operatiewe hulpmiddel vir die bepaling van geskiktheid vir mediese fonds vergoeding vir chirurgie.

B) Vetsug is ‘n epidemie wat regoor die wêreld strek, en die globale sterftesyfer verhoog. Genetiese vatbaarheid vir die ontwikelling van vetsug het herkenbare betekenis. Polimorfismes binne die Melanokortien 4 Reseptor (MK4R), ‘n G-protein gekoppelde-reseptor, verantwoordelik vir die na-maaltyd vesadigingssein, word geassosieer met monogeniese vetsug. Die voorkoms van vetsug in Suid-Afrika is besig om drasties te verhoog, maar geen ondersoek na die oorsaak is al geloods nie. Ons het dus be-oog om ‘n inisiële bepaling van die voorkoms van die MK4R polimorfisme binne ‘n Suid-Afrikaans verteenwoordigende goep te doen. Bloed is van ‘n gemengde Liggaams Massa Indeks (LMI) groep van 259 volwassse individue getrek, en hul DNS is geëkstraheer. Die MK4R-geen was deur PKR vermenigvuldig van die DNS, die amplikon nukleotiedbasis volgorde bepaal en die volgorde data geanaliseer vir polimorfismes. ‘n Polimorfisme voorkoms van 13.51% was binne die pasiente wat oor die LMI reeks, wat van ondergewig (19.6) tot uiterse-vetsug (126.0) strek, gevind. Addisioneel tot die MK4R polimorfismes, wat alreeds geïdentifiseër is, is twee nuwe polimorfismes, naamlik R7H en S36T, waargeneem. Vier haplotipes is ook geïdentifiseër. MK4R mutasie frekwensie bleik om etnies-afhanklik te wees, alhoewel die hipotese van verskillende etniese agtergronde wat wisellende mutasie penetrasie toon, nie bevestig is nie. Die verwagte neiging met betrekking tot MK4R polimorfisme funksionele effek en geassosieerde patogenisiteit was nie gevolg in die lig van ons resultate nie. Die vraag of MK4R polimorfismes bydrae tot die ontwikkeling van vetsug, is onbestwisbaar, maar die huidig aanvaarbare neiging van hul presiese rol is nie korrek nie, en moet uitgedaag word.

Literature Review – Part 1

Chapter 1: Obesity Treatment

1.1 Introduction, Classification and the South African Epidemic

Obesity (body mass index [BMI] > 30 kg/m²) has been recognized as a chronic disease by the World Health Organization (WHO). It is characterized by alterations in metabolic function, which result in an increase in total body fat mass as well as an accumulation of visceral adipose tissue [1]. Excess energy is available to fuel obesity in the modern human population because energy expenditure has decreased and calorie intake has risen.

In accordance with this, it has been shown that in order for a normal weight person to put on weight they must consume 100 kcal over and above their minimum daily calorie requirements; individuals that are overweight and obese however, need to consume up to 225 kcal more than a normal lean individual to achieve satiety [2], it is inevitable therefore that these individuals will gain weight. Obtaining a balancing of the calories consumed with those that are used during exercise is thus essential for obesity prevention [3].

Obesity is however not only dependent on the balance between energy intake and energy expenditure. Research has illustrated the importance of the genetic aspect of the disease and in particular the genetic susceptibility of certain individuals to obesity development. Thus obesity can be described as a multi-factorial disease, meaning both genetic and environmental factors contribute to its expression [4]. The prevalence of obesity is increasing in most African countries, particularly in individuals living in urban areas.

Ethnicity has a major impact on obesity as well as on co-morbid diseases, for example ischemic heart disease (IHD) and atherogenesis are less prevalent within the black South African population; the reason posed for protection against IHD is that serum lipid profiles are more favourable when compared to other racial groups, they have low cholesterol, high ratios of high-density

lipoprotein cholesterol and low homocysteine values [5]. Additionally differences in lifestyle will impact differently on various genetic backgrounds [5].

It has been incorrectly perceived by the general public and the media alike that developing countries do not have an obesity problem. South Africa is one of many developing countries where obesity is becoming increasingly prevalent [5]. South African citizens observed to be underweight and obese are sometimes part of the same family; this can be explained by the apparent correlation of small size in full term pregnancies to the advancement of features of the metabolic syndrome later in life [5]. These include obesity, type 2 diabetes, elevated blood pressure, dyslipidemia and an increased mortality resulting from disease of the cardiovascular system [5]. Development of the metabolic syndrome can be attributed to intrauterine undernutrition of the foetus [5]; however patterns of growth and premature birth are also emerging as important risk factors in the advancement of obesity and the metabolic syndrome as these individuals reach adulthood [3].

Currently, in several parts of South Africa, the prevalence of combined overweight and obesity (BMI > 25) has reached significant proportions within the economically active adult population (18-65 years); black women 75%; black men 49%; coloured women 66%; coloured men 45.7%; Indian women 37%; Indian men 36%; white women 42% and white men 56% [5]. Information on the South African obesity landscape showed that in a random sample of 13,089 individuals mean BMI figures were 22.9 kg/m^2 and 27.1 kg/m^2 for men and women respectively [6]. Overweight/obesity (BMI ≥ 25 kg/m^2) information showed that 29.2% of men and 56.6% of women fell into this category. Figures investigating abdominal obesity were 9.2% and 42% for men and women respectively, with white urban and non-urban African women being the highest [6].

Factors influencing the variation in BMI and waist circumference were age, level of education, population group and area of residence, a higher incidence of obesity was identified within urban African women [6]. Women with no education were found to have lower BMI's than those that had been schooled,

due to more manual labour based occupation in the latter group, however those women that had received tertiary education had lower BMI's than those that had had some schooling [6]. The investigators state that obesity management is particularly important within women that fall into the older age groups and urbanized, older white and higher educated men within the South African population [6].

South Africans very rarely fell into the underweight category, but it was more common within men (12.2%) than in women (5.6%) [6]. Perception of body weight showed that individuals underestimate or are oblivious to their weight; perception data showed that 9.7% and 22.1% of men and women from the study recognize themselves as being overweight, the collected anthropometric data show these measures to be 29.2% and 56.6% respectively [6]. Within differing ethnic groups, white women had a better and more accurate perception of themselves being overweight or obese as opposed to their black counterparts [6]. This may have to do with the fact that within the African, South African culture, being overweight represents prosperity, happiness and health [6]. Early-onset childhood obesity is a problem particularly within South African women, with 10% of women being obese between the ages of 15 and 24 [6].

Urbanization has been recognized as a major cause of the increased rate of obesity development within previously rural African communities [6]. When looking at nutritional status and food intake patterns, the more urbanized these individuals became, the less concern for diet there was [6]. The adult South African population (age ≥ 15 yrs) are characterized by overnutrition when looking at dietary intake, with an increase of calories from total fat [6]. Therefore within adult South Africans, specifically in African women, there is a milieu of a high rate of abdominal obesity and overweight [6]. Education and challenging certain cultural perspectives with regard to obesity are necessary steps that need to be undertaken in order to manage the South African epidemic [6].

Along with the increasing prevalence of obesity, co-morbid conditions associated with the disease are also on the rise. These include type 2 diabetes, hyperlipidaemia (defined as: increased serum triglycerides; decreased HDL cholesterol or alternatively use of pharmaceuticals that cause a lowering of lipid content), hypertension, obstructive sleep apnoea, heart disease, stroke, asthma, back and lower extremity weight-bearing joint degeneration, cancer in many forms and depression [7]. In individuals that present with morbid obesity and thus qualify for bariatric surgery, diabetes and hypertension illustrate strong correlations with body weight and the waist to hip ratio [www.asbs.org]. Globally these co-morbidities are the cause of 2.5 million deaths per annum [7].

Obesity can be divided into three classes (Table 1), as was put forth in the 1998 NIH (National Institute of Health) Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults [8].

Table 1: The Three Classes of Obesity according to 1998 NIH Clinical Guidelines.

Class	**BMI Cut-off**
Class I	30.0 kg/m^2 - 34.9 kg/m^2
Class II	35.0 kg/m^2 - 39.9 kg/m^2
Class III	≥ 40 kg/m^2

The rise of obesity is exponential and has been described as an epidemic within an epidemic [8]. Between 1986 and 2000 the prevalence's of obesity (BMI ≥ 30 kg/m^2) doubled, morbid obesity (BMI ≥ 40 kg/m^2) quadrupled and super obesity (BMI ≥ 50 kg/m^2) increased by 5-fold within adults in the US [8]. From 1986 - 2001 patients that qualified for surgical treatment have increased in pre-operative weight; BMI increased from 45.2 ± 7.8 to 49.8 ± 9.5 respectively at a significance level of $p < 0.0001$ [www.asbs.org]. Even more alarming is the fact that similarities are being observed within the paediatric

population [8]. Within the morbidly obese population life expectancy on average is decreased by 9 years in females and 12 years in males [8].

The medical management of obesity includes lifestyle management, as well as pharmacological (Table 2) and surgical approaches (Figure 1).

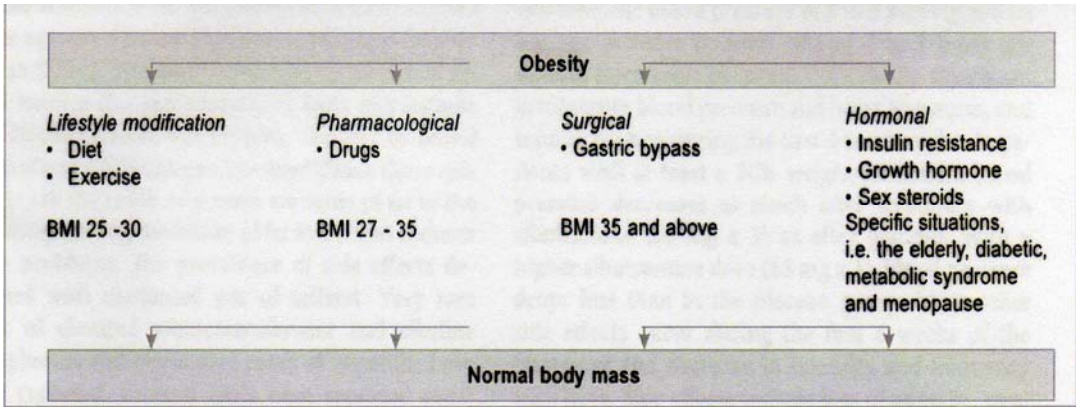


Figure 1: Strategies for the Treatment of Obesity. (van der Merwe. MIMS Primary Care 2007: 229-236.)[9].

Table 2: Principles of drugs for weight control.

Agents	**Action**
Dexamphetamine	Releasing Agent (NA, DA)
Phentermine	Releasing Agent (NA, DA)
Fenfluramine	Releasing Agent (5-HT)
Sibutramine*	Re-uptake inhibitor (5-HT, NA) Central α_2 clonidine-like effect
Orlistat*	Peripherally acting lipase inhibitor
Rimonabont	Cannabinoid receptor, antagonist
Exanitide	GLP-1 glucagon-like peptide agonist
*Available in South Africa	

(van der Merwe. MIMS Primary Care 2007: 229-236.)[9]

1.2 Bariatric Surgery

1.2.1 Surgical Procedures

There are three surgical procedures falling under three distinct classes that are currently being utilized worldwide; gastric bypass with a standard, long or very long Roux limb (restrictive and malabsorptive) (Figure 2 & Table 2); laparoscopic adjustable gastric banding (restrictive) (Figure 3 & Table 2); and biliopancreatic diversion (BPD) or duodenal switch (primarily malabsorptive) [8] (Figure 4 & Table 3). BPD shows association to greater weight loss post-op, but complications such as liver failure and metabolic bone disease as well as lengthened hospital stay are seen when compared to other surgical strategies [10].

Table 3: Operative mortality and weight loss outcomes of the three main surgical procedures currently being used for obesity treatment.

	Percentage of excess weight loss		Operative mortality (≤ 30 days)
	Mean	95% confidence interval	95% confidence interval
Gastric banding	47.5%	40.7%-54.2%	0.1%
Gastric bypass	61.6%	56.7%-66.5%	0.5%
Biliopancreatic diversion or duodenal switch	70.1%	66.3%-73.9%	1.1%

(Buchwald *et al.* JAMA. 2004; 292(14):1724-1737.)[7]

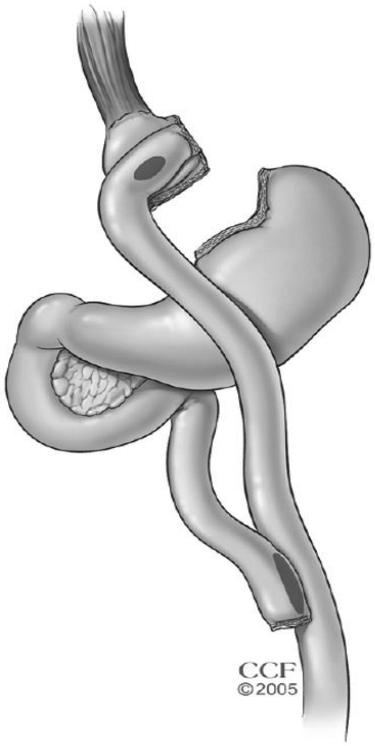


Figure 2: Roux-en-Y gastric bypass.

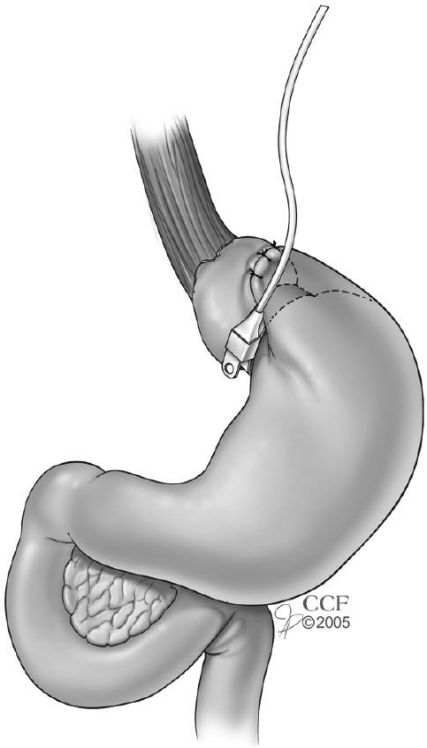


Figure 3: LapBand® or adjustable gastric banding.

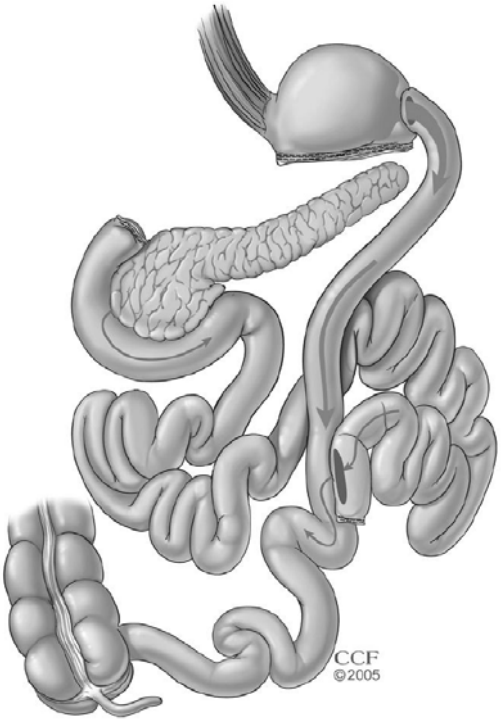


Figure 4: Biloipancreatic diversion or duodenal switch.

Figures 2, 3 & 4 – Sugerman *et al.* Obesity Sugery. Dec 2007; 251-254 [11].

Additional operative procedures that are under current investigation incorporate negligible malabsorption however have positive effects on the metabolic syndrome by mechanisms involving neurological brain-gut peptides [12].

The choice of the procedure should be customised according to the patients' BMI, risks during surgery, metabolic complications, co-morbid conditions, preference and surgeons' recommendations [10]. In patients that are aged, severely obese and present with severe co-morbidities a two-staged approach has been proposed to decrease risk; this involves the initial placement of an adjustable gastric band to achieve initial weight-loss and co-morbidity improvement (the risk of band insertion far outweighs that of any of the malabsorptive procedures); once post-banding goals are achieved a conversion to a malabsorptive strategy can then be employed to maintain weight-loss [12].

All procedures substantiate the use of laparoscopy as opposed to open operation. Wound difficulties (6.6% open; 3.0% laparoscopic) and incisional hernias are more common following open procedures [12]. Laparoscopy is favoured because of fewer post-op complications and a shorter hospitalisation period [10]. Along with this technique is the use of gastric transection as opposed to the use of staple lines, which has eradicated revisional surgical approaches to correct staple line malfunction [www.asbs.org]. Conversely, in the long-term, anastomotic narrowing was more common in those individuals that had laparoscopic procedures [10]. Additionally small-bowel obstruction (3.1% laparoscopic; 2.1% open) and gastrointestinal haemorrhaging (1.9%; 0.6% respectively) were more prevalent following laparoscopic techniques. The proposed reason for increased small-bowel obstruction was internal hernia [12]. Other complicities measured showed no difference between the two techniques and these included pulmonary emboli, leakage and pneumonia [12]. Peri-operatively patients should be treated with both antibiotic and antithromboembolic agents [10]. Pulmonary embolism fatality is 0.2%; however strategies such as shorter operative times, use of compression stockings and

speedy post-operative mobilisation could circumvent the use of anticoagulants [10].

Table 4 summarizes the risk of complications, nutritional deficiencies, mortality rate and benefits regarding weight loss across the three main surgical procedures.

Table 4: Comparison of Surgical Procedure Risks.

Roux-en-Y Gastric Bypass (RYGB) vs. Adjustable Gastric Banding (AGB) vs. Biliopancreatic Diversion +/- Duodenal Switch (BPD/DS)

Risks	**RYGB (%)**	**LAGB (%)**	**BPD, BPD-DS (%)**
Conversion to open procedure	0-8	0.3-1	0-26
EARLY POST-OPERATIVE COMPLICATIONS			
Major and Minor	4.2 – 30	0.8 - 12	2.6 - 22.5
Bleeding	0.4 – 4	0.1	5 - 10
Leak	0 – 4.4	0.5 – 0.8	2.5 – 3.2
Wound infection	0 – 8.7	0.1 – 8.8	2.5 – 18.7
DVT	0 – 1.3	0.01 – 0.2	0.5 – 2.5
PE	0 – 1.1	0.1	0.9
LATE COMPLICATIONS			
Major and Minor	8.1 – 47	6 - 26	1.5 – 60
Anastomotic stricture	2 – 16	N/A	1.7 – 7.6
Marginal ulcer	0.7 – 5.1	N/A	1.6(DS)
Bowel obstruction	1.1 – 10.5	0	1.5
Re-operation rate	9.8 – 13.8	4 - 19	0 – 12.5

BAND-RELATED COMPLICATIONS			
Prolapse	N/A	2 - 25	N/A
Erosion	N/A	0 – 3	N/A
Gastric outlet obstruction/ Pouch dilation	N/A	0.2 – 14	N/A
Tube or port malfunction	N/A	0.4 – 7	N/A
Band intolerance	N/A	0.4 – 3.1	N/A
NUTRITIONAL DEFICIENCIES			
Iron	6 – 52	NR	23 – 44
Vitamin B12	3 – 37	NR	22
Fat soluble vitamins	Rare	NR	5 – 69
Calcium	Rare	NR	25 – 48
Protein malnutrition	Rare	NR	3 – 18
Mortality Rate	0 – 2; mean: 0.5	0 – 0.7; mean: 0.1	0 – 2.5; mean: 0.8
BENEFITS			
Excess weight loss (5 years)	68 – 80	44 - 68	65 – 61.5
Excess weight loss for BMI > 50	51 – 69	47 - 49	77
Durability	49 – 75 EWL at 10 – 14 years	35 EWL at 12 years	77 at 18 years

(van der Merwe, SA Cardiology & Stroke Summer 2008: 25-35.)[13]

Table 5 shows the common complications seen following gastric bypass and duodenal switch over a time period from 0 weeks to 5 years.

Table 5: Common complications of bariatric surgery and time course.

Procedure	Complication	0-2 wks	2-4 wks	4-8 wks	2-6 mnths	6-12 mnths	1-5 yrs
GASTRIC BYPASS AND DUODENAL SWITCH	Leak	++					
	PE	++	+	+	+		
	MI	+	+	+			
	Pneumonia	+					
	Wound Infection	+++	+				
	SBO	+	+	+	+	+	+
	Stomal Stenosis		+	+++	+		
	Nausea		++	++	++	+	
	Incisional hernia (open)				++	++	+
	Cholelithiasis				+	+	
	Nutritional complications Hypoglycemia			+	+	++	+++
	Suboptimal weight loss						+

Curr Prob Surg 2008;45:68-137.)[14]

Table 6 illustrates the early post-operative complications that are life threatening and can lead to mortality if they occur.

Table 6: Early Postoperative Complications: Life-threatening and death.

Buchwald: **Restrictive procedures – 0.1%** **Gastric Bypass – 0.5%** **BPD – 1.1%**			
	Risk Profile		
DVT & Pulmonary Embolism	0.5% - 4%		
Anastomotic Leak	2-4%	Biphasic: Early: 24–48 hrs Later: 5-10 days	Rapid clinical deterioration Minimal or no symptoms; unexplained tachycardia, tachypnea, fever, hypoxia, extreme anxiety
Myocardial Infarction	0.5% - 1%		

(Curr Prob Surg 2008;45:68-137.)[14]

Table 7 shows other early post-operative complications that are not necessarily fatal, but do need serious attention if observed.

Table 7: Other Early Postoperative Complications.

	Risk Profile	Timescale
Pulmonary Dysfunction e.g. • **Atalectasis** • **Pneumonia** • **Lobar collapse** • **Severe respiratory failure requiring prolonged ventilation**	Up to 5%	
Wound Infection	5 - 20%	5 – 20 days post-op
Small bowel obstruction (early and late occurrence	2 – 8%	Months to Years

Curr Prob Surg 2008;45:68-137.)[14]

The conclusion from the Swedish Obese Subjects (SOS) study sums up the overall effect of bariatric surgery in the obese patient and it states, “Bariatric surgery for severe obesity is associated with long-term weight loss and decreased overall mortality” [15].

A large prospective study conducted by Adams *et al.* in a cohort of more than 8000 patients showed that positive clinical outcomes post-gastric bypass lead to significant disease-specific reductions in mortality (Table 8).

Table 8: Long-term mortality after gastric bypass surgery.

	Deaths per 10,000 person years		% change	p value
	Control	Surgery		
Any cause	57.1	37.6	40% decrease	p < 0.001
Coronary artery disease	5.9	2.9	56% decrease	p = 0.006
Diabetes	3.4	0.4	92% decrease	p = 0.005
Cancer	13.3	5.5	60% decrease	p < 0.001
Accidents and suicide	6.4	11.1	58% increase	p = 0.04

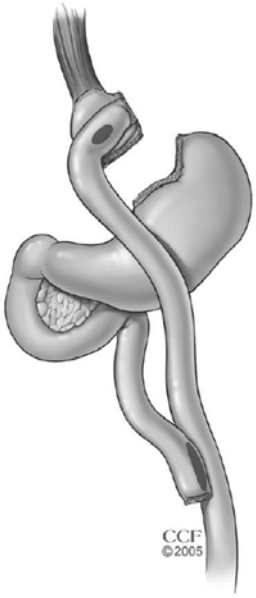
(Adams *et al.* NEJM. 2007; 357(8):753-761)[16]

An explanation for the reduction in mortality owing to cancer is that following post-op weight loss, screening for cancer may be improved leading to early detection and thus an enhanced survival within the gastric bypass group [16]. An approximation of the number of lives saved after 7.1 years of follow-up was 136 per 10 000 gastric bypass procedures [16]. A proposed reason for the increase in long-term mortality due to accidents and suicide in the surgery group is that alcohol dehydrogenase levels are decreased post-op, which leads to reduced acetylation of alcohol and subsequent lowered alcohol tolerance.

Post-operatively there is a clear emerging relationship between extent of weight-loss and co-morbidity improvement. This is however somewhat hindered by the duration of disease before treatment [12]. All bariatric surgical procedures do show successful outcomes and should be utilized as early as

possible within the context of obesity development and especially where alternative treatment regimes have failed.

1.2.2 Gastric Bypass



This procedure combines both restrictive and malabsorptive elements and can be performed using either open or laparoscopic techniques. With regard to the restrictive aspect, a gastric pouch is fashioned, which when filled with food results in satiety [8]. The volume of the pouch is considered imperative in the RYGB procedure [10]; it is reduced to 5% of its normal volume and food consumed bypasses approximately 95% of the stomach, the whole duodenum and 20cm of the upper jejunum [12]. Creation of the pouch involves the use of small staples (3.5 mm) as well as dissection at the lesser curvature of the stomach [10].

A gastrointestinal bypass incorporates the malabsorptive part of the operation and the length of the bypassed intestinal tract directly ascertains the amount of macronutrient malabsorption [8]. The Roux limb is created at different lengths according to the BMI of the individual; in patients that have a BMI < 50 its length is between 75-100cm, however in individuals whose BMI is ≥ 50 length of the limb should be between 100 and 250 cm [10]. The trend pertaining to length of the biliopancreatic limb is that its length is kept similar in all RYGB procedures [10]. With regard to gastrojujenostomy, prevention of stenosis

formation is best achieved when using stapled anastomoses as opposed to suturing [10]. 12-24 months post-op, expansion of the stomach pouch occurs which decreases the impact of the restrictive component of the procedure, subsequently the “rerouting” constituent of the procedure comes into play to avoid weight gain [12]. Ingested food is rapidly transported and bypassing of the peristaltic forces, acid and pepsin of the stomach induces maldigestion [12]. The fact that undigested food-stuffs are literally “dumped” into the small intestine is cause for speculation that mechanoreceptors and chemoreceptors are responsible for post-prandial nimiety and satiety respectively [12].

Patients lose in excess of 45 kg and approximately 35% of initial BMI post-op [8]. The longer-limb procedures are used in patients that fall under the super obese (BMI ≥ 50 kg/m^2) and result in similar weight reductions to those individuals that are less obese and undergo “shorter-limb” bypasses [8]. In the long-term, weight loss is seen to reach stability within 1-2 years and the patients can regain up to 9 kg [8].

The procedures’ complications include a morbidity rate of approximately 5% due to pulmonary emboli, anastomotic leak, bleeding and wound infection [8]. In the long-term, complications such as the dumping syndrome, stomal stenosis, ulcers, staple line disturbance, hernias [8], gastric dilation and nutritional deficiencies [10] are seen. Within the first post-op year bone loss is also seen to occur, however alleviation ensues with the balancing of vitamin D concentration [12]. Life-long vitamin supplementation is needed to combat deficiencies; endoscopic dilatation, conversion or perforation by dilatation are used in stenotic treatment; sclerotherapy is utilised to prevent dilation of the gastrojujenostomy and anti-ulcerative treatment is also ensued in those patients that develop ulcers [10]. Table 9 summarizes the benefits and complications of the gastric bypass.

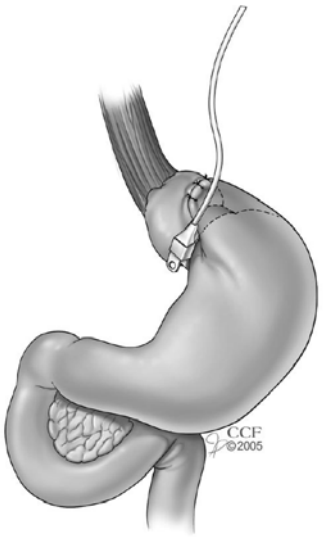
Table 9: Benefits and Possible Complications of the Roux-en-Y Gastric Bypass.

BENEFITS	
• Greatly controls food intake	
• Significantly reduces caloric absorption	
• Major weight loss	
• Completely and easily reversible	
• No plastic "Foreign Body" material	
• Can be performed laparoscopically in most cases	
POSSIBLE COMPLICATIONS	
• Abscess	• Bile reflux
• Leak	• Fistulas
• Atelectasis	• Death
• Wound infection / seroma	• Pulmonary embolus
• Dumping syndrome	• Deep vein thrombosis
• Acute gastric dilatation	• Roux-Y obstruction

(van der Merwe, SA Cardiology & Stroke Summer 2008: 25-35.)[13]

The procedure can be reversed or amended depending on the complication [8], however reversal results in excessive weight regain. Those individuals that do not lose expected amounts of weight post standard gastric bypass can be converted to a very long-limb Roux-en-Y procedure [8]. Adjustment of limb length could result in malnutrition; to combat this, subcutaneous or intramuscular injection of nutritional support causes adaptation of non-bypassed intestine [12].

1.2.3 Laparoscopic Adjustable Gastric Banding



The procedure is undergone by the placement of a band on the upper-most part of the stomach closest to the oesophagus and results in the construction of a small stomach pouch and stoma [8]. An advantage of the banding system is that it is adjustable, and should be adjusted correctly up to 6 times per annum to ensure success [8]. Patients that have optimal band tightening post-op have increased satiety when compared those individuals whose bands are looser; speculations regarding this include the bands effect on mechanoreceptors directly below it and their effect on satiety [12].

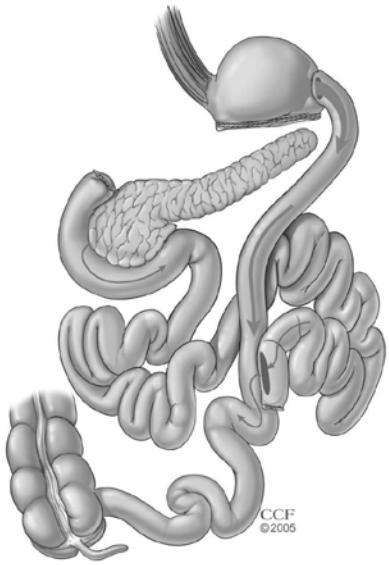
Patients are seen to lose approximately 25% BMI 2 years post-op [8]. As with gastric bypass the morbidity is approximately 5% [8]. Long-term complications include malfunction of the band as well as infection resulting in erosion and necrosis of the stomach [8]. Additionally pouch dilation, closure of the stoma and port-associated problems are evident difficulties post-op [10]. Gastric erosion is preventative of further weight-loss and the band should be removed as soon as possible following identification of this complication [10]. The procedure is completely reversible and patients that do not lose desired amounts of weight have the option of undergoing any of the other procedures available [8]. Table 10 summarizes the benefits and complications of gastric banding.

Table 10: Benefits and possible complications associated with Laparoscopic Adjustable Gastric Banding.

BENEFITS:
• Simple and relatively safe
• No altering of the natural anatomy
• Short recovery period
• Completely and easily reversible
• Major complication rate is slow
• No opening or removal of any part of the stomach or intestines
POSSIBLE COMPLICATIONS
Surgical and short-term
• Splenic injury
• Conversion to open procedure
• Esophageal injury
• Wound infection
• Acid reflux
Long-term
• Reservoir deflation / leak
• Failure to lose weight
• Persistent vomiting
• Band slippage / Erosion

(van der Merwe, SA Cardiology & Stroke Summer 2008: 25-35.)[13]

1.2.4 Biliopancreatic diversion and duodenal switch



Both of these procedures entail a sectional gastrectomy with the creation of a stomach pouch that is somewhat bigger than those in the other procedures (100 – 150 mL) [7]. Circumvention of leaving non-functional intestinal sectors is achieved by division of the intestinal tract into two limbs, termed the enteric and biliopancreatic limbs that are joined together before the valve of the ileum and cecum [7]. The common limb, where these two limbs join, should be above 50 cm, but less than 100 cm in length [10].

35% decrease in BMI is achieved post-operatively, and weight loss in some individuals illustrates total stability without increase once lowest weight is achieved [7]. The procedure decreases the area of intestinal mucosa presented for nutrient absorption and endorses discriminatory malabsorption of fat [12]. A 5% morbidity is observed for the procedures [7]. Complications in the long-term include diarrhoea, nutrient deficiencies and patients that have undergone biliopancreatic diversion may present with dumping [5], as well as hypoalbuminemia [10] and protein malnutrition [12]. Treating continuing diarrhoea and hypoalbuminemia can be done intra-operatively by lengthening the common canal [10]. With regard to reversal, the gastrectomy section of the procedure cannot be reversed, however the intestinal link can be restored [5]. For individuals failing to reach target weight, it has been observed that

creating a shorter common channel can achieve improvement in some patients [7].

1.2.5 The Intragastric Balloon

Intragstric Balloons (IGB) have been used in obesity treatment for 20 years [17]. Air balloons introduced in the 1980s were unsuccessful due to their high complication rate and placement difficulties [17]. The Bioenteric® Intragastric Balloon (BIB®) was first introduced in 1991 and is a smooth, saline-filled (400-700 ml), spherically shaped device that is made from silicone [18] and is the most widely used balloon to treat obesity [17]. It is inserted under conditions of either general anaesthetic or conscious sedation, using endoscopic operating techniques and is placed in an inflated state within the stomach [18]. Its design proposes to result in a decrease of weight due to reduced food intake [18]. The only other air balloon in use is called the Heliosphere®; other balloons that were designed in the 1980s have now been superseded [17].

The intragastric balloon procedure is no longer regarded as a safe and viable option for bariatric surgery (van der Merwe – verbal comm.). Complications encountered when using this technique include balloon rupture, balloon migration, intestinal obstruction, peptic ulceration, stomach rupture and severe oesophagitis [18]. However safety assessment across a pooled group of studies showed that the majority of complications associated with BIB® were minor and an early removal rate of 4.2% was observed [17]; severe complications were uncommon, but there were 26 digestive tract obstructions and 4 gastric perforations, with 2 mortalities out of 3429 patients [17].

Another downfall of the balloon is that it is not adjustable following initial inflation, unlike the LAGB, and that weight loss is minimal [18]. The device also has to be removed at six months to avoid any additional complications and as a result has been abandoned by centres focusing on long-term weight loss [18].

Although the BIB® can induce a weight loss that corresponds to BMI changes of 4.0 – 9.0 kg/m^2, short term results were best in those patients that did not

have BED and fell within the BMI range of 30.0 – 40.0 kg/m^2 [19]. It is however a useful tool to be used in the preparation of super-obese patients for bariatric surgery [18, 19, 20]. Meta-Analysis found that weight loss parameters at removal of the BIB®, across 3608 patients, when assessing effectiveness were 14.7 kg, 12.2% of initial weight, 5.7 kg/m^2 and 32.1% of excess weight [17]. Efficacy studies when comparing BIB® treated patients against a placebo group showed that the balloon group performed better, the differences in weight reduction were 6.7 kg, 1.5% of initial weight, 3.2 kg/m^2 and 17.6% of excess weight [17].

In another study assessing effectiveness, safety and tolerability of the intragastric ballon in combination with a low-calorie diet, the patients' mean weight loss 6 months after balloon insertion corresponded to a mean BMI reduction of 5.23 kg/m^2 [20]. Twelve months after balloon removal more than half (51.6%) of the patients had gained weight with an average weight gain of 9.43 kg [20]. Seven out of the 38 (18.4%) patients assessed presented with complications [20]. Early side effects within 1 week post-op included nausea (71.1%), vomiting (57.9%), and 23.7% of the patients suffered from epigastralgia [20].

A study done in Singapore reported BIB® intolerance as a major problem with 20% of their study group requiring early removal procedures due to nausea and discomfort [18]. The other 80% of the group experienced repeated intolerance episodes during the 6 month post-insertion period, despite oral pharmaceutical treatment [18]. Mean weight loss 6 months following BIB® insertion was 4.4 kg [18]. One year after the procedure the mean weight loss for the study group when compared to pre-op weight was 1.5kg and not significant; with four patients weighing more than they had before the procedure [18]. There was also no noticeable effect on co-morbidities [18]. Weight regain post BIB® removal is a major downfall of this form of treatment.

These results lead the investigators to conclude that the BIB® does not perform favourably in Asian patients and that most of the study subjects regained the weight they had lost following BIB® removal [18]. Most of the

weight lost by these patients while the device was in place was actually an effect of intolerance and not gastric restriction and some patients began weight regain preceding BIB® removal [18]. They state that patients eligible for BIB® should rather have bariatric surgery if possible, as the latter procedure results in improved results in terms of weight loss [18]. The BIB® study conducted here deemed it as a last resort for weight reduction and even in preparation of a patient for bariatric surgery, in that the investigators would much rather use a very low calorie diet, followed by the insertion of a LAGB and avoid BIB® insertion in Asian patients altogether [18]. Other investigators state that the BIB® is described as being useful for short-term weight loss, however its effect in the long-term is questionable [17].

1.2.6 Overall weight loss and mortality

Meta-analytical data shows that mean post-operative weight loss for 10172 patients undergoing a variety of surgical procedures was 61.2% [7]. Weight loss usually reaches its greatest extent after 18-24 months post-op, and most studies report that weight loss in the long-term never increases to beyond 50% of the patients' initial body weight [www.asbs.org]. However when looking at mortality within a 30 day follow-up period across the various surgical procedures a 0.1% mortality for restrictive (banding), 0.5% mortality in gastric bypass and 1.1% mortality in patients undergoing biliopancreatic diversion or duodenal switch procedures [7] was observed. In contrast to this, morbidly obese individuals all with significantly increased mortality risk pre-op show a total alleviation of this risk following successful surgery [www.asbs.org].

1.2.7 Background to Gut Hormones

The centre for appetite control is the paraventricular nucleus of the hypothalamus and all hormonal interactions and signal perceptions occur here [2]. Figure 5 shows how hormones from the gut and adipose tissue are integrated within the hypothalamus to regulate control of an individuals' food intake.

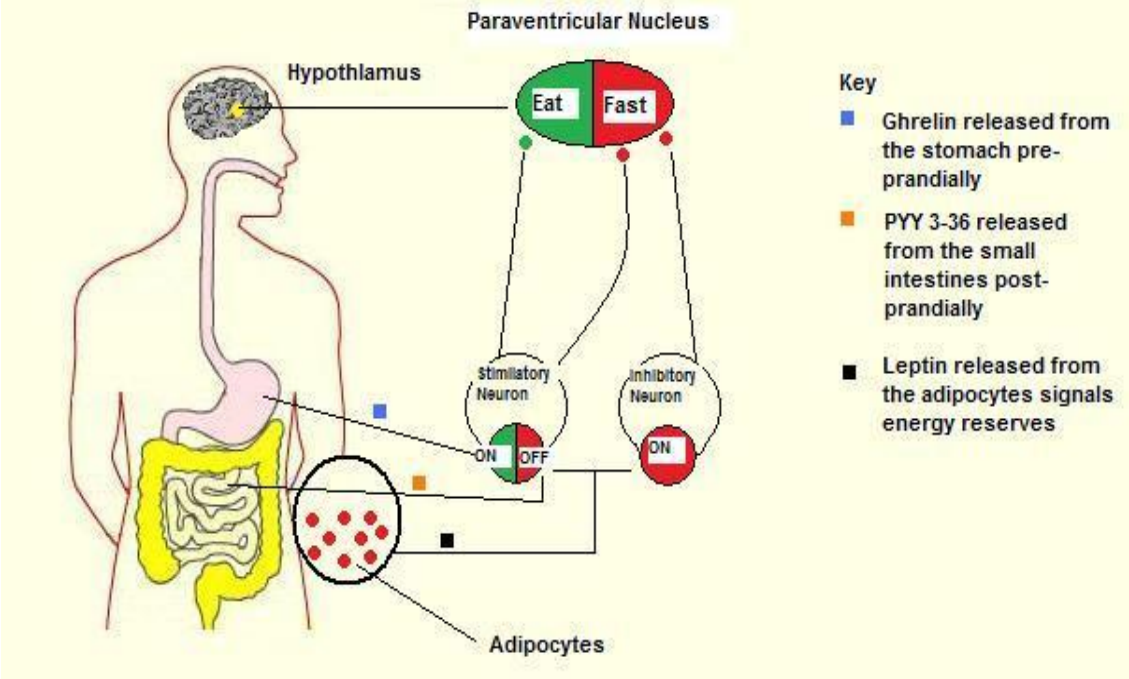


Figure 5: Hormones released from the gut (ghrelin and PYY) and adipose tissue (leptin) exert their appetite regulatory effects at the paraventricular nucleus of the hypothalamus and result in either stimulation or inhibition of food intake (Drawing prepared by Murray Logan).

Ghrelin is a hormone that originates from the stomach pre-prandially and has an orexigenic effect by causing the production of elevated levels of appetite stimulating hormones such as neuropeptide Y (NPY) [2]. Its levels are influenced by nutrients from the gut and not by gastric distension [21]. In murine models ghrelin stimulates gastric emptying and causes a decline in gastric acid secretion [21]. Calorie consumption is hypothesized to be the main regulator of plasma ghrelin [22]. Tying in with this, is the fact that an increase in plasma ghrelin levels is seen in individuals that have lost weight while dieting, which implicates it in the bodies' adaptive response to regain the lost weight [23]. Ghrelin may also have a possible physiological role, the reason being that increases in plasma ghrelin can be controlled by altering the schedule at which an individual eats [22]. A strong positive correlation between plasma ghrelin levels and body weight has been seen [22]. Contradictory to this an

inverse relationship between ghrelin levels and BMI was illustrated, with significantly lower levels in obese individuals; a proposed hypothesis to this is that normal weight individuals' ghrelin profiles promote a positive energy balance [21] whereas obese persons uncontrolled ghrelin concentrations hinder a "healthy" energy balance. Murine studies demonstrate that ghrelin is responsible for both short term orexigenic effects as well as long-term effects on body weight; continuous administration of ghrelin causes an increase in body weight by inducing a decrease in metabolic rate and fat breakdown [23]. Ghrelin also causes the release of Growth Hormone (GH) from the pituitary gland due to its agonistic interactions with the GH secretagogue receptor (GHS-R) within the hypothalamus [22].

Speculations about whether or not ghrelin concentrations within RYGB patients actually have an effect on weight loss have been made because of its inconsistent concentrations within study subjects [2]. Rejection of previous ideas that stipulated that ghrelin was responsible for weight loss because it does not increase in concentration within RYGB patients as it does in individuals on a diet, has recently occurred [2]. Investigation of ghrelin concentrations across population study groups illustrated that within LAGB, RYGB and obese control subjects' ghrelin levels were lower when compared to lean controls [2], which questions the impact of the hormone on post-op appetite regulation. In contrast to these statements le Roux and Bloom state that it is of utmost importance to compare octanoylated ghrelin (active form) to total ghrelin when looking at *in vivo* ghrelin profiles, as conclusions drawn from studies investigating non-active ghrelin levels and the hormones orexigenic effect could be misleading.

Peptide YY (PYY) is produced within the colon post-prandially; it has an anorexic effect by inhibiting the release of NPY [2] and inducing α-Melanocyte Stimulating Hormone (α-MSH) release [16]. Therefore its main function is to cause a sated feeling and to prevent further eating following a meal [2]. It reaches its peak level ± 2 hrs post-prandially and this peak level differs depending on the constitution of the food as well as calorie count [22]. Known physiological effects of the hormone include a decrease in gastric emptying,

slowing down of nutrient transit within the gastrointestinal tract (GIT) (causes longer absorption time resulting in satiety after reduced amount of food intake) and a decline in eating [2]. When investigating PYY levels in non-obese versus obese individuals being fed meals from high to low calorie content, the obese subjects are seen to have depleted serum concentrations [24]. Furthermore it took an increase in caloric content of a meal to raise the plasma PYY concentrations of obese individuals to those seen in normal weight controls [24].

Obese individuals are deficient in PYY and this sheds light onto possible reasons as to why they have reduced satiety post-prandially and the cause of their morbidly obese phenotypes [24]. Administration of PYY to subjects resulted in a marked reduction of eating habits [24]; to add, a 30% reduction in calorie intake was seen when PYY $_{3-36}$ (N-terminally truncated form) was administered to normal weight subjects [22]. Batterham et al reiterated this point by showing a reduction in calorie intake by 30 and 31% within lean and obese individuals 2 hours post administration of PYY $_{3-36}$, as well as having a reducing effect on the extended 24 hr calorie intake on the same subjects. In addition, post-PYY administration ghrelin levels were reduced [25]. Intravenous (IV) administration to morbidly obese individuals is a viable treatment strategy as the anorexigenic effect of PYY is maintained within these individuals [22], however it is somewhat invasive and a hassle for patients. Future research should be directed at ascertaining means by which endogenous PYY concentrations can be increased within the obese individual.

Glucagon-like peptide 1 (GLP-1) is an incretin and thus causes a discharge of insulin following a meal, induces β-cells of the pancreas to increase their productivity and might also have an anorexic effect [2]. Gastric bypass patients show large increases in GLP-1 post-prandially [2]. Pancreatic polypeptide (PP) has an anorexic effect and promotes means of exercise to burn energy [2].

1.2.8 Post-operative Gut-Hormone Profiles

Gastric bypass was initially designed to cause malabsorption and thus weight loss; however adaptation of the gut to the changes made occurs a few weeks post-op [26]. Remarkably weight loss continues following this adaptation due to reduced eating behaviour [26]. In conjunction with this, post-op, patients feel hungry less frequently, eat fewer times per day and of their own accord eat less calorie dense foods [23]. If for some reason the procedure had to be reversed the phenotypic characteristics of obesity, namely weight gain and extreme eating behaviour return [26].

Following gastric bypass the plasma levels of gut hormones undergo significant alterations. Ghrelin, which is secreted from the stomach and has an orexigenic effect, shows decreased concentration post-op [12]. Moreover the expected ghrelin level fluctuations associated with meals and the 24 hour "ghrelin cycle" were absent from gastric bypass patients [23]. The surgery isolates ghrelin producing cells from coming into contact with nutrients traversing the intestine [23], which could be a major factor in ghrelin signalling. The extremely low levels of ghrelin in gastric bypass patients when compared to lean and obese controls, is said to be achieved through a process termed downregulation [23]. An explanation of this is that post-op; individuals' stomachs and part of the duodenum are permanently empty thus inducing a permanent signal for ghrelin production and an eventual suppression of ghrelin production [23]. Thus an accepted hypothesis is that, post gastric bypass, patients lose weight partly because of the subsequent suppression of ghrelin [23]. Pre-op, a resistance to the anorexigenic effects of leptin and insulin is prevalent within the obese population [25]. It is suggested that post gastric bypass leptin and ghrelin quantities may decline, and could consequently lead to a sensitization of leptin pathways [12]; this would ultimately cause an anorexigenic milieu and circumvent the hedonic feeding behaviour associated with elevated *in vivo* ghrelin concentrations.

PYY and GLP-1 have an anorexigenic effect, as well as the latter hormone having a positive effect on insulin levels [12]. Post-op levels of these two

hormones are increased after the consumption of a meal [12]. The levels of these hormones could thus be responsible for post-operative improvements in weight and co-morbid conditions, as well as an increased improvement in appetite regulation [2].

Gastric bypass patients' are also seen to have enhanced insulin profiles, thus indicating that the control of glucose and fat metabolism is greatly improved [2].

When comparing gastric banding and bypass patient cohorts, both groups exhibit similar post-op weight loss, however gut hormone profile alterations are only observed within the latter group [2]. Therefore it is evident that the surgical interventions performed in RYGB are responsible for the alterations in hormone secretion [2].

It is obvious that many additional changes occur post-gastric bypass, other than the necessary anatomical alterations. The hormonal alterations observed are strongly hypothesized to be responsible for the subsequent weight loss, long-term sustained weight loss, improvement of the metabolic syndrome and co-morbid diseases [21]. A long-term goal would be to investigate the happenings following surgical intervention with regard to increasing/decreasing hormone levels and eventually eliminate the need for invasive surgery by mimicking these outcomes through pharmacological means [26].

1.2.9 Patient Care

The Diabetes Surgery Summit held in Rome, Italy in March 2007 [27] established international consensus guidelines on the clinical use of surgery for the treatment of diabetes and recognized the requirement for research in the field of interventional diabetology.

The NIH Consensus Conference came to an important conclusion which states that "patients judged by experienced clinicians to have a low probability of success with non-surgical measures, as demonstrated, for example, by

failure in established weight control programs or reluctance by the patient to enter such a program, may be considered for surgical treatment" [www.asbs.org]. Additionally, it is considered conventional clinical practice that the patient should have made attempts to lose weight by dietary intervention pre-op [www.asbs.org]. Variables including gender, race, as well as the physical and constitutional characteristics of an individual could affect outcomes and which operative procedure is chosen [7].

Pre-op the patient must be advised as to how they will have to adapt their lifestyle with regard to diet and physical activity as well as clinical outcomes and recovery processes post-op [7]. They should also be assessed on life-history, physical and laboratory aspects [7]. Laboratory testing should comprise of a full blood count, liver, kidney, coagulation and thyroid factors, thyroid stimulating test, lipid profile evaluation, an oral glucose screening test (in diabetic patients) and an analysis of arterial blood gas [10]. Figure 7 shows the list of biochemical tests that are performed on a patient assessed at a Bariatric Centre of Excellence (BCE). They also undergo an abdominal sonar and DEXA scan to assess body composition.

BGV*	☐	Fasting Glucose
BLIPOP*	☐	Fasting Lipogram
BUE*	☐	U & E
BCMP*	☐	Ca + Mg + Phos
BLFT1*	☐	Liver Functions
BPROBNP	☐	Pro-BNP
BFE	☐	Ferritin + Iron Studies
BALB*	☐	Albumin
BVIT	☐	Vit B12
BRFOL	☐	RBC Folate
CVITD	☐	25 (OH) Vit D
BHBA	☐	HbA1C
BCRP	☐	CRP
HFBC	☐	FBC
BURAC	☐	Urate (Uric Acid)

Figure 6: Biochemical test sheet showing which biochemical parameters are assessed following patient consultation at the Bariatric Centre of Excellence (BCE), Unitas hospital, Pretoria.

There should be a specialised team in place that are available whenever necessary, these individuals include the surgeon, nurse, dietician and where needed, other specialists [7]. Surgeon competency requires completion of 75-100 cases; high-throughput bariatric surgery centres have remarkably better post-op outcomes when compared to low-throughput centres when looking at all parameters including hospitalisation length, complications, costs and mortality rates [12]. This implies that experience and extreme specialization is needed before “neo-surgeons” are competent enough to perform these surgical procedures.

Additionally the psychological health of the patient is associated with post-operative success and individuals’ pre-op motivation shows a direct correlation to beneficial weight loss following gastric bypass [10]. Therefore, looking at the patients’ mental health is also imperative in the circumvention of any unfavourable post-op results [7]. Psychological studies have shown that there are two specific recommendations pertaining to psychological health pre-op illustrating association to post-op outcome; the first being that the more

concerned the individuals are regarding their weight the higher the probability of weight-loss and secondly psychiatric disturbance affects post-op outcome negatively [www.asbs.org]. Other contraindications include alcohol or drug abuse. Identification of a high risk patient can be done by identifying one or more of the points made in Table 12. A summary of the contra-indications to bariatric surgery are presented in Table 13.

Table 11: Criteria used to identify a high risk patient

• **BMI > 50**
• **Age > 45**
• **Male gender**
• **Life-threatening co-morbidity, esp. DM and HT**
• **ASA Class III or IV**
• **Obstructive sleep apnea**
• **Risk factors for PE/DVT**

(Ballantyne GH *et al.* Obes Surg 2004;14:1042-1050.) [28]

(Dallal RM *et al.* Obes Surg 2004;14:47-53.) [29]

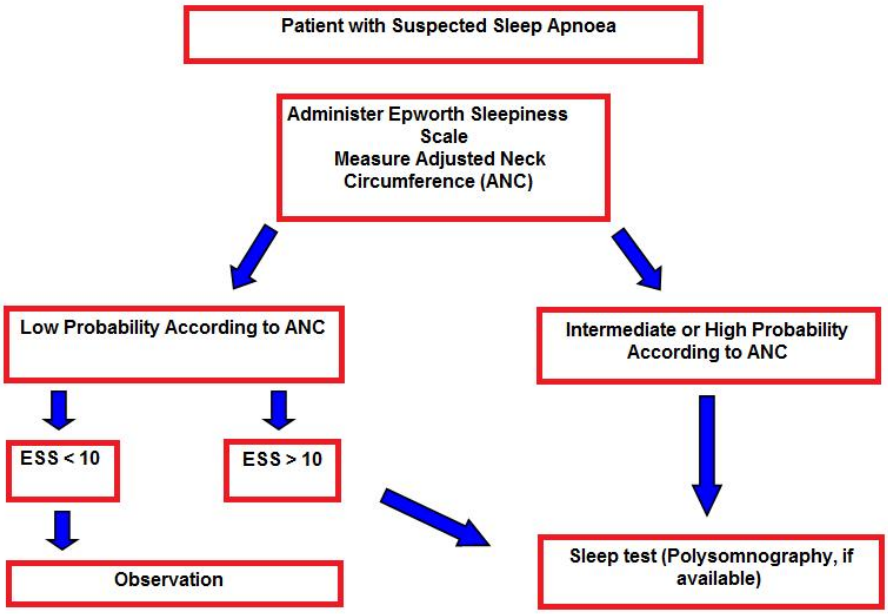
(Cooney RN *et al.* Obes Surg 2003;13: 29-36.) [30]

Table 12: Contraindications to Bariatric Surgery

• **Severe cognitive dysfunction and psychoses**
• **Advanced liver disease with portal hypertension**
• **Unstable CAD and uncontrolled severe OSA**
• **CA with less than 5 year survival**
• **Smoking with severe hyperactive pathways**

(Mayo Clinic Proceedings Oct 2006 suppl.Vol 81 No. 10) [31]

Figure 8 illustrates the method of investigation if a patient is suspected of having sleep apnoea-hypopnea syndrome.



(Sousa, AGP *et al.* Obesity and Sleep apnea. Obesity Reviews (9) No 4. 2008, 340-354.) [32]

Figure 7: Approach to an obese patient with suspected sleep apnoea-hypopnoea syndrome. (Drawing prepared by Murray Logan).

Anaesthesiology of the obese patient is of utmost importance and the anaesthesiologist must be familiar with all complications that can occur intraoperatively [7].

Post-op the patients should have at least 3 follow-up visits in the first year and should be assessed for the remainder of their lives [7]. Follow-up also includes consultation with specialists such as the surgeon, dietician, psychiatrist and psychologist as well as others where deemed necessary [10]. Diet supplementation due to micronutrient deficiencies (Table 14), physical activity and other lifestyle changes should also be put into place [7]. Table 15 shows the recommendations for micronutrient deficiencies post bariatric surgery.

Table 13: Micronutrient Consequences of Bariatric Surgery.

Deficiency of Vitamins	Deficiency of Minerals
• Thiamine • Vitamin B12 • Folic Acid • Vitamin D	• Iron • Calcium

(Reproduced with permission from Prof. Tessa van der Merwe.)

Table 14: Treatment Recommendations for Micronutrient Deficiencies.

Deficiency	Treatment
Thiamine	100 mg IV or IM for 7–14 days followed by 10 mg PO daily for 1 – 3 months
Vitamin B12	Crystalline vitamin B12 : 250-500 µg p.o. b.d. or monthly 100-200 mcg IM injections for all patients for 3 months followed by 3-6 months IMI injection (Max. 1000 µmg)
Folate	5g/d until deficiency corrected (as folic acid), then general multivitamin supplement with a dose of 1-2g/d
Vitamin D	Vitamin D tablets 400 IU b.d (total daily dose 800 IU)

Iron	150-300mg elemental iron in 3 divided doses x 4 months of supplemental ferrous iron (as sulfate, gluconate, or fumarate) Parenteral IV iron infusion (when intolerant to PO supplementation)
Calcium	1000 mg elemental calcium (Calcium citrate)

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With regard to post-op assessment of outcome, weight-loss and the preservation thereof, nutritional condition, co-morbidity status and quality of life [10] are essential parameters that need to be focused on. Weight-loss results from the SOS study show that maximal weight-loss post-op occurs after 1-2 years; the figures for each surgical subgroup are as follows: gastric bypass, 32 ± 8%; banding, 20 ± 10% [15]. Weight was regained in all surgical subgroups, but then reached a plateau 8-10 years post-op; final measurements of weight-loss after 15 years of follow-up for each group was 27 ± 12%, 18 ± 11% and 13 ± 14% respectively [15]. When looking at the control group there was a mere ± 2% in weight alteration during the time of the study [15]. Post-operative failure can be described as reoccurrence of co-morbidities and weight-loss; this is seen in approximately 10% of individuals admitted to international Bariatric Centres of Excellence. Careful consideration should be placed on whether or not a procedure is deemed a failure or not, there is a common delusion that if a patients' post-op sustained BMI is above 30 kg/m^2 the surgery has failed [12]. Evidence shows that sustained weight loss of the bariatric surgery patient correlates with an increased decline in morbidity and mortality when compared to individuals of the population of the same BMI having not undergone surgery [12]. Post-op weight-loss failure is the most challenging complication to treat, because re-

operation difficulties are higher than those of primary procedures [12]. Re-operative guidelines have not been developed, however emphasis is placed on determining the probable cause of failure and basing salvation procedures on this [12].

Mortality associated with bariatric surgery when compared to control group patients illustrates once again that surgical approaches lead to a prolongation of life (Table 15)

Table 15: Mortality Associated With Bariatric Surgery: Comparison Of Data From Two Studies.

Variable	Sjöström et al		Adams et al	
	Surgery group	Control group	Surgery group	Control group
Mean follow-up (yr)	10.9		7.1	
No of subjects	2010	2037	7925	7925
Female sex (%)	82	82	84	84
Mean age (yr)	46.1	47.4	39.5	39.3
Mean BMI	41.8	40.9	45.3	46.7
Deaths				
Total number	**101**	**129**	**213**	**321**
Early occurrence (%)	0.25	0.10	0.53	0.52

(Sjostrom L, Narbo K *et al.* NEJM 2007; 357 (8);748)[15]

(Adams *et al.* NEJM. 2007; 357(8):753-761)[16]

Note: Definition of early occurrence of death after surgery:

- Sjöström *et al.*: within the first 90 days
- Adams *et al.*: within 1 year

Women who undergo weight-loss procedures are advised to adhere to strict birth control during the post-op period of prompt weight-loss [www.asbs.org]. The reason being that malnutrition could seriously hinder natural development of the foetus [www.asbs.org]. Post-op, fertility is seen to be elevated [www.asbs.org], which again stresses the importance of birth control in these

women. Maternal obesity during gestation rapidly increases the advancement of the condition within the child post-natally [12]. Investigation of a total of 113 children who were born to mothers that had undergone bariatric surgery showed a decline in obesity development [12]. If a gastric bypass patient conceives following the procedure, it is advised that they are closely monitored by all specialists involved [www.asbs.org], to ensure normal foetal growth and development.

In the LAGB approach, band adjustment post-op is an essential part of follow-up [10]. Initially the band should be filled after 2-8 weeks with an injection of 1-1.5ml of saline [10]. Following the initial filling, adjustment of the band should be done on an individual basis based on assessment of gastric complication, satiety, weight-loss and eating activities [10].

1.2.10 Post-operative co-morbidity improvement

Generally amendment of all co-morbid conditions is seen post-operatively. However this is somewhat reliant on the type of procedure used, and has lead to the conclusion that restrictive procedures do not have the “resilience” with regard to co-morbidity improvement as compared to the malabsorptive techniques [12]. Table 16 compares co-morbidity resolution across the three major operative procedures currently used.

Table 16: Mean percentages of patients with post-op resolution of co-morbidities.

Major Comorbidity	Operations		
	Adjustable Gastric Band	Gastric Bypass	Biliopancreatic Bypass
Diabetes	**48** (9; 29 – 67)	**84** (26; 77 – 90)	**99** (9; 87 – 100)
Dyslipidaemia	**59** (6; 82 – 89)	**97** (6; 94 – 100)	**99** (3; 98 – 100)
Hypertension	**43** (12; 30 – 56)	**68** (20; 58 – 77)	**83** (7; 73 – 94)
Sleep apnoea	**95** (5; 89 – 100)	**80** (13; 68 – 92)	**92** (6; 82 – 100)

(Buchwald *et al. JAMA*. 2004;292(14):1724-1737.)[7]

* **mean percentage of patients** (no. of studies; 95% CI)

Improvement of type 2 diabetes as well as impaired glucose tolerance is seen across all surgical procedures, however significant differences were seen with regard to post-op diabetes outcome across the different classes of operative methods [7] (Table 17). Similar results were seen with regard to improvement or resolution of the other three co-morbidities that were focused on in this study [7].

Results of a Medline search for diabetes analysis post bariatric surgery looked at 621 studies with a total of 135 246 patients included and found:

- Diabetics comprised 22.3% of population
- 86% Resolution of diabetes in first 2 years after surgery
- 62% remained free of diabetes more than 2 years after surgery
- Weight loss overall was 38.4kg or 56.0% of excess body weight loss

Table 17: Resolution of diabetes in bariatric surgery.

Surgery	Time to resolution	Comments
LAGB	2 years	• Weight loss dominant predictor of resolution • Comparison of LAGB & controlled pharmacotherapy & lifestyle changes shows 73% vs. 13% resolution respectively*
RYGB & BPD	Days to weeks before substantial weight loss	• 30% of patients require no medical therapy at time of discharge (3 days post-op) • Other 70% discontinues pharmacotherapy within 1 month

(*Dixon *et al.* JAMA 2008) [33]

When comparing the mortality rate of patients that were treated pharmacologically for diabetes to those individuals who underwent gastric bypass, a three-fold increase in the former groups' mortality rate was observed [www.asbs.org]. Demonstration that there is a direct positive correlation in blood glucose control and quantity of weight loss has been achieved contemporarily [34]. These investigators suggest that surgical intervention should be considered at earlier treatment stages of diabetes within obese individuals [34].

Within the morbidly obese patient effective weight loss is achieved following bariatric surgery [7]. When looking at co-morbidities, diabetes hyperlipidaemia, hypertension and obstructive sleep apnoea are either completely resolved or show marked improvement post-operatively [7].

When focusing on each individual co-morbidity, approximately 85% of patients illustrated improvement of diabetes following surgical intervention. Resolution of diabetes, on occasion, occurs within a few days post-op before subsequent

weight loss [7] (Table 17). In compliance with this statement, RYGB and BPD are said to put in motion anti-diabetic mechanisms that are independent of weight-loss [33] (Table 18). Conversely the diminution of diabetes was accredited to weight loss following LAGB [33] (Table 17). These statements are re-enforced by the observation that the prevalence of diabetes resolution is dependent on surgical procedure, with the malabsorptive (biliopancreatic diversion or duodenal switch) and combination malabsorptive/restrictive gastric bypass producing a significantly increased improvement when compared to restrictive gastric band insertion [7]. In the long-term, bariatric surgery causes a reduction in mortality from type 2 diabetes; in RYGB patients there was a 92% decrease of diabetes-associated deaths [33].

Table 18: Potential mechanisms of action for diabetes resolution after RYGP/BPD.

• Increased insulin sensitivity
• Enhanced β-cell activity/function
• Enhanced nutrient stimulatory secretion of glucagon-like peptide by distal small bowel
• Compromised ghrelin production (including decrease in hedonic appetite stimulation at level of nucleus accumbence and amygdala)
• Effect of relative and rapid calorie withdrawal

The reason posed for the observed difference in co-morbidity improvement within the differing operative groups is that certain operative procedures have a marked effect on gut hormone profiles and thus effect the time to and extent of diabetes resolution [7]. The RYGB procedure for example, elevates insulin sensitivity and also could enhance β-cell function [33] (Table 18).

The means by which diabetes resolution is achieved post-malabsorptive/restrictive gastric bypass are speculated to be an increase of glucagon-like peptide 1 (GLP-1) secretion, a decrease of ghrelin generation

as well as the significance of eliminating the upper section of the GIT [33] (Table 18).

In a study comparing the improvement of type 2 diabetes in patients that were randomly assigned to two treatment groups, the results showed that 13% of individuals that underwent medical/lifestyle modification had complete diminution of the disease as opposed to 73% remission of diabetes in patients that received LAGB as well as medical/lifestyle modification [33] (Table 17). The latter group also encountered greater declines in blood glucose, Haemoglobin $A1_c$ ($HbA1_c$), insulin resistance, anti-diabetic medication usage and numerous aspects of the metabolic syndrome [33].

Because of the success of operative strategies on the resolution of diabetes as well as evidence that non-surgical approaches infrequently lead to significant and long-term reductions in weight, surgical approaches are being seriously considered in the treatment of diabetes [33]. However malabsorptive/restrictive surgical procedures do present with increased risk following surgery, thus more research is needed before or if they become a standard strategy in diabetes treatment [33]. Ultimately research endeavours are underway to reveal post-op alterations that facilitate recovery of diabetes, with an end goal of creating a pharmacological agent that mimics the effects of surgery, but eradicates the risk surrounding surgery [33].

Improvement of hyperlipidaemia was also more prevalent following malabsorptive surgery [7]. In contrast to the improvement of these two conditions, resolution of hypertension was seen to be independent of the surgical procedure performed [7]. With sustained weight loss, enhancement of cardiac function which includes a reduction in ventricular wall thickness as well as a decline in chamber size is prevalent [www.asbs.org]. In contrast to these statements blood pressure was observed to decline within 5 years post-op, but to then undergo subsequent increase within 10 years [12]. This was speculated to be due to weight regain within patients that underwent restrictive operations [12].

With regard to improvements in obstructive sleep apnoea the results were prolific, in that 80% or more of the patients showed recuperation [7]. Physiologically, patients demonstrate beneficial conversion of blood contents with regard to improvement in respiratory function, which in turn have positive effects on neuronal control of respiration. These physiological alterations are due to an increase in diaphragmatic movement; this is caused by a decrease in intra-abdominal pressure post successful bariatric surgery [7].

Binge eating disorder or symptoms thereof are extremely prevalent within the obese patient population, whether or not these difficulties are relieved post-op is essentially dependant on the type of surgical procedure employed [10]. Table 19 shows what effect the RYGB has on the Quality Of Life (QOL) of a patient post-op.

Table 19: Roux-en-Y Laparoscopic gastric bypass Change in Quality of Life (QOL).

Greatly Improved	58%
Improved	37%
No Change	5%
Diminished	0%
Greatly Diminished	0%

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1.2.11 Pro-BNP as a Clinical Marker for Cardiac Disease

Risk scoring systems are becoming increasingly popular within the pre-op work-up of obese patients, with the aim of:

1) Simplifying the calculation of pre-operative risk
2) Dealing with health economic pitfalls (medical aid remuneration)

Clinically it would be beneficial to identify a biochemical test that is accurate enough to indicate early cardiac failure within the obese patient. Although the best methods are in the form of an angiogram or Electrocardiogram (ECG), a number of studies have identified B-type natriuretic peptide (BNP) as an accurate indicator of left ventricle (LV) wall tension and thus cardiac failure. BNP is released from the LV as a result of an increase in myocardial wall tension. The amino-terminal portion of pro-BNP (NT-pro-BNP) which is released in equal amounts to BNP, but has a longer half-life, could be used to exclude systolic LV-dysfunction in high-risk cardiac patients because of its good negative predictive value [35]. Although values of NT-pro-BNP of <150pg/ml virtually exclude left ventricle failure, it has been seen that NT-pro-BNP values may be decreased by obesity [35]. Since heart disease is highly prevalent in obesity, it would be beneficial to determine whether NT-pro-BNP can, despite its reduction in obesity, be used to exclude cardiac failure risk in the obese patient population. Validation of the use of Pro-BNP testing in pre-operative obese patient work-ups will be useful as it is a more streamlined strategy and is far simpler than sonar, ECG and stress ECG.

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Literature Review – Part 2

Chapter 2: The Melanocortin 4 Receptor (MC4R) and Obesity

2.1 Introduction and Classification of the MC4R

Melanocortins play a role in pigmentation, inflammation, energy homeostasis [1] and sexual function [2]. The MC4R is involved in both energy homeostasis and sexual function, particularly erectile function [2]. The receptor plays a vital role in the leptin pathway. Leptin is encoded by the human *ob* gene, and is secreted by adipocytes. It binds to the leptin receptor which is located within the hypothalamus [3].

MC4Rs are expressed in a number of locations within the central nervous system (CNS). They are however expressed in a concentrated manner within the paraventricular nucleus of the hypothalamus (PVH) [4]. MC4R is a part of the family A super-family of G-protein coupled receptors (GPCRs) [6]. It consists of a single polypeptide that contains seven α-helical transmembrane domains, an extracellular N-terminus, three extracellular loops, three intracellular loops and an intracellular C-terminus [5] (Figure 1).

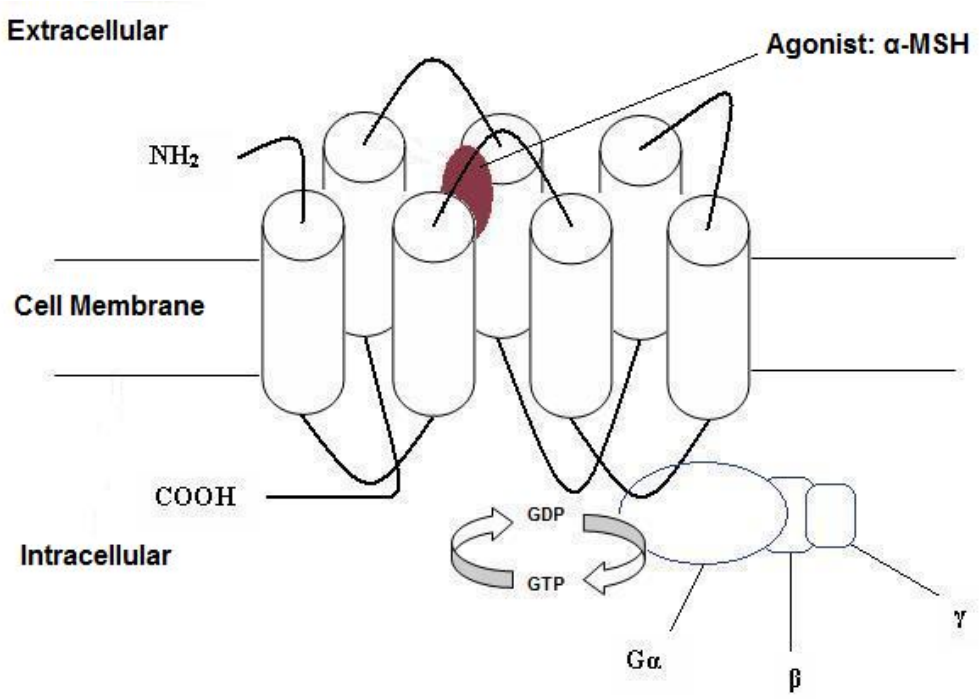

Figure 1: The structure of MC4R showing the seven α-helical transmembrane domains, the three extracellular and three intracellular loops, extracellular N-terminus, intracellular C-terminus and the coupling of the receptor to a heterotrimeric G-protein. (Drawing prepared by Murray Logan).

The MC4R polypeptide chain consists of 332 amino acids which are encoded by a gene that contains a single exon and is located on chromosome 18q22 [7]. Its primary function is to regulate food intake following the binding of α-MSH (α-melanocyte stimulating hormone) which is an anorexigenic ligand. α-MSH is an MC4R agonist and causes the production of a satiety signal through the activation of the cyclic Adenosine Monophosphate (cAMP) second messenger system [7]. (Figure 2)

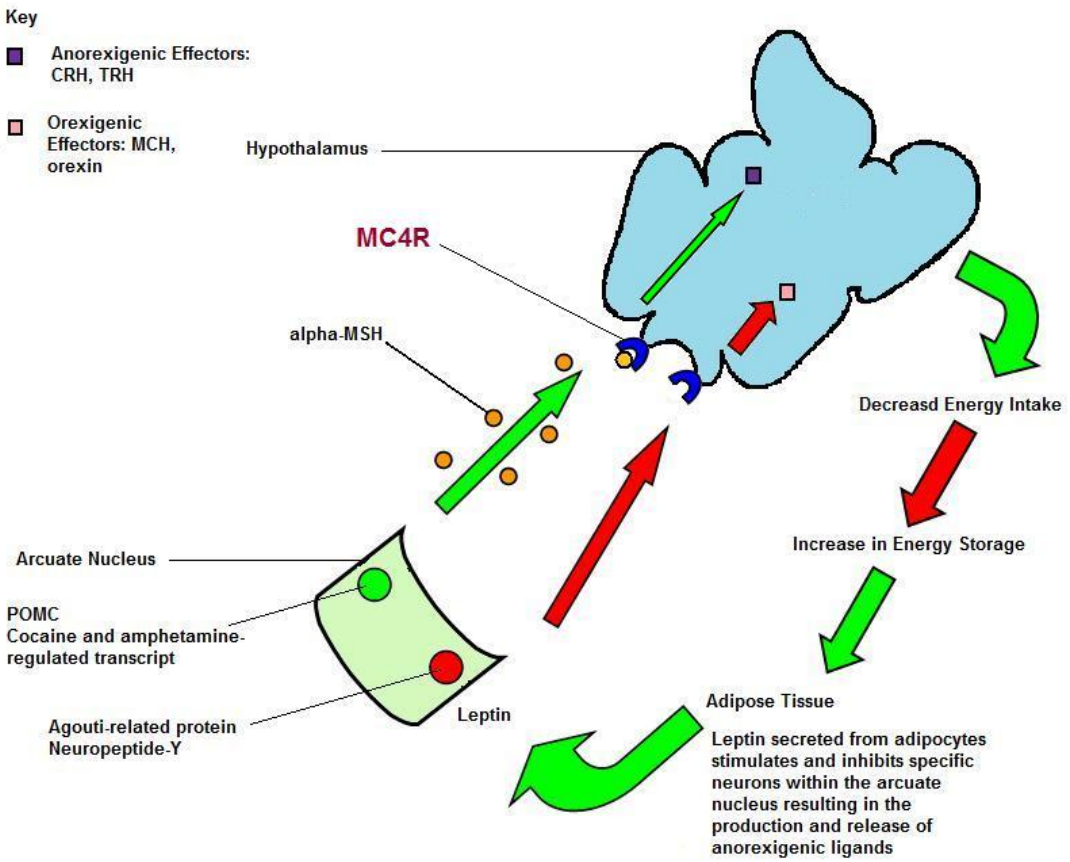

Figure 2: Events occurring within the arcuate nucleus and hypothalamus involving the MC4 Receptor (purple text) and its role in energy homeostasis and satiety signalling. Green arrows represent promotion and red arrows represent inhibition of satiety. The downstream anorexigenic effectors (purple square) of α-MSH are CRH and TRH; the downstream orexigenic effectors (pink square) of AGRP (MC4R antagonist and inverse agonist) and Neuropeptide-Y are MCH and orexin. (Drawing prepared by Murray Logan).

α-MSH is produced by a subset of neurons within the arcuate nucleus of the hypothalamus. It is derived from a precursor protein known as POMC, which is post-translationally processed by pro-hormone convertases into melanocortins -3 and -4, α-, β-, γ-MSH and adrenocorticotropin (ACTH) (Figure 3). Prohormone pro-opiomelanocortin (POMC) is processed in a tissue specific manner and can thus be used by many cell types to perform a wide variety of physiological functions [2].

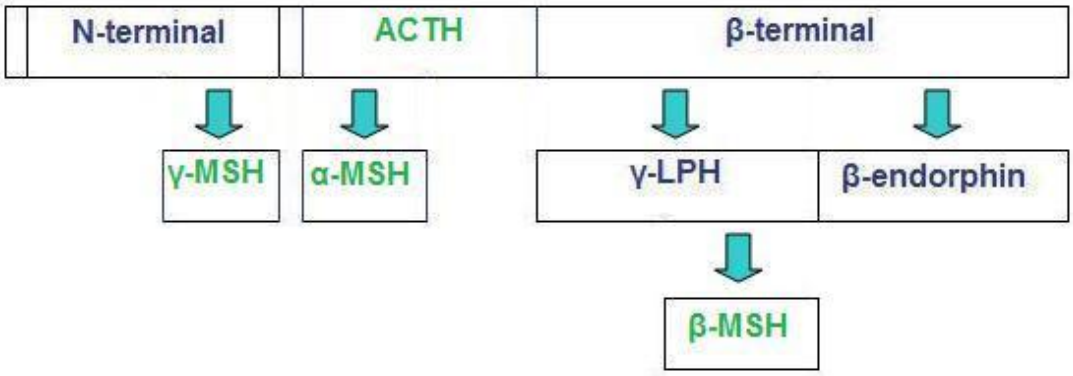

Figure 3: The POMC gene product and its post-translational products produced by pro-hormone convertase processing. The melanocortins: α, β, γ-MSH and ACTH (green text) are produced following processing. (Drawing prepared by Murray Logan).

Agouti-related protein (AGRP) is an MC4R antagonist, and when bound produces an orexigenic signal [7]. AGRP also acts as an inverse agonist; this term can be defined as a ligand that blocks the action of an agonist as well as suppressing the constitutive action of the receptor [5]. AGRP thus inhibits the constitutive signal produced by MC4R [7]. Regions of the MC4R that are responsible for the inverse agonistic effect of AGRP on constitutive cAMP signalling are the N-terminus, transmembrane domain residues D90 and D298 and the distal region of the MC4R C terminus [5]. Long-term [7] or basal regulation [5] of energy homeostasis is suggested to be under the control of the constitutive signal produced by the N-terminal region of the receptor.

Ligand binding occurs within the opening of the transmembrane domain and confers receptor conformation alteration and activation [6]. A model explaining pharmacological interactions of the MC4 receptor and its associated ligands illustrates that subunits form oligomers; however the individual subunits act as mutually exclusive “tandemly operated” conformations [9]. One of the subunits will lock in the ligand, and the other will attain a more loosely bound conformation between it and the ligand [9], these interactions are of utmost importance when investigating potential pharmaceutical agents targeted at the MC4R.

2.2 Mutational prevalence and inheritance mechanisms

Obesity is most commonly polygenic. However, monogenic forms of obesity do exist, and affected genes described thus far include leptin, the leptin receptor, POMC, pro-hormone convertase-1 and melanocortin receptor (MCR) gene defects [3]. 40-70% of an individuals' body weight is genetically determined, the remainder by the type, quality and quantity of food that is consumed [10]. Investigation of monogenic obesity disorders and disregarding their rarity is and important step in the destigmatization of the disease in declaring that it is not solely determined by an individuals' behaviour, but that there is undoubtedly a biological basis for obesity development [6].

MC4R deficiency is one of the most common human monogenic disorders [11]. MC4R mutations have a population prevalence of at least 1 in 2000 (0.05%)[11, 12], are found in 0.5%-1% of obese adults [11, 12] and are accountable for 6% of all severe cases of the disease starting in childhood [5, 11, 12, 13]. This low percentage indicates that the mutations have a rather low epidemiological significance; however with regard to the individual they should be considered as highly significant [14]. The frequency of MC4R mutations is dependent on the ethnicity of the study group [15]. Carriers of MC4R mutations will pass these on to their offspring with an 82% frequency and individuals that carry mutations that affect function have a 4.5 fold increased risk of developing obesity as opposed to non-carriers [15]. MC4R deficiency is not associated with a modification in thyroid or reproductive function [15].

A hypothesis posed is that mutations within the MC4 receptor gene are responsible for an "early onset, highly penetrant" form of obesity, and other genes or genotype and environment (G + E) interactions are responsible for the later onset forms of the disease [16]. However this early onset hypothesis has been questioned and it appears that it is not specific to MC4 mutation carriers [16]. In a pedigree analysis, family members of the proband were obese; however they were not carriers of MC4R mutations [14]. In an epidemiological study group of 4068 subjects, heterozygous, nonsynonymous MC4 mutations that caused the receptor to have impaired function did not

confer obesity on the carriers [17]. In familial studies, relatives of probands (heterozygous carriers; morbidly obese) are also obese, but they are homozygous wild-type [17, 18]. Therefore it is evident that other genetic and/or environmental factors must contribute to obesity within these individuals. At the other end of the scale, there have been cases where a family member is the carrier of the same mutation as the proband, but is only moderately overweight or sometimes even lean [17]. This again illustrates the diverse pattern of expressivity observed with mutations within this receptor, as well as the fact that loss of function mutations within the MC4R gene do not necessarily lead to obesity [17].

This being said it cannot be excluded that MC4 polymorphisms might have a moderate effect on the development of the obesity, if not fully responsible for it, and could predispose the individual to obesity and the development thereof when in conjunction with another environmental or genetic "trigger" [17]. These observations highlight the importance of G (including MC4R deficiency) + E interactions in the pathogenesis of obesity.

The accepted mode of inheritance of MC4R mutations is co-dominance with modulation of penetrance and expressivity [6]. This inheritance pattern explains why homozygous carriers are known to be more obese than heterozygotes [20]. Two factors have been proposed to explain discrepancies in MC4R mutation phenotypic penetrance. Firstly differing ethnic backgrounds which illustrate varying penetrance and secondly, whether or not the mutation leads to a receptor-function defect [20]. In conjunction with this the severity of the phenotype of mutant MC4R carriers was more extreme in those individuals that had complete loss of receptor function when compared to those with partial loss of function. This observation was consistent when observing both homo- and heterozygotes [19]. It was also proposed that discrepancies in the amount of functional MC4R have a direct causative effect on the control of body weight in humans [19]. Transmission of mutations leading to either loss of function or reduced function occurred at a rate of 81.8%. In addition, all mutations were transmitted to offspring in favour of the wild-type alleles [15].

Arguments for an autosomal dominant segregation pattern have also been proposed [15]. It would seem however that this theory is questionable as observed phenotypes do not confirm its assumptions.

Dominant-negative effects of mutant receptors are rare. However, in one case heterodimerization of a mutant (Asp90Asn) and wild-type receptor was observed, which resulted in a receptor that was fully expressed on the cell surface but was unable to signal [15]. The reason for this is that the mutated receptor prevents the normal receptor from acheiving its agonist-activated state upon binding of α-MSH [9]. Mutations that result in intracellular retention of receptors could also have a dominant negative effect, in that disruption of cellular function or even cellular degeneration could occur because of the build-up of mutant proteins within the cell [15].

An argument against the dominant negative hypothesis was put forward following transfection studies of mutant and wild-type receptors in the same cell. This showed that there was no difference in signal transduction by mutant receptors. Instead, haploinsufficiency [9, 21] was proposed as the most likely explanation for the clinical phenotype of heterozygous mutation carriers. This can be seen in heterozygotic MC4R knock-out (KO) mice as they present with a phenotype that falls between the two extremes of homozygotic mutants (morbidly obese) and wild-type individuals [15].

A mutation that causes an amino acid change of N26S found to be homozygous in some probands, illustrated a recessive pattern of inheritance, as heterozygous relatives were not obese [22]. Certain mutations within the MC4R gene produce a receptor that is indistinguishable from the wild-type receptor with regard to their signalling properties [22]; this obviously deems the mutations non-pathogenic. Because of the variability of expression of obesity in MC4R mutation carriers it has been suggested that MC4R may interact with other genetically produced elements in its control of appetite and body weight, as well as having different effects under varying environmental conditions [13].

A direct correlation has been observed between BMI in obese individuals and MC4R mutations [8]. Individuals that harbour mutations that totally abolish MC4R function have a higher BMI than those that have mutations that retain some of the functional aspects of the receptor [8]. Consequently, persons that are homozygotic for MC4R mutations have higher BMI's than those that are heterozygotic for the same mutation [8].

Some mutant MC4R's are able to respond to ligand (NDP-MSH) binding with a subsequent increase in cAMP production, which shows that these mutants are able to undergo G-protein coupling and effector activation [20]. In contrast to the severe early onset obesity seen in children with MC4R mutations, adults seem to have a BMI that is more middle of the range than at the upper end of the scale. A compelling study investigated this phenomenon using a pedigree analysis, and found that the proband, who was a fourteen year old boy who had severe early onset obesity, had received the mutation in the MC4R gene region from his father [23]. The father, who had a BMI in the range of 30-40 kg/m^2, reported having had severe obesity during his childhood, but as he aged he did not progress to a morbidly obese stage [23]. This observation illustrates that obesity associated with polymorphisms within the MC4R gene region is most prominent in younger individuals in the form of severe early onset obesity; however as these individuals age, it becomes less pronounced [23]. These findings were confirmed by Hinney and colleagues when looking at MC4R heterozygotic mutation prevalence with impaired receptor function in a group of German children and adolescents. They pose similar reasons for their observations as Valli-Jaakola *et al.*, as well as saying that other causes that could be either genetic or environmental come into play as individuals' age and thus "drown out" the MC4R effect within the obese population [17]. To reiterate the point, Farooqi and colleagues reported that the intensity of hyperphagia and hyperinsulinemia decrease with age in obese children carrying MC4R mutations. These observations suggests that when searching for mutations within this gene in an adult population group, they may be more prevalent within patients that have a BMI that is more "moderate" and not in the so-called morbidly obese end of the spectrum [23] or that their prevalence might be higher within the extremely obese and thus they are "missed" when

performing population based studies [17]. These ideas need to be tested and confirmed or disproven.

2.3 Phenotypic Classification

Obesity is the result a positive energy balance, meaning energy intake exceeds energy expenditure and this leads to the storage of surplus calories as fat [15]. Human MC4R mutation carriers do not have an impairment in the expenditure of energy; therefore obesity in these individuals is most likely due to their hyperphagic state [15]. The symptoms of MC4R mutation carriers include an increase in fat mass, linear growth and lean mass, extensive hyperinsulinaemia [1], increase in bone mineral density (BMD) [14], hyperphagia in early childhood and possibly binge eating disorder (BED) [24]. They also present with an elevated prevalence of the metabolic syndrome which includes an increased peripheral fat mass ratio, more individuals with type 2 diabetes, dyslipidemia and hypertension at escalated levels [25]. There is however no effect on reproductive function or fertility. It has been stated that a specific clinical phenotype cannot be used to predict MC4R mutation presence within severely obese individuals [16], which makes it challenging to relate clinical outcome with mutation identification and prevalence. Farooqi and colleagues observed that increased linear growth was especially prominent within paediatric cases that harboured homozygous N62S mutations. Leptin may have an anti-osteogenic function that is controlled by the MC4R pathway, which could be an explanation as to why in humans there is an increase in BMD in MC4R deficient individuals [15].

Although MC4R mutations correlate with hyperphagia, some mutations do not confer a loss-of-function on the receptor [15]. Hyperphagia and hyperinsulinemia are seen to decrease with regard to their severity as an obese MC4R mutation carrying individual matures to adulthood [15, 22]. This indicates that certain appetite control mechanism(s) change following adolescence and puberty [15]. In some cases, including an individual with a homozygous null MC4R mutation, hyperinsulinaemia was absent [15]. Lubrano-

Berthelier and colleagues also emphasized the point that an altered glucose metabolism did not show a correlation to MC4 mutation prevalence.

MC4R mutational effects on bone density, linear growth and eating habits are said to be most noticeable during phases of body growth, the reason being that differences in these aspects were not observed in a cohort of adult, severely obese MC4R mutation carriers [16]. Similarly BED was not prevalent within adult carriers of MC4 mutations [16]. Contrary to this, BED was identified in all subjects carrying MC4R mutations, but only in 14.2% of a control group that did not have MC4R gene alterations [24]. These results are not in agreement with each other and lead to scepticism as to whether BED is a clinical characteristic of MC4R mutation carriers.

When looking at variations in other genes (POMC and LEPR – leptin receptor) involved in the leptin / melanocortin pathway, the phenotypic eating behavioural differences seen between MC4R variant carriers and non-carriers were not observed, implying that MC4R polymorphisms cause a disturbance in the regulation of eating behaviour [25]. Farooqi *et al.* (2003) confirmed this when they found that the severity of the functional defect of the receptor shows a positive correlation to food intake at a test meal by the mutation carrier.

In addition to their role in appetite control, melanocortin receptors also affect peripheral metabolism [15]. Murine intracerebroventricular (ICV) infusion of a non-selective MCR4 agonist caused a decline in abdominal fat, enhancement of insulin effects on peripheral glucose metabolism and glucose production from the liver [15]. The glucose metabolism effects were blocked when rodents were pre-treated with certain anti-sense oligo's that knockdown MC4R. This illustrates that the MC4Rs were in fact arbitrating the effect of the non-specific MC4R agonist [15]. Additionally, intense ICV administration of MC4R agonists results in an increase in glucose production by the liver by amplifiying gluconeogenesis [15]. In accordance with these findings, Wikberg and Mutulis emphasize the fact that MC4Rs also seem to control body energy expenditure, thermogenesis, oxygen consumption and levels of uncoupling

proteins in brown adipose tissue. They also have been linked to the control of glucose uptake, insulin sensitivity, lipid uptake, lipid synthesis and fat accumulation in white adipose tissue via the sympathetic nervous system [9].

Potoczna and colleagues concluded that alterations within the MC4R gene have an influence on co-morbidities and the outcomes of certain curative strategies in severely obese patients. In this study, carriers and non-carriers of MC4R mutations were compared when considering improvement of the obesity and co-morbidities following laparoscopic gastric banding. Mutations were not separated into non-pathogenic/common or pathogenic /rare polymorphisms. The results revealed that (a) carriers lost less weight; (b) there was less improvement in components of the metabolic syndrome; (c) they had dilated oesophagi; (d) vomiting was increased; and (e) they had five-fold more gastric complications than non-carriers [25]. Metabolic syndrome improvement was significant in non-carriers as 80% of these patients showed remission, whereas persistence of the metabolic syndrome persisted in MC4R mutation carriers over a three year observational period [25]. However, if patients underwent a subsequent gastric bypass procedure due to complications following gastric banding, weight loss patterns in MC4R variant carriers and non-carriers were very similar [26]. However, gastric bypass conversion occurred in fourfold more carriers when compared to non-carriers [25]. MC4R variant carriers are known for their assertive, uncontrollable overeating habits that are more prolific than non-carriers that binge [25]. This characteristic could be the root of why they have more complications post-op and metabolically [25]. The authors thus hypothesized that because of the aggressive eating habits of MC4R variant carriers, the restrictive gastric band's function was superseded; this resulted in fast insulin release, increased FFA's and triglycerides, an increased tendency towards fatty liver, decreased insulin breakdown and thus hyperinsulinaemia [25]. These observations have however been strongly refuted as BED was not a specific characteristic of obese adult MC4R mutation carriers (Lubrano-Berthelier *et al.* 2006), and basing treatment outcome on pathogenic mutation presence requires too many assumptions. If a correlation of this sort were to be substantiated in the future, there would have to be far more clinical and

laboratory evidence to demonstrate its significance. Furthermore, the fact that non-pathogenic/common mutations were included in the analysis together with pathogenic/rare mutations, makes it difficult to interpret the data.

In studies in which melanocortin has been administered therapeutically, effects on blood pressure, heart rate, inflammation, levels of sodium excretion (natriuresis) and erectile function have been observed in addition to the desired effects on energy usage and food ingestion [1]. Targeting the melanocortin system by therapeutic means result in significant improvements in obesity, diabetes and cachexia [1].

Male and female adult subjects harbouring MC4R mutations have an elevated risk of obesity; quantification of this finding in relation to BMI revealed an increase of ~4 and ~9.5 kg/m^2 respectively when compared to wild-type relatives [26]. According to Lubrano-Berthrlier *et al.* 2006, a specific phenotype of MC4R mutation carriers has not been observed and therefore prediction of an MC4R mutation cannot be made based on phenotypic observation. However, these authors state that MC4R mutations are a significant cause of severe human obesity within both early and late onset forms of the disease.

2.4 Functional Impact of MC4R Mutations

A correlation between the severity and onset of obesity with the degree of MC4R dysfunction exists; mutations that cause intracellular receptor retention are the most pertinent in light of this association [16]. This characteristic is thus proposed to be the best predictor of the onset and severity of obesity in MC4R mutation carriers. In accordance with these findings, these authors propose a phenotype-genotype relationship within MC4R mutation carriers [16].

Functional defects within the MC4R gene that are responsible for obesity result in decreased or absent ligand binding, decreased cell surface receptor expression because of intracellular retention of mutant receptors, incorrect protein folding (GPCR) which results in the receptor never being released from the endoplasmic reticulum [27] and a reduction in signal transduction [7].

Mutant receptors that are retained within the cell have been suggested to impede the cell surface expression of wild-type receptors [20]. Mutations occurring between the first transmembrane domain (TM1) and the beginning of TM4 lead to impairment of NDP-MSH and AgRP binding and cause a reduction in cAMP generation [21]. Functional assays of MC4R mutations found that decreased basal activity was the most common defect (79% of mutants); EC_{50} for α-MSH activation was altered in 64% of mutants and membrane expression was significantly reduced in 50% of mutants [16].

When looking at the onset and severity of the obesity, all mutations identified showed a positive relationship. However, those mutations that resulted in intracellular retention of the receptor illustrated the strongest correlation [7, 16] and appear to be the most common mechanism by which MC4R mutations effect function [21]. Polymorphisms that cause the receptor to be truncated before the C-terminal tail, which carries the signal for cell surface expression [21], result in intracellular retention of the mutant protein [15]. In addition, cell surface expression is affected if mutations result in an alteration of receptor folding, which modifies the receptor's tertiary structure resulting in cellular retention [15]. These mutations therefore appear to be the best forecasters of onset and severity of obesity in carriers of MC4R mutations, [7] and it has been suggested that intracellular retention should be used to functionally classify MC4R mutations [16].

In agreement with this suggestion, it has been found that intracellular retention of mutant MC4Rs is predominantly responsible for early onset obesity (70%) (defined as BMI > 30 kg/m^2 at age 20); the same parameter measured in patients with late onset of obesity was not as prevalent (23%) [16]. In addition, another study found that ± 80% of childhood obesity heterozygous MC4R mutations impede receptor cell surface expression [15].

The most common defect found in individuals with MC4R mutations is a reduction in the constitutive activity of the receptor [7]. Normal constitutive receptor activity results in basal cAMP generation in the absence of an agonist [15]. N-terminal sequences are responsible for this constitutive activity

and it is thus compromised if mutations arise within this domain [15]. Mutations of this nature have only been identified within obese individuals, which implies that loss or decline in basal MC4R activity could impact body weight regulation [15]. Certain gain-of-function mutations associated with enhancement of the receptor's constitutive activity have also been identified [15].

In 2008 a total of 100 mutations within the MC4R gene had been described [6]. 30% of these comprise frameshift or nonsense mutations and 70% are missense mutations that impair signalling through cyclic AMP *in vitro* [6]. 70% of all inherently occurring MC4R missense mutations result in the disruption of cell surface expression of the receptor and thus lead to obesity [6].

Mutations within the MC4R gene do not always cause obesity; this statement is based on *in vitro* findings that have illustrated normal receptor functioning in obese individuals and conversely loss-of-function mutations within the non-obese [27]. Common polymorphisms observed within MC4R that do not effect the functioning of the receptor or are at similar frequencies in individuals that are not obese should be omitted from MC4R-obesity studies to prevent inapt clinical decisions and avoid confusion [16]. Tao *et al*. also state that functional characterization of a defective MC4R caused by mutation within the gene encoding it is of utmost importance when concluding that the mutation is in fact responsible for the obesity observed. This illustrates the importance of functional studies once polymorphisms have been identified, especially when investigating therapeutic potential for mutant receptors. Table 1 provides a summary of the majority of MC4R mutations identified thus far, and the effects that these have on receptor function.

Table 1: MC4R mutations and their effects on receptor function *

Mutation	**Functional effect / comment**	**References**
Y35X (110 A>T)	• Complete receptor loss-of-function • Reduced cell surface expression	17; 32
I102S	• Decreased ligand binding	18; 27; 29

	• Reduced endogenous agonist effects • Variable penetrance & expressivity in pedigree analysis	
V103I	• Most common missense mutation • No functional effect • Similar in frequency in obese & non-obese • Increased receptor constitutive activity • Negative association with obesity/BMI • Protection against obesity – gain-of-function • Frequently found in haplotypes • Association with favoured cholesterol/triglyceride levels • 2-fold reduced AgRP/antagonist binding	9; 13; 17; 29; 30; 31; 32
I125L	• Similar in frequency in both obese and non-obese • Functionally similar to wild-type receptor	17
W147C	• Decreased extended strand structure of polypeptide • Located in TM4 • Alters receptors structural integrity	33
Q43X	• Truncated N-terminal peptide – truncated from TM1 • Abolishes ligand binding	33
A175T	• Partial receptor activity • Associated with early-onset & family history of obesity • Insulin resistance • Hindered cAMP production	33
S19fsX51	• Caused by 1-bp deletion at nucleotide 448 (A); results in a frameshift after codon 18 & a stop at codon 51	33

	• Alters constitutive activity	
I317V	• C-terminal region • Effects receptor trafficking	33
S127L	• AgRP inhibition not hindered • Defect in ligand binding and/or signal transduction • Associated with early-onset childhood obesity in all cases	23; 29
Y35C & M218T	• Normal receptor functioning • Did not segregate with obesity in a pedigree analysis	31
D37X (100 Ains)	• Truncated receptor	18
R165Q/W	• 15-90 fold reduced endogenous agonist activity • 2-9 fold reduced synthetic ligand activity • R165Q strong correlation to development of early-onset obesity; also effects receptor cell surface expression	18; 21; 29
I170V	• Normal receptor function vs. • Decreased cell surface expression	34 vs. 16
N97D; L106P; C271Y	• Greater responses to endogenous agonist β-MSH when compared with other agonists • Cell surface expression impaired	21; 29
S58C	• Strong correlation to development of early-onset obesity; also effects receptor cell surface expression	14; 18; 29

	• Variable penetrance & expressivity in pedigree analysis	
T11S; S30F; D37V; I169S; A175T; T178M; P200L; V253I; N274S	• Similarities to wild-type receptor • A175T & V253I - impaired ability of adenylate cyclase signalling; normal ligand binding	21; 29
T150I; Y287X; I130T	• Reduced endogenous agonist effects • Y287X – nonsense mutation, premature termination, cell surface expression impaired	21; 29
T112M; I137T; A244E; G252S; I316S	• Reduced effects when bound to some, but not all endogenous agonists • I316S – impairment of signalling by selectively affecting the affinity of agonist vs. antagonist (affinity for NDP-MSH reduced; AgRP affinity not affected). Receptor tertiary structure effected.	21; 29; 35
L250Q	• Intracellular end of TM6 – constitutive activity increased • Reduced cell surface expression	29; 35
I251L	• Thought to have increased constitutive activity, however shown to be false observation	29
F202L	• Identified in normal weight individuals • Decrease in basal/constitutive receptor activites	27
H158R	• Increased basal/constitutive receptor activity	17; 37

	• Intracellulary retained (Lubrano-Berthelier *et al.* 2003) vs. • Similar characteristics to wild-type receptor (Hinney *et al.*) • **Note:** differing functional study methods give differing results therefore standardized procedure should be agreed upon *	
P78L; V95I; I125K; P299H; TM5Del; TM6Ins	• Reduced cell surface expression • Unable to establish expressional & functional stability • I125K – ligand binding impairment	21; 29
750 – 751 GA Del	• Truncation at TM6 • Addition of 33 amino acids • Reduced cell surface expression • Homozygous carrier experienced excessive weight gain before 6 months of age	35
T101A	• Reduced signal transduction following normal cell surface expression & agonist binding	17
CTCT Del at codon 148	• Frameshift & premature termination codon • Partial response to α-MSH – ligand binding affinity impairment	21
N62S	• Partial response to α-MSH – ligand binding affinity impairment	21
A Ins at codon 112	• Inefficient translation, rapid degradation of receptor • Premature termination • Cell surface receptor expression	21

** The results represented here are based on various functional studies; there is however a lack of standardization in the methodology used to functionally characterize mutant MC4Rs. This is the reason why differences are seen when the same mutation is functionally characterized in two or more studies. A standardized method should therefore be agreed upon when performing in vitro functional studies.*

A patient with complete loss of MC4R function was compared with leptin receptor-deficient patients and the results indicated that MC4R mediates the anorexigenic effects of leptin in the early stages of life; however it is not the mediator of leptin effects on other aspects such as linear growth and alternate endocrine functions [36]. It was also observed that total MC4R dysfunction does not cause hyperinsulinaemia or have an effect on the hypothalamic-pituitary-adrenal axis, thyroid hormone levels or lipid metabolic profiles [36]. Ultimately, because leptin and MC4R deficient individuals present with a similar early-onset increase in weight, it is suggested that the total effect of leptin on body weight is dependant on activation of the MC4R pathway [36]. Studies such as these will continue to provide clarity on the functional effects of the melanocortin pathway, and will ascertain which aspects are useful for the treatment of obesity.

2.5 Concluding Remarks

Obesity has become a major healthcare problem in the last 30 years and is a chief player in the increasing global mortality rate. Surgical procedures are proving to be the best treatment option for weight loss and co-morbidity resolution. Identifying genetic mechanisms that contribute to the development of the disease and using them to implement therapeutic strategies at both pharmacological and surgical levels is likely to be the way of the future. Genetically-induced malfunction of proteins that are involved in appetite regulation and energy homeostasis could be used as markers in diagnostic strategies as well as predictors of therapeutic outcome. Genetic markers such as MC4R and other obesity associated alleles could therefore be used as additional parameters to assess viability of treatment options as well as

therapeutic outcome in obesity management. This is obviously a long-term goal; however the outcomes of this study in conjunction with many others will increase the use of genetic markers in the battle against obesity and its co-morbidities.

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Objectives

The main objectives of this study were divided into *2 sections*:

A) To determine the significant changes that occur within South African obese patients who are managed by the Bariatric Centres of Excellence following surgical (Roux-en-Y gastric bypass) intervention

B) To assess the presence of MC4R gene polymorphisms and their correlation with BMI within a South African group of individuals

Chapter 3: Early Changes Following Bariatric Surgery in South Africa.

This chapter's format is written in accordance with the "Instructions for Authors" of the journal Obesity Surgery.

3.1 Abstract

Background: Bariatric surgery for the treatment of obesity has shown much promise. The Roux-en-Y gastric bypass is a procedure that combines both restrictive and malabsorptive elements. Post-operative weight loss and co-morbidity improvements previously achieved are over and above those which are seen during life style modification and drug therapy.
Aim: To provide a descriptive, retrospective analysis on a typical cohort of patients undergoing baritric surgery in the form of Roux-en-Y gastric bypass, with reference to anthropometric and biochemical parameters. A secondary aim was to determinine whether pro-BNP could be used as a marker to exclude cardiac failure in the obese patient population.
Methods: 330 patients (2005-2007) with a mean BMI of 45.87 ± 0.63 were characterised pre-operatively with regard to clinical, anthropometric and DEXA measurements. 130 were matched for the same parameters post-operatively over a 9-12 month observation period. The data was analysed statistically using paired t-tests and regression analyses.
Results: Significant post-operative improvements were observed with regard to patients' weight loss and co-morbidity improvement. Positive and significant correlations of anthropometric measures to biochemical parameters ensued. Risk factor scoring methodology produced an average total score of 17 points / 36.
Conclusions: Average post-op weight loss at 9-12 months (half-way mark in terms of weight loss) follow-up was 20% of initial pre-op weight. Co-morbid diseases and anthropometric measurements illustrated significant changes following surgery. Risk factor scoring is a valuable pre-op tool for assessing medical aid re-imbursement eligibility for surgery.

Keywords: Roux-en-Y gastric bypass, co-morbidities, BMI, diabetes, NASH, dyslipidaemia, hypertension, Pro-BNP, weight-loss, clinical staging.

3.2 Introduction

The medical management of obesity includes lifestyle management, as well as pharmacological and surgical approaches. Each can be applied to a different degree depending on local resources. Surgical treatment of obesity includes both restrictive and malabsorptive procedures [1]; choice of the procedure should be customised according to the patients' BMI, risks during surgery, metabolic complications, co-morbid conditions, preference and surgeons' recommendations [2]. All procedures substantiate the use of laparoscopy as opposed to open operation as with the former fewer post-op complications and a shorter hospitalisation period ensue [2].

Procedures that have been the most successful are those that combine both restrictive and malabsorptive elements, namely the Roux-en-Y gastric bypass [1] and Figure 1. This procedure involves the establishment of a small stomach pouch with it being reduced to 5% of its normal volume [3], resulting in restriction of food intake [1]. The intestine is cut and the lower section of the intestine is attached to the stomach pouch [1]. The result is bypassing of approximately 95% of the stomach, the whole duodenum and 20cm of the upper jejunum [3]. The absorption of nutrients and calories of ingested foodstuffs is then greatly reduced [1]. Following this surgical procedure individuals are seen to lose significant amounts of weight in excess of 45 kg and approximately 35% of initial BMI post-op [4]. They also experience a phenomenal improvement in co-morbid conditions post-op, the most notable of which is a rapid resolution of diabetes even before weight loss [1].

In 2007 alone over 400 bariatric surgeries were performed within the South African Centres of Excellence with a morbidity of 6-8% and a mortality of < 1%. This illustrates a clear growth in this field, implies that the bariatric surgery is a hugely successful modality of treatment for the morbidly obese and exemplifies that it has earned its right as a treatment strategy. The

implementation of metabolic surgery for the resolution of diabetes or so-called second phase metabolic surgery where the BMI cut-off will have less significance and the goal will be to resolve metabolic disease is becoming increasingly eminent.

In a meta-analysis performed by Buchwald et al. (Table 1) showing the rates of resolution of various co-morbid diseases post-surgery drastic improvement of diabetes, dyslipidaemia, hypertension and sleep apnoea was seen. The biliopancreatic bypass being the most advanced procedure showed the best post-op improvements. The key, take home message from this study is that this is essentially what we are moving toward and that emphasis should be placed on the effect of the operative procedure on resolution of co-morbid conditions. Sjöström *et al.* found that severely obese patients who underwent gastric bypass surgery and achieved and maintained a 20-30 kg weight loss over 13 years, also benefited by a 30% reduction in total mortality attributable to a cause specific reduction in both ischemic heart disease and obesity-related cancers. This study was a prospective large cohort randomized matched study and illustrates the importance of bariatric surgery with regard to long-term mortality.

In the largest retrospective cohort study on long-term mortality outcome data performed by Adams et al. (Table 2) it was observed that mortality due to any cause was reduced by 40% post-operatively, there was a 56% decline in death due to coronary artery disease, a 92% decrease in diabetes associated fatalities and a 60% reduction in cancer related deaths. There was however a 58% increase in mortality attributable to accidents and suicide, the cause of which still needs to be determined.

Gastric bypass was initially designed to cause malabsorption and thus weight loss; however adaptation of the gut to the changes made, occurred a few weeks post-op [8]. Remarkably weight loss continued following this adaptation due to reduced eating behaviour [8]. In conjunction with this, post-op, patients feel hungry less frequently, eat fewer times per day and of their own accord eat less calorie dense foods [9]. If for some reason the procedure had to be

reversed the phenotypic characteristics of obesity, namely weight gain and extreme eating behaviour returned [8].

Following gastric bypass the plasma levels of gut hormones undergo significant alterations. Ghrelin, which is secreted from the stomach and has an orexigenic effect shows decreased concentration post-op [3]. Moreover the expected ghrelin level fluctuations associated with meals and the 24 hour "ghrelin cycle" were absent from gastric bypass patients [9]. Peptide YY (PYY) and Glucagon-like peptide 1 (GLP-1) have an anorexigenic effect, as well as the latter hormone having a positive effect on insulin levels [3]. Post-op levels of these two hormones are increased after the consumption of a meal [3]. The levels of these hormones could thus be responsible for post-operative improvements in weight and co-morbid conditions, as well as an increased improvement in appetite regulation [8]. Gastric bypass patients' are also seen to have enhanced insulin profiles, thus indicating that the control of glucose and fat metabolism is greatly improved [8].

Patients are selected for bariatric surgery based on BMI and co-morbidity criteria. In 1991 the NIH Consensus Conference's criteria was that of a BMI of ≥ 40 kg/m^2 or BMI 35 kg/m^2 – 39.9 kg/m^2 as well as presentation of serious co-morbidities [10]. This has recently been challenged by reports that state that surgical procedures within patients that present with a BMI of 25-35 have similar success with regard to weight loss and co-morbidity eradication [2]. In accordance with this Dixon and colleagues state that surgical intervention should be considered at earlier treatment stages of diabetes within obese individuals.

When looking at the main co-morbidities associated with obesity, cardiovascular disease is on the short-list, this gives reason to find a reliable clinical risk marker. Although the best methods are in the form of an angiogram or ECG, a number of studies have identified B-type natriuretic peptide (BNP) as an accurate indicator of left ventricle (LV) wall tension and thus cardiac failure. BNP is released from the LV as a result of an increase in myocardial wall tension. The aminoterminal portion of pro-BNP (NT-pro-BNP)

which is released in equal amounts to BNP, but has a longer half-life, could be used to exclude systolic LV-dysfunction in high-risk cardiac patients because of its good negative predictive value [12]. Although values of NT-pro-BNP of <150pg/ml virtually exclude left ventricle failure, it has been seen that NT-pro-BNP values may be decreased by obesity [12]. Since heart disease is highly prevalent in obesity, it would be beneficial to determine whether NT-pro-BNP can, despite its reduction in obesity, be used to exclude cardiac failure risk in the obese patient population.

Thus the aim of this study was to provide a descriptive, retrospective analysis on a typical cohort of patients undergoing baritric surgery in the form of Roux-en-Y gastric bypass, with reference to anthropometric and biochemical parameters. A secondary aim was to determinine whether pro-BNP could be used as a marker to exclude cardiac failure in the obese patient population.

3.3 Materials and Methods

3.3.1 Sample Size and Study Population

The group comprised of 330 morbidly obese (BMI – 45.87 ± 0.63) individuals that qualified for surgical procedures according to defined clinical criteria. Pre-op data concerning vital clinical, biochemical and anthropometric measurements were recorded as part of routine clinical practice. 130 of the patients underwent Roux-en-Y gastric bypass surgery and were matched for the same parameters post-operatively over a follow-up period of approximately 1 year (9-12 months) **.

*** The reason the post-op group number was 200 patients less than the initial pre-op group, was that the 200 patients were still awaiting surgery and due to time constraints of the study, data could only be captured on the 130 patients that had undergone surgery. It is also important to note that a post-gastric bypass follow-up period of 9-12 months is recognized as a half way mark with regard to weight loss and the results will be interpreted with this point in mind.*

For Pro-BNP analysis, 50 patients with a mean BMI of 37.23 ± 0.94 with normal cardiac function, assessed by ECG and echocardiograms, were assayed for NT-pro-BNP levels.

3.3.2 Techniques and Measurements

Patients were assessed according to the following parameters both pre- and post-operatively as part of routine clinical practice:

Anthropometric:

Waist circumference, Hip circumference, Height, Weight, BMI, Neck circumference, Blood Pressure, Pulse Rate.

Biochemical:

Fasting Glucose, Fasting Lipogram (cholesterol, triglycerides, HDL and LDL-cholesterol), Urea, Creatinine, Calcium, Magnesium, Phosphate, Liver Functions (albumin, alanine transaminase - ALT, aspartate transaminase - AST, gamma glutamate transferase - GGT), Pro-BNP, HbA_{1C}, C-reactive Protein (CRP).

Special Investigations:

DEXA scan (assessment of osteoporosis, body fat, bone mineral content and density).

A database of the measurements was created on a retrospective basis.

A published reference for risk factor scoring ([1] and Table 3) was used to determine eligibility for surgery. Its significance and relevance was also investigated in the context of this study.

Pro-BNP was analysed using an NT-pro-BNP kit (Immune assay N-terminal Roche Elecsys and MODULAR ANALYTICS).

3.3.3 Statistical Analysis

The statistical analysis programme GB-stat v8.0 was used to analyse the database measurements. Paired t-tests, regression analyses and correlation

co-efficients were the methods used to investigate pre to post-op alterations and various relationships within the data respectively.

3.4 Results

3.4.1 Anthropometric and Clinical Outcomes

The changes in anthropometric and clinical parameters among the 130 patients that underwent bypass surgery were highly significant (Table 4).
Waist circumference decreased by 14% (pre-op: 137 ± 2.65 cm; post-op: 118 ± 2.19 cm; $p < 0.0001$) on average within the first 3 months post-op, this figure decreased to 17% (114 ± 3.29 cm; $p < 0.0001$) after 9-12 months.
When looking at ***hip circumference*** during the same time periods the measurements decreased by 12% (pre-op: 148 ± 2.02 cm; post-op: 131 ± 2.2 cm; $p <0.0001$) and 15% (126 ± 4.04 cm; $p < 0.0001$) respectively;
Weight decreased by 14% (pre-op: 137 ± 3.4 kg; post-op: 118 ± 3.82 kg; $p < 0.0001$) and by 20% (110 ± 5.2 kg; $p < 0.0001$);
BMI decreased by 14% (pre-op: 48.2 ± 1.16 kg/m^2; post-op: 41.6 ± 1.4 kg/m^2; $p < 0.0001$) and by 19% (39.1 ± 1.92 kg/m^2; $p < 0.0001$) respectively.
Clinical ***blood pressure*** readings were as follows over the same follow-up periods of < 3 and 9-12 months;
Systolic 9% decrease (pre-op: 144 ± 1.75 mmHg; post-op: 131 ± 1.25 mmHg; $p < 0.0001$) and 6% decrease (136 ± 2.53 mmHg; $p = 0.06$);
Diastolic 10% decrease (pre-op: 88 ± 1.24 mmHg; post-op: 79 ± 1.1 mmHg; $p < 0.0001$) and 7% decline (82 ± 1.6 mmHg; $p = 0.1$).

3.4.2 Biochemical Outcomes

Physiological alterations in biochemical parameters post-op showed vast improvements and were substantially significant (Table 5). The same follow-up time intervals (3 and 9-12 months) were used for the biochemical measurements respectively.
Average ***fasting glucose*** declined by 5% (pre-op: 5.4 ± 0.1 mmol/L; post-op: 5.14 ± 0.08 mmol/L; $p = 0.009$) and by 10% (4.85 ± 0.1 mmol/L; $p = 0.0001$);

Cholesterol decreased by 14% (pre-op: 5.18 ± 0.095 mmol/L; post-op: 4.45 ± 0.098 mmol/L; $p < 0.0001$) and by 13% (4.51 ± 0.12 mmol/L; $p = 0.0001$);
Triglycerides decreased by 20% (pre-op: 1.73 ± 0.11 mmol/L; post-op: 1.39 ± 0.06 mmol/L; $p = .005$) and by 21% (1.36 ± 0.11 mmol/L; $p = .004$);
HDL-cholesterol decreased by 4% (pre-op: 1.15 ± 0.036 mmol/L; post-op: 1.11 ± 0.03 mmol/L; $p = 0.009$ and then showed a 5% increase (1.21 ± 0.06 mmol/L; $p = 0.1$);
LDL-cholesterol was reduced by 18% (pre-op: 3.42 ± 0.08 mmol/L; post-op: 2.8 ± 0.09 mmol/L; $p < 0.0001$) and by 17% (2.83 ± 0.11 mmol/L; $p = 0.0006$);
GGT declined by 19% (pre-op: 34.6 ± 2.24 U/L; post-op: 28.02 ± 2.12 U/L; $p = 0.01$) and by 36% (22.31 ± 2.1 U/L; $p = 0.0003$) respectively.

3.4.3 Regression and Correlation Investigation

3.4.3.1 Comparison of Weight and BMI

When looking at the relationship of % subcutaneous (SC) fat to weight and BMI, a positive correlation was found in both (Figure 2). The 2-tailed probability (p)-value for the latter relationship, however was 100-fold more significant ($p = 0.5$; $r \geq 0.1$ vs. $p = 0.005$; $r \geq 0.3$).

3.4.3.2 Neck Circumference Trend

Neck circumference is used as a clinical marker for sleep apnoea at a cut-off value of > 41.5 cm. This measurement is also used as part of the risk factor score. Neck circumference was correlated with indices of the metabolic syndrome and Non-alcoholic steatohepatitis (NASH) to determine its clinical relevance. It had a strong and highly significant correlation to both Fasting Glucose ($p = 0.005$; $r \geq 0.2$) and $HbA1_c$ ($p = 0.0001$; $r \geq 0.4$) (Figure 3); a positive correlation to triglyceride levels ($p = 0.03$; $r \geq 0.1$) and a strong, negative, significant relationship to HDL-cholesterol ($p < 0.0001$; $r \geq -0.4$) (Figure 3). When correlated with liver enzyme measurements a positive relationship with both ALT ($p < 0.0001$; $r \geq 0.3$) and AST ($p = 0.06$; $r \geq 0.1$) ensued, however the former relationship illustrated a 600-fold more

significance than the latter. When correlated with kidney enzyme measures, a positive and significant relationship with both Urea ($p = 0.0006$; $r \geq 0.2$) and Creatinine ($p < 0.0001$; $r \geq 0.3$) was observed. (Figure 3)

3.4.3.3 Waist Circumference Relationships

Waist circumference is used as a clinical marker for visceral adiposity at cut-off values of > 88 cm in women and > 102 cm in men. This study investigated various correlations of this parameter with biochemical and clinical measurements. Waist circumference correlated positively with both fasting glucose ($p = 0.01$; $r \geq 0.2$) and $HbA1_c$ ($p = 0.002$; $r \geq 0.3$) (Figure 4), however showed a more significant relationship with the latter. A positive and significant relationship with triglyceride quantities ($p = 0.005$; $r \geq 0.2$) was observed (Figure 4); the correlation between waist and HDL-cholesterol was negative and highly significant ($p < 0.0001$; $r \geq -0.3$) (Figure 4). The relationship between waist circumference and indicators of NASH (ALT and GGT) showed a positive relationship with both however the former was highly significant ($p = 0.0005$; $r \geq 0.2$) as compared to the latter ($p = 0.01$; $r \geq 0.2$) (Figure 4). Investigations of the relationship between waist and blood pressure (BP), a positive and highly significant correlation was seen for both systolic ($p < 0.0001$; $r \geq 0.3$) and diastolic ($p < 0.0001$; $r \geq 0.3$) measurements (Figure 4).

3.4.3.4 Risk Factor Scoring

The mean percentages of scoring for the different categories of the risk factor score represented in Table 3 were as follows: Total – 46.9% (17 points / 36); History – 50% (6 points / 12); Clinical – 80% (8 points / 10) and Special Investigations – 28.6% (4 points / 14).

3.4.3.5 Pro-BNP Outcomes

Within the other investigatory sub-group various indices correlated positively with pro-BNP measurements (67.6 ± 11.4 pg/ml; normal range <150 pg/ml):

age (46.6 ± 1.2 yrs; $p < 0.02$); weight (103.4 ± 2.6 kg; $p < 0.05$); waist circumference (110.5 ± 1.9 cm ± 0.8, $p < 0.01$) and height (166.7 ± 1.04 cm; $p< 0.04$), however Pro-BNP remained within the normal range in a morbidly obese sample group (BMI – 37.3 ± 0.9 kg/m^2) that had normal cardiac function.

3.5 Discussion

3.5.1 Post-operative Outcomes

Post-operatively there were highly significant and encouraging improvements in anthropometric, clinical and biochemical variables. The weight loss results of 27kg (*pre-op: 137 kg ± 3.4; post-op: 110 kg ± 5.2; p < 0.0001*) 9-12 months post-surgery compare to results achieved by other centres of the world. This 20% weight loss achieved implies that the patients of this study are well on their way to the expected weight loss of 32-35% after 2 years (full weight loss is expected at 18-24 months post-op) (Figure 5). This studies' short term results are comparable to those of the SOS study [6] whose patients had a 27% sustained weight loss over a 15 year post-op follow-up period. Therefore this studies' patients at the half-way mark of 9-12 months post-surgery are on track in terms of weight loss (Figure 5).

Clearly in this sample of bariatric surgery patients there was a consistent decrease in post-op waist, weight and BMI. It has been illustrated that a weight loss of 10% correlates with a 30% reduction in visceral adiposity [13] [14]; by inference therefore it can be assumed that in this cohort there could be a potential 50-60% reduction in visceral adiposity. The blood pressure decrease of ~ 7% (*systolic: pre-op: 144 mmHg ± 1.75; post-op: 136 mmHg ± 2.53; p = 0.06* and *diastolic: pre-op: 88 mmHg ± 1.24; post-op: 82 mmHg ± 1.6; p = 0.1*) is equal to that which is achieved with a 2-3 drug medication schedule.

All biochemical parameters' improvements were either equal to or exceeding results that have been achieved with drug therapy. The improvements of liver

function in individuals that presented with NASH pre-op cannot be achieved when using drug therapy only. Average fasting glucose measurements fell from just below the reference threshold (3.5 – 5.5 mmol/L) with a pre-op reading of 5.4 ± 0.1 mmol/L to a 9-12 month post-op measure of 4.85 ± 0.1 mmol/L ($p = 0.0001$) indicative of an improvement in the patients overall glucose metabolism. Average cholesterol measurements (Reference – 3.0 – 5.2 mmol/L) were pre-operatively 0.02 mmol/L below the upper limit of the reference range and decreased after 9-12 months to 4.51 ± 0.12 mmol/L ($p = 0.0001$). Additional lipogram measures showed significant improvements with average triglyceride levels decreasing by 0.37 mmol/L ($p = .004$) after 9-12 months to within reference range (0.3 – 1.7 mmol/L). Average HDL-cholesterol levels showed a 5% (pre-op: 1.15 mmol/L ± 0.036; 1.21 mmol/L ± 0.06; $p = 0.1$) increase 9-12 months post-surgery and LDL-cholesterol levels declined from an average pre-op value of 3.42 ± 0.08 mmol/L which is 0.02 mmol/L above the reference range to an average value that is well within the expected range (2.83 ± 0.11 mmol/L; $p = 0.0006$). The fasting lipogram measurements illustrate that an overall trend towards the resolution of the patients' dyslipidaemia is initiated post gastric bypass. The argument that the use of statins result in vast improvements of cholesterol metabolism is one that arises regularly, however it is important to note that at the level of morbid obesity that these patients present with, drug therapy alone would not achieve desired cholesterol level improvements.

GGT measurements also showed highly significant improvements post surgery with a 36% decrease (pre-op: 34.6 U/L ± 2.24; post-op: 22.31 U/L ± 2.1; $p = 0.0003$) in enzyme levels over the 9-12 month follow-up period. Thus the patients that presented with NASH are subject to an improvement in liver function following bariatric surgery. It is important to mention here that the increased pre-op GGT levels were not in fact due to increased alcohol consumption, reason being that in the regression analysis ALT correlated highly significantly with waist circumference ($p = 0.0005$), thus indicating that these patients did present with NASH.

3.5.2 Regression Analyses

The comparison of % SC fat to weight and BMI illustrated clearly that weight in its own right is not a good marker for obesity as the correlation was non-significant ($p = 0.5$). BMI however showed a highly significant correlation ($p = 0.005$) with % SC fat thus emphasizing the fact that the height of a patient must undoubtedly be taken into consideration.

Both neck circumference as a marker for sleep apnoea and insulin resistance and waist circumference as a marker for visceral adiposity showed positive correlations with indices of the metabolic syndrome and NASH. A positive correlation between waist circumference and blood pressure readings was also observed. Thus the assessment of these measures is of utmost importance in the clinical work-up of an obese patient. The results clearly indicate that if a patients' neck or waist circumference is above the determined cut-off values it is likely that they will present with one or more of the obesity-associated co-morbidities namely; type II diabetes, dyslipidaemia, NASH and hypertension. These correlations will undoubtedly assist the clinician in making a more efficient and accurate diagnosis before receiving blood tests results.

Neck circumference also correlated positively and significantly with urea ($p = 0.0006$; $r \geq 0.2$) and creatinine ($p < 0.0001$; $r \geq 0.3$) levels which could be an indirect measurement for increased muscle bulk. Urea and creatinine levels remained within their normal reference ranges (3.4 – 7.4 mmol/L and 53 – 97 umol/L respectively), thus indicating that the patients did not have impaired renal function.

3.5.3 Risk Factor Scoring

Generally patients at a total risk factor score of 18 and above are considered eligible for medical aid re-imbursement for surgery. This studies sample population had an average total score of 17 which implies that approximately half (47%) are highly eligible medical aid re-imbursement. The breakdown of

the averages in each category of the risk factor score showed that the clinical analysis of the patient is of utmost importance (average score: 8 points / 10). It is also important to mention that the average special investigatory score was in fact so low because the DEXA scan bed used could only support a weight of 122 kg thus some patients could not be assessed.

Table 6 represents results from a recent study that was done in the same centre in which this study was conducted and shows the percentage of patients that are obese due to the varying causes covered in the risk factor score (n = 108). This reiterates the point that pre-operative patient assessment across all of the listed categories is of utmost importance in order for healthcare professionals to make correct treatment decisions. Therefore the risk factor score assessment shows much future clinical potential.

3.5.4 Pro-BNP Measurements

The pro-BNP analysis illustrated that NT-pro-BNP levels remained within normal ranges in obese patients with normal cardiac function and even may be decreased within this population. Therefore Pro-BNP level assessment is a reliable marker to exclude cardiac failure in the obese patient.

3.5.5 Concluding Remarks

Within the patient cohort the average post-op (110 ± 5.2 kg) weight at 9-12 months follow-up or half-way mark was 20% of the initial pre-op (137 ± 3.4 kg) weight ($p < 0.0001$). Anthropometric and clinical measurements showed significant post-op improvements. Biochemical parameters illustrate improvements of the metabolic syndrome post-surgery over and above what is achievable with drug therapy or behaviour modification.

Therefore bariatric surgery is clearly a powerful tool for the induction of weight loss and the resolution of co-morbidities within the morbidly obese patient and thus has very far reaching health economic implications. Accurate risk factor scoring to determine medical aid re-imbursement eligibility for surgery still

needs further analysis, but appears promising. Using only BMI for the clinical staging of obesity appears to be insufficient; because of the altering of gut-peptides (ghrelin and PYY) post-surgery which results in improved glucose and lipid metabolism, the bariatric surgery model may well be a very useful future tool to investigate pathophysiology in the obese patient and re-determine the clinical staging of the disease.

3.6 Tables

Table 1: Mean percentages of patients with post-op resolution of co-morbidities.

Major Co-morbidity	Operations		
	Adjustable Gastric Banding	Gastric Bypass	Biliopancreatic Bypass
Diabetes	48	84	99
Dyslipidaemia	59	97	99
Hypertension	43	68	83
Sleep apnoea	95	80	92

Buchwald et al. *JAMA*. 2004;292(14):1724-1737 [10].

Table 2: Long-term mortality after gastric bypass surgery.

	Deaths per 10,000 person years		% change	p value
	Control	Surgery		
Any Cause	57.1	37.6	40% decrease	p<0.001
Coronary artery disease	5.9	2.9	56% decrease	p=0.006
Diabetes	3.4	0.4	92% decrease	p=0.005
Cancer	13.3	5.5	60% decrease	p<0.001
Accidents & suicide	6.4	11.1	58% increase	p=0.04

Adams et al. *NEJM*. 2007; 357(8):753-761 [7].

Table 3: Risk factor score sheet used as part of the clinical work-up of a patient referred to the Bariatric Centre of Excellence to determine eligibility for medical aid re-imbursement for surgery. The score sheet is submitted to relevant medical aid schemes in order to secure re-imbursement for operative procedures and post-op hospitalization.

HISTORY SCORE		RESULT
Family history	Score 1	
Childhood obesity	Score 1	
Weight gain since 18yr > than 10 kg	Score 1	
Post-partum weight gain > 5%	Score 1	
Infertility / PCOD / Menopause	Score 1	
Cessation of smoking	Score 1	
Depression or Treatment of Depression	Score 1	
Endocrinopathy - related to obesity	Score 1	
Epilepsy or Treatment of Epilepsy	Score 1	

History of Eating Disorders	Score 1	
Failure of Obesity Pharmacotherapy	Score 1	
Medication resulting in obesity	Score 1	
Total score: (12)		
CLINICAL SCORE		**RESULT**
Waist circumference	Score 2*	
Male: > 102 ; Female: > 88		
BMI; 35 & Comorbid disease (Surgery)	Score 1	
BMI; 30 & Comorbid disease (Pharmacotherapy)		
BP; > 130/85 (or on anti-hypertensives)	#Score 2*	
Neck circumference ; > 41.5 cm	Score 1	
Cardiomegaly / Vascular disease	Score 1	
Hepatomegaly / Gallstones	Score 1	
Sleep disordered breathing / aspiration	Score 1	
Arthropathy / Chronic back pain	Score 1	
Total score: (10)		
SPECIAL INVESTIGATIONS		**RESULT**
Fasting glucose > 5.6 mmol/L (or treatment for IGT or DM)	#Score 2*	
Fasting TG > 1.7 mmol/L (or on hyperlipidaemics)	#Score 2*	
HDL Females:<1.3;Males:<1.0 mmol/L (or on hyperlipidaemics)	#Score 2*	
LDL > 3.5 mmol/L (or on hyperlipidaemics)	#Score 2*	
Elevated ALT / GGT	Score 1	
Elevated pro-BNP	Score 1	
Total score: (9)		
SPECIALIST LEVEL INVESTIGATIONS		
C-Reactive Protein	Score 1	
Growth hormone: stimulated < 3ug/L	Score 1	
Oesophageal erosions or related	Score 1	
Dexa % fat: Females >35%; Males >20%	Score 1	
MRI L4/5 > 30% expected	Score 1	
Total score: (5)		

RISK FACTOR SCORE	Total score 36	
** IDF CRITERIA for Metabolic Syndrome have been weighted as 2*		
# Note that patients on treatment will automatically have maximum score		
Category A: Reimbursement on specialist motivation only **Risk Score: 0-10**		
Category B: Reimbursement for Bariatric Centre of Excellence on motivation only **Risk Score: 11-18**		
Catecory C: Reimbursement for Bariatric Centre of Excellence without additional motivation **Risk Score: 19-36**		
THIS RISK SCORE IS COPYRIGHTED TO PROF M-T VD MERWE		
SURGICAL RISK FACTOR SCORE		
Gender: Male	Score 1	
Waist > 120cm	Score 1	
Age: > 65yrs	Score 1	
Pulmonary Embolism (prev. incident)	Score 1	
CVA (prev. incident)	Score 1	
Total Score: (5)		

Table 4: Average anthropometric and clinical outcomes across 130 patients showing significance and percentages of decreases in various measurements post gastric bypass. (The percentages represented in red show percentage loss or decrease of the parameter post-operatively).

Parameter	PRE	POST	
		< 3 months	9-12 months
Waist (cm)	137 ± 2.65	118 ± 2.19 **** **-14%**	114 ± 3.29 **** **-17%**
Hip (cm)	148 ± 2.02	131 ± 2.2 **** **-12%**	126 ± 4.04 **** **-15%**
Weight (kg)	137 ± 3.4	118 ± 3.82 **** **-14%**	110 ± 5.2 **** **-20%**
BMI (kg/m^2)	48.2 ± 1.16	41.6 ± 1.4 **** **-14%**	39.1 ± 1.92 **** **-19%**
BP systolic (mmHg)	144 ± 1.75	131 ± 1.25 **** **-9%**	136 ± 2.53 **-6%**
BP diastolic (mmHg)	88 ± 1.24	79 ± 1.1 **** **-10%**	82 ± 1.6 **-7%**

*p < 0.05; **p < 0.01; ***p < 0.005; ****p < 0.001

Table 5: Average biochemical outcomes across 130 patients showing significance and percentages of decreases/increases in various measurements post gastric bypass. (The percentages represented in red show percentage loss or decrease of the parameter post-operatively).

Parameter	PRE	POST	
		< 3 months	9-12 months
Fasting Glucose (mmol/L)	5.4 ± 0.1	5.14 ± 0.08 ** **-5%**	4.85 ± 0.1 **** **-10%**
Cholesterol (mmol/L)	5.18 ± 0.095	4.45 ± 0.098 **** **-14%**	4.51 ± 0.12 **** **-13%**
Triglyceride (mmol/L)	1.73 ± 0.11	1.39 ± 0.06 *** **-20%**	1.36 ± 0.11 *** **-21%**
HDL (mmol/L)	1.15 ± 0.036	1.11 ± 0.03 ** **-4%**	1.21 ± 0.06 **+ 5%**
LDL (mmol/L)	3.42 ± 0.08	2.8 ± 0.09 **** **-18%**	2.83 ± 0.11 **** **-17%**
GGT (U/L)	34.6 ± 2.24	28.02 ± 2.12 * **-19%**	22.31 ± 2.1 **** **-36%**

*p < 0.05; **p < 0.01; ***p < 0.005; ****p < 0.001

Table 6: Percentages of patients that are obese due to various causes that are listed in the risk factor score.

Cause	**Percentage**
Childhood obesity	76.64%
Weight gain since 18yrs > 10kg	88.79%
Postpartum weight gain	48.15%
Medication due to obesity	80.37%
Family history	97.22%
Depression	59.26%

Endocrinopathy	31.48%
Epilepsy	1.85%
History of eating disorders	12.04%
Failure of obesity pharmacotherapy	80.56%
Infertility/PCOD/Menopause	35.19%
Cessation of smoking	25.00%
IHD/Ischemia/Cardiovascular Disease	18.52%
NAFLD/NASH, Hepatic disease, Gallstones	25.93%
Sleep apnoea/Disordered breathing	72.22%
Arthopathy, Lower back pain	81.48%

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3.7 Legends for Figures

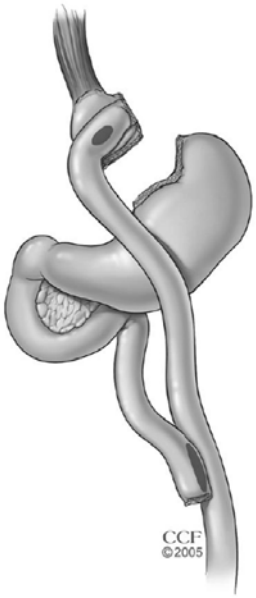


Figure 1: The Roux-en-Y gastric bypass (Sugerman *et al.* Obesity Sugery. Dec 2007; 251-254).

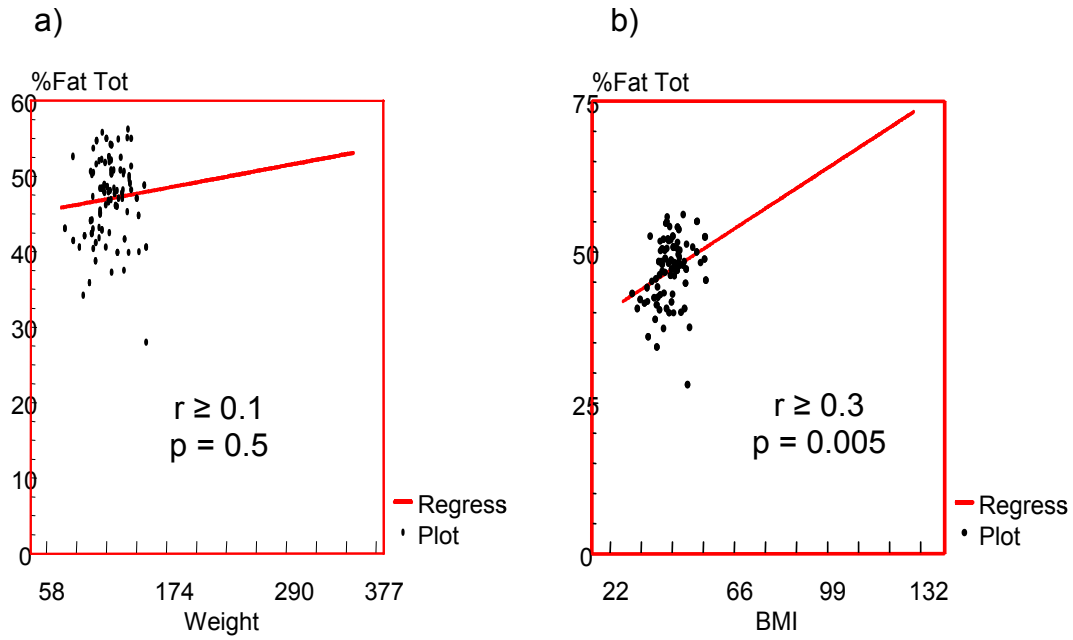
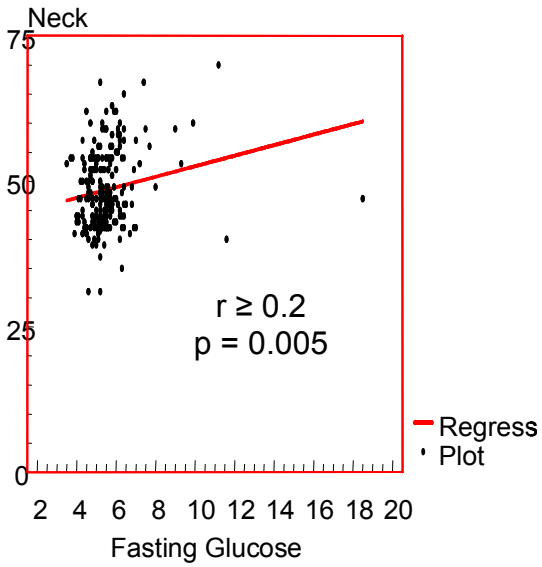
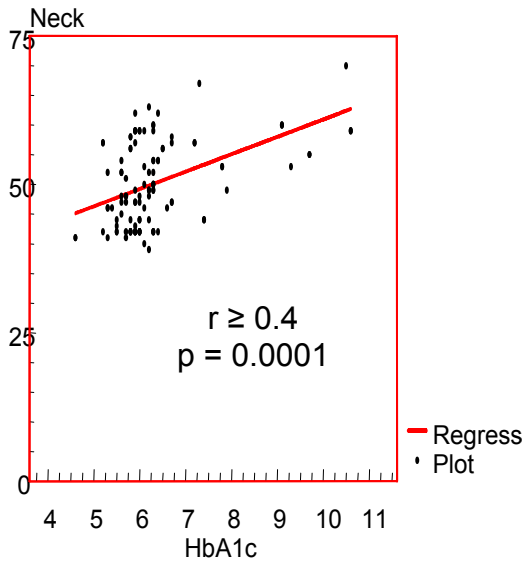


Figure 2: Linear regression analyses of the relationship between % SC fat and: a) weight; b) BMI. Both show a positive correlation, however the relationship between % SC fat and BMI is 100-fold more significant.

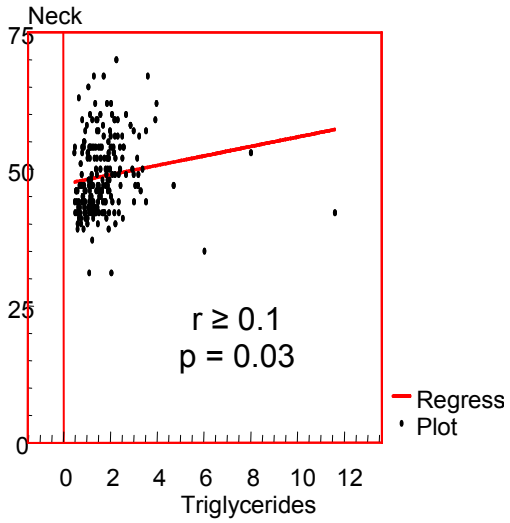
a)


Neck
75
50
25
0
r ≥ 0.2
p = 0.005
Regress
Plot
2 4 6 8 10 12 14 16 18 20
Fasting Glucose

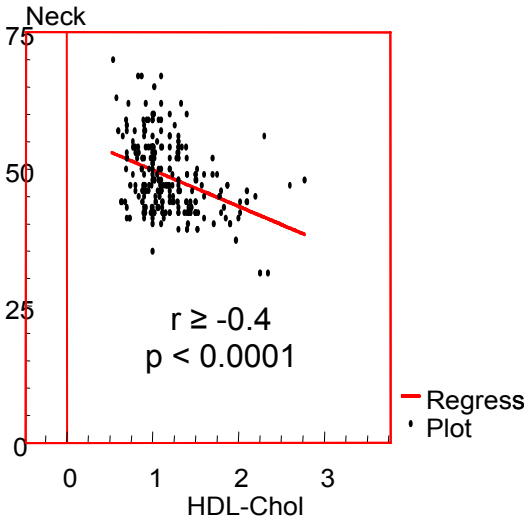
b)


Neck
75
50
25
0
r ≥ 0.4
p = 0.0001
Regress
Plot
4 5 6 7 8 9 10 11
HbA1c

c)


Neck
75
50
25
0
r ≥ 0.1
p = 0.03
Regress
Plot
0 2 4 6 8 10 12
Triglycerides

d)


Neck
75
50
25
0
r ≥ -0.4
p < 0.0001
Regress
Plot
0 1 2 3
HDL-Chol

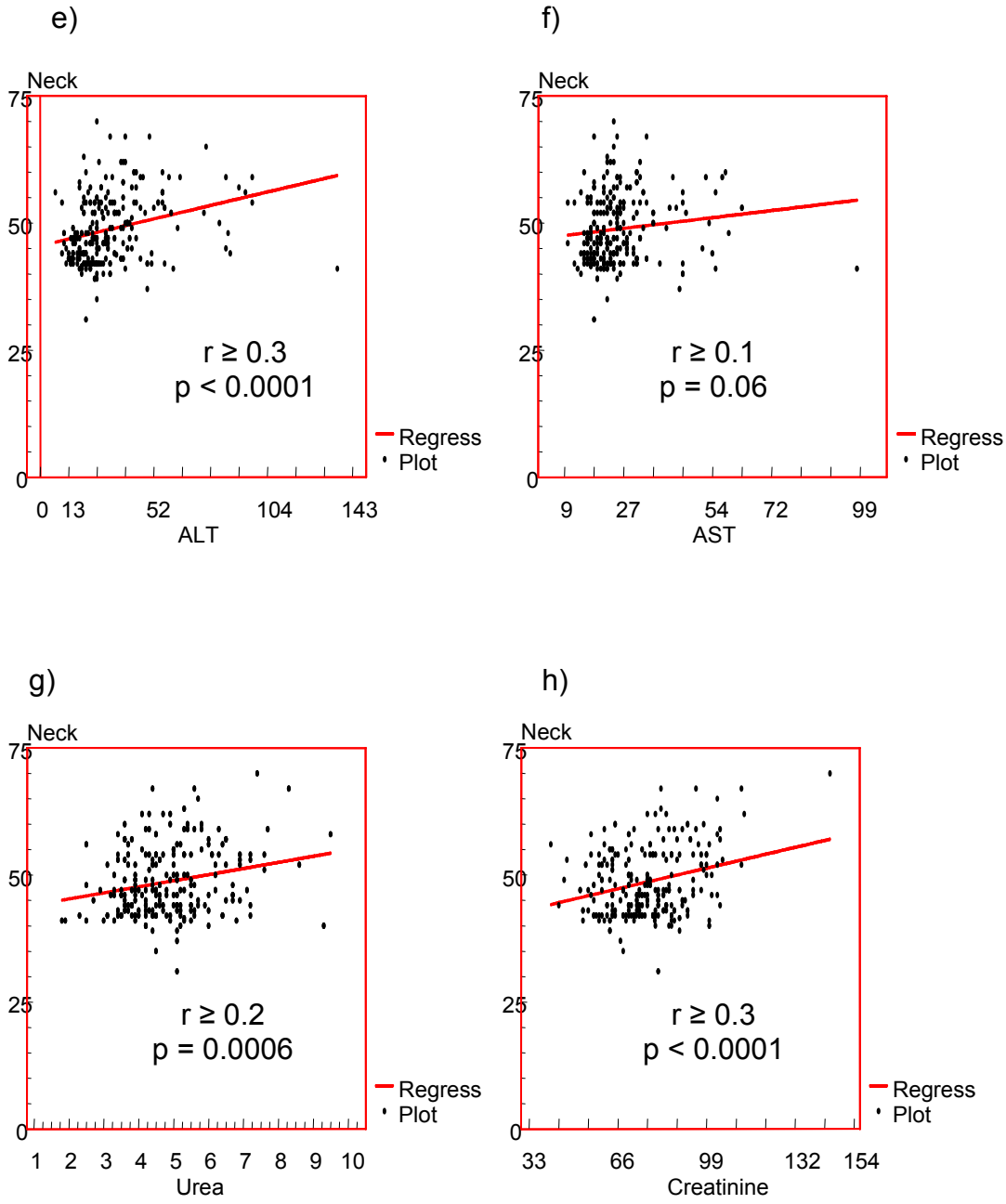
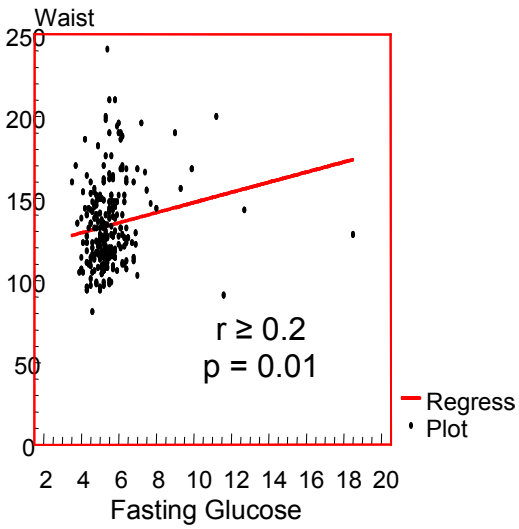
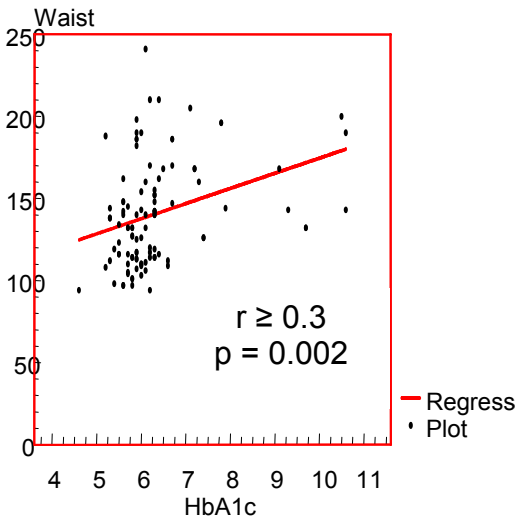


Figure 3: Linear regression analyses of the relationship between Neck Circumference and: a) Fasting Glucose; b) $HbA1_c$; c) Triglycerides; d) HDL-cholesterol; e) ALT; f) AST; g) Urea; h) Creatinine. Significance (p-value) and correlation co-efficients (r-value) are represented for each figure.

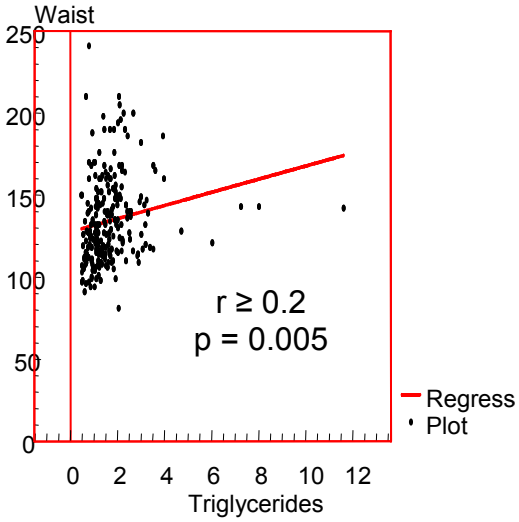
a)


Waist
250
200
150
100
50
0
2 4 6 8 10 12 14 16 18 20
Fasting Glucose
r ≥ 0.2
p = 0.01
Regress
Plot

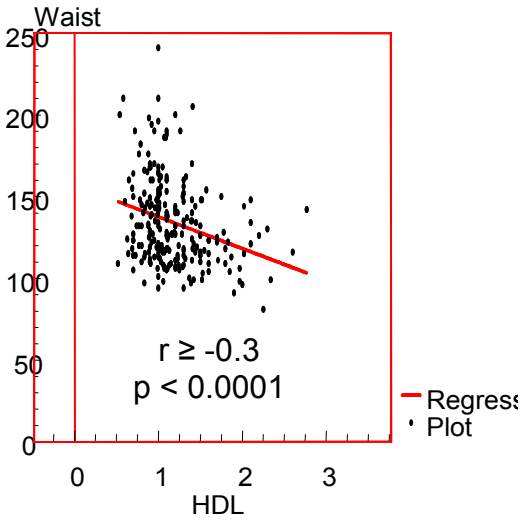
b)


Waist
250
200
150
100
50
0
4 5 6 7 8 9 10 11
HbA1c
r ≥ 0.3
p = 0.002
Regress
Plot

c)


Waist
250
200
150
100
50
0
0 2 4 6 8 10 12
Triglycerides
r ≥ 0.2
p = 0.005
Regress
Plot

d)


Waist
250
200
150
100
50
0
0 1 2 3
HDL
r ≥ -0.3
p < 0.0001
Regress
Plot

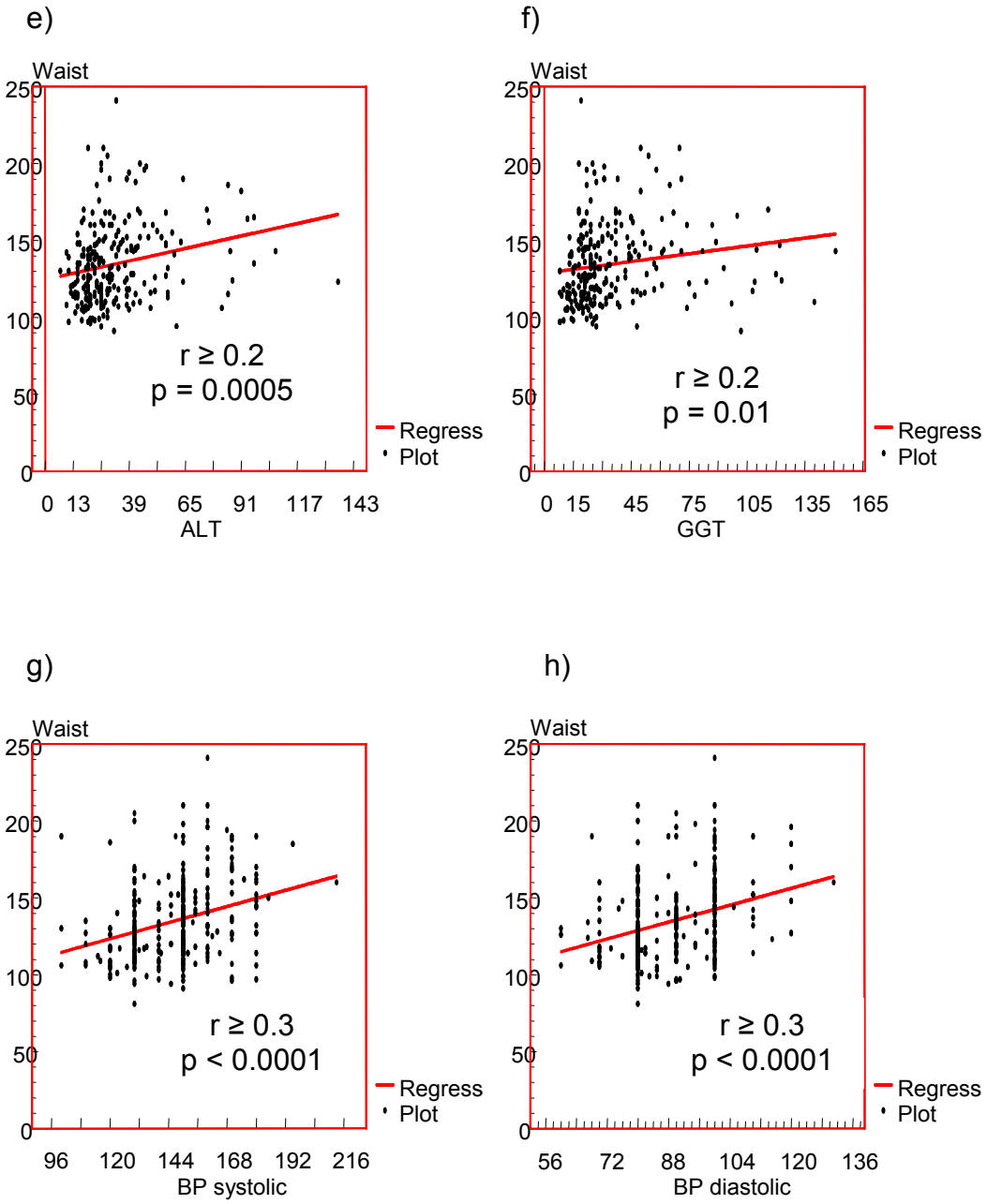
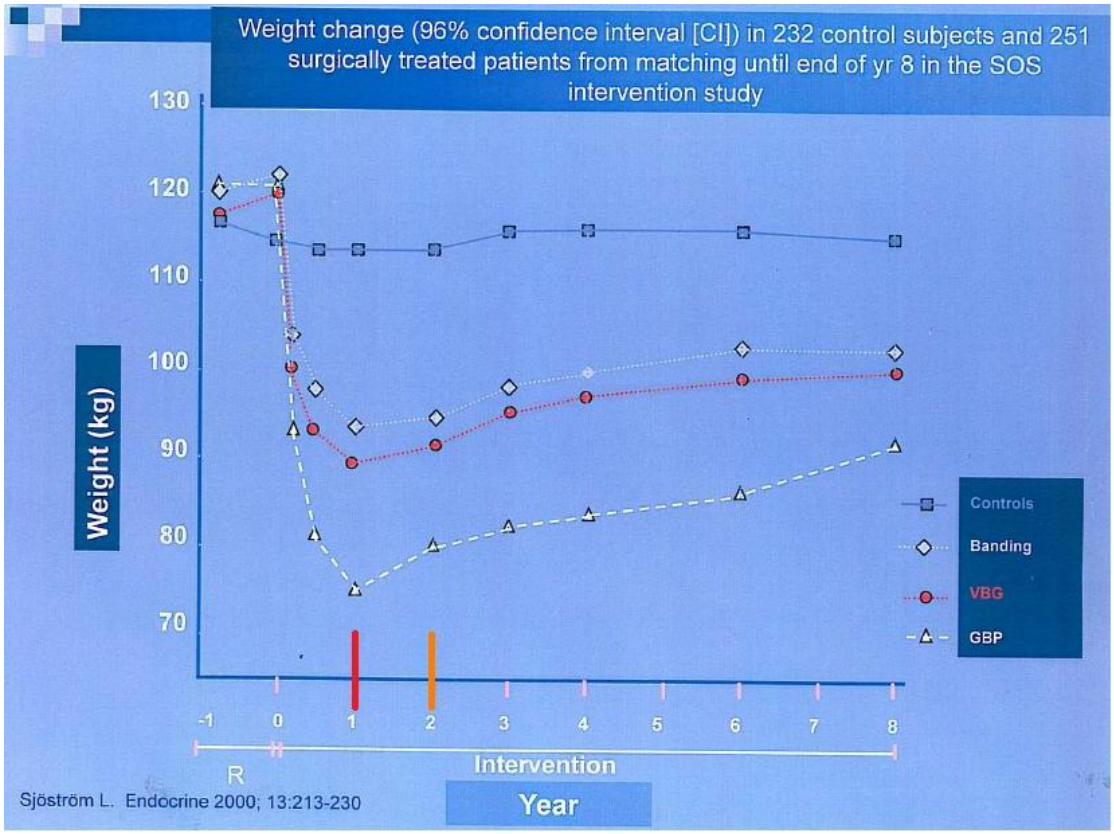


Figure 4: Linear regression analyses of the relationship between Waist Circumference and: a) Fasting Glucose; b) $HbA1_c$; c) Triglycerides; d) HDL-cholesterol; e) ALT; f) GGT; g) Systolic BP; h) Diastolic BP. Significance (p-value) and correlation co-efficients (r-value) are represented for each figure.



(Sjöström L. Endocrine 2000; 13:213-230. [15])

Figure 5: Graph representing weight change within 232 control subjects and 251 surgically treated (Banding, Vertical Banded Gastroplasty – VBG and Gastric Bypass – GBP) patients from the SOS intervention study. Marked off on the x-axis are the half-way mark for post-op weight loss (red) which is approximately 1 year (this was the cut-off point in this study) and the 2 year mark (orange) which is when full post-op weight loss is expected.

3.8 Summary of Results

- *Anthropometric and Clinical Outcomes:*

    - Waist circumference, hip circumference, weight and BMI changes 3 months and 9-12 months post-op were all statistically significant ($p < 0.0001$).

- Systolic and diastolic blood pressure changes 3 months post-op were both significant ($p < 0.0001$), however at 9-12 months post-op there was a slight increase in blood pressure, rendering both parameters not significant ($p = 0.06$ and $p = 0.1$ respectively).

- *Biochemical Outcomes:* (time intervals: 3 months; 9-12 months)

  - Average fasting glucose declined significantly; $p = 0.009$ and $p = 0.0001$ for the time intervals respectively.

  - Average lipogram (cholesterol, triglycerides and LDL) all showed significant changes post-op.

  - HDL showed a 5% increase at 9-12 months post-op almost reaching statistical significance ($p = 0.1$).

  - GGT changes declined significantly across both time intervals; $p = 0.01$ and $p = 0.0003$ respectively.

- *Regression and Correlation Investigations*

  - The relationship of % SC fat to weight and BMI was positive in both, however % SC fat to BMI was 100-fold more significant ($p = 0.005$) than % SC fat to weight ($p = 0.5$).

  - *Neck circumference:*

    - Strong and highly significant correlation to fasting glucose ($p = 0.005$; $r \geq 0.2$) and $HbA1_c$ ($p = 0.0001$; $r \geq 0.4$).

    - Positive correlation to triglycerides ($p = 0.03$; $r \geq 0.1$).

- Negative, significant relationship to HDL ($p < 0.0001$; $r \geq -0.4$).

- Positive relationship to both ALT ($p = 0.0001$; $r \geq 0.3$) and AST ($p = 0.06$; $r \geq 0.1$).

- Positive and significant relationship with both Urea ($p = 0.0006$; $r \geq 0.2$) and Creatinine ($p < 0.0001$; $r \geq 0.3$).

- *Waist Circumference:*

  - Positive relationship to fasting glucose ($p = 0.01$; $r \geq 0.2$) and $HbA1_c$ ($p = 0.002$; $r \geq 0.3$).

  - Positive correlation to triglyceride levels ($p = 0.005$; $r \geq 0.2$)

  - Negative correlation to HDL ($p < 0.0001$; $r \geq -0.3$).

  - Positive relationship with ALT ($p = 0.0005$; $r \geq 0.2$) and GGT ($p = 0.01$; $r \geq 0.2$)

  - Positive and highly significant relationship to systolic ($p < 0.0001$; $r \geq 0.3$) and diastolic ($p < 0.0001$; $r \geq 0.3$) blood pressure measurements.

- *Mean percentages of scoring for risk factor score categories:*

  - Total – 46.9% (17 points / 36)

  - History – 50% (6 points / 12)

  - Clinical – 80% (8 points / 10)

- Special Investigations – 28.6% (4 points / 14)

- *Average pro-BNP measurement:* (67.6 ± 11.4 pg/ml) remained within the normal range in a morbidly obese sample group (BMI – 37.3 ± 0.9 kg/m^2) that had normal cardiac function.

- Pro-BNP correlated significantly and positively with age ($p < 0.02$), weight ($p < 0.05$), waist ($p < 0.01$) and height ($p < 0.04$).

3.9 Conclusions

- *Post-operative Outcomes:*

  - Weight loss results of 27 kg on average 9-12 months post-surgery compare to results achieved by other centres of the world.

  - Patients are well on their way to the expected weight loss of 32-35% after 2 years.

  - By inference it can be assumed that there was a potential 50-60% reduction in visceral adiposity.

  - ~ 7% blood pressure decrease is equal to that which is achieved with 2-3 drug medication.

  - All biochemical measurements' improvements were equal to or would exceed the results that have been reported previously with drug therapy in other studies.

- Average fasting glucose measurements fell from 5.4 mmol/L pre-op to 5.14 mmol/L at 3 months post-op and then to 4.85 mmol/L after 9-12 months post-op, which indicates an improvement in glucose metabolism.

- Fasting lipogram measurements show that the resolution of the patients' dyslipidaemia occurs post-op.

- GGT measurement improvements (36% decrease) post-op showed liver function improvement and a resolution of non-alcoholic steatohepatitis (NASH).

- *Regression Analyses:*

  - % SC fat to weight and BMI reiterates the point that taking the height of a patient into account is vitally important during the pre-op work-up.

  - If neck (marker for sleep apnoea and insulin resistance) and waist (marker for visceral adiposity) circumference are above the determined cut-offs it is likely that patients will present with one or more of the obesity-associated co-morbidities; type 2 diabetes, dyslipidaemia, NASH and hypertension.

  - Positive and significant correlations of neck circumference with urea and creatinine could be an indirect measure for increased muscle bulk; urea and creatinine levels remained within their normal reference ranges, thus indicating that there was not impaired renal function.

- *Risk Factor Scoring*

    - A risk factor score of 18 is a good and safe indicator for health economic safety and the level at which medical aid re-imbursement is suggested for surgery.

    - The sample had an average total score of 17 which implies that approximately half (47%) are highly eligible for medical aid re-imbursement for surgery.

    - Clinical analysis of the patient is of utmost importance (average score: 8 points / 10).

- *Pro-BNP Measurements*

    - NT-pro-BNP levels remained within normal ranges in obese patients with normal cardiac function and even may be decreased in this population.

    - Therefore pro-BNP assessment is a reliable marker to exclude cardiac failure in the obese patient.

- Average post-op weight loss (110 ± 5.2 kg) at 9-12 months follow-up or half-way weight loss mark, was 20% of initial pre-op weight (137 ± 3.4 kg) ($p < 0.0001$).

- Anthropometric and clinical measurements showed significant post-op improvements.

- Biochemical parameters illustrate improvements of the metabolic syndrome post-surgery over and above what has previously been achieved with drug therapy or behaviour modification in other studies.

- Accurate risk factor scoring to determine eligibility for surgery needs further analysis, but appears promising.

- Using only BMI for the clinical staging of obesity appears to be insufficient and the bariatric surgery model may well be a very useful future tool to investigate pathophysiology in the obese patient and re-determine the clinical staging of the disease.

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Chapter 4: Melanocortin 4 Receptor (MC4R) Polymorphisms in a South African Study Group

4.1 Abstract

Introduction: Obesity is a global epidemic that results in significant morbidity and mortality. Genetic predisposition to obesity is well recognized. Polymorphisms in the melanocortin 4 receptor (MC4R) gene, which codes for a G-protein coupled receptor responsible for post-prandial satiety signalling, have been associated with monogenic obesity. The prevalence of obesity in South Africa is increasing; however the possible role of MC4R mutations has not been studied in this regard.

Aim: To perform a retrospective pilot study on the presence of MC4R polymorphisms and their correlation with body mass index (BMI) in three unrelated groups of South African individuals across a BMI range of 19.6 kg/m^2 (underweight) to 126.0 kg/m^2 (super-obese).

Materials and Methods: Blood was drawn from 259 adult individuals and DNA was extracted from the buffy coat. The MC4R gene was PCR amplified, the amplicon sequenced and the sequences were compared to a reference wild-type sequence.

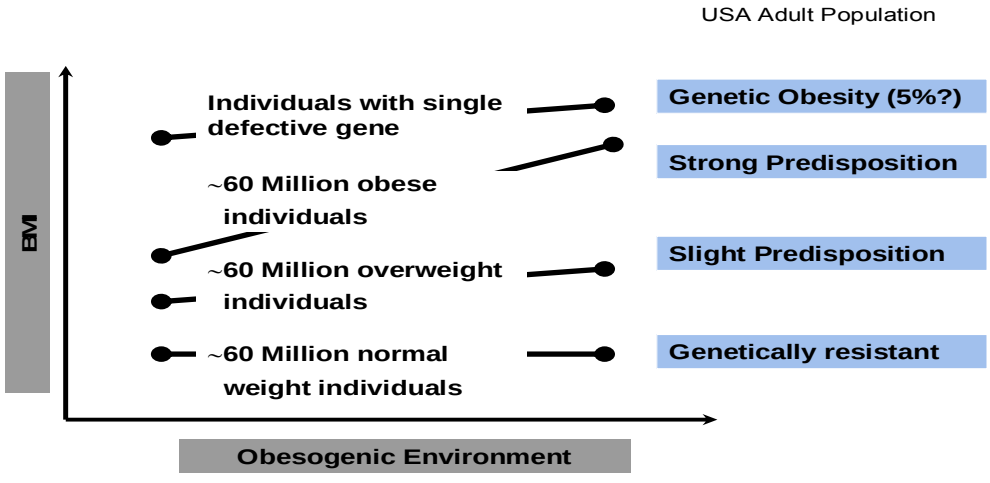
Results: MC4R polymorphisms were identified in 35 of the 259 (13.51%) subjects from the three groups, the majority (23) of which were previously identified non-pathogenic polymorphisms. However, 10 were polymorphisms that have previously been implicated in the pathogenesis of obesity, while two novel polymorphisms, namely R7H and S36T were identified. Four haplotypes were also identified.

Conclusions: The role of MC4R polymorphisms in the development of obesity is indisputable. However, this studies' findings demonstrate that the presence of pathogenic/rare MC4R polymorphisms is not necessarily correlated with BMI. As a secondary outcome, MC4R pathogenic/rare polymorphism frequency differed across ethnic groups in this pilot study, which comprises a limited sample size.

4.2 Introduction

Obesity (body mass index (BMI) > 30 kg/m²) has been recognized as a chronic disease by the World Health Organization (WHO). It has reached epidemic proportions and South Africa has not been spared [1]. The prevalence of obesity is increasing in most African countries, particularly in individuals living in urban areas. Currently, in several parts of South Africa, the prevalence of combined overweight and obesity (BMI > 25) in economically active (18 – 65 years) black-African women has reached the alarming figure of 75% [1].

The genetic susceptibility of certain individuals to the development of obesity has been well documented (Figure 1). In this respect, 40-70% of body weight is genetically determined, while the remainder is determined by the type, quality and quantity of food consumed [1]. Although obesity is most commonly polygenic, rare monogenic forms of obesity do exist, and the affected genes described thus far include leptin, the leptin receptor, pro-opiomelanocortin (POMC), prohormone convertase-1 and the melanocortin receptor genes. Melanocortins play additional roles in pigmentation, inflammation and sexual function [2].



Bouchard, Rankinen. Ob Management. 2005

Figure 1: Polygenic genetic susceptibility to obesity.
(Bouchard C, Rankinen T. Obesity Management 2005; 1(3): 100-104) [3]

Appetite is regulated by many processes that occur either before or after the consumption of a meal. One of these processes involves a part of the central melanocortin system. A subset of neurons within the arcuate nucleus of the hypothalamus express a precursor protein known as POMC, which is post-translationally processed by prohormone convertases into the melanocortins, α-, β-, γ-melanocyte stimulating hormone (MSH) and adrenocorticotropin (ACTH). Cell surface melanocortin receptors are then activated following melanocortin binding [4]. The melanocortin 4 Receptor (MC4R) is a G-protein coupled receptor whose primary function is to regulate food intake following the binding of the agonist α-MSH, which in turn causes the production of a satiety signal through the activation of the cyclic AMP (cAMP) second messenger system [5]. Agouti-related protein (AGRP) is an MC4R antagonist [6] and inverse agonist [9], and when bound produces an orexigenic signal [6].

MC4R deficiency is one of the most common human monogenic disorders [7]. MC4R mutations have a population prevalence of at least 1 in 2000 (0.05%), are found in 0.5%-1% of obese adults [7, 8] and are accountable for 6% of all severe cases of the disease starting in childhood [7, 8, 9, 10]. This low percentage indicates that the mutations have a rather low epidemiological significance; however with regard to the individual they should be considered highly significant [11]. The frequency of MC4R mutations is dependent on the ethnicity of the study group [12], and differing ethnic backgrounds contribute to variance in the penetrance of MC4R mutations [4]. Therefore the role of ethnicity is one that should not be overlooked when performing MC4R mutation studies. Carriers of MC4R mutations will pass these on to their offspring with an 82% frequency and individuals that carry pathogenic mutations have a 4.5 fold increased risk of developing obesity as opposed to non-carriers [12].

MC4R mutations result in an increase in fat mass, lean mass, linear growth, extensive hyperinsulinaemia [13], increase in bone mineral density [10], hyperphagia in early childhood and possibly binge eating disorder (BED). The role of MC4R mutations in BED is controversial [14, 15]. They also present with an elevated prevalence of the metabolic syndrome which includes an increase

in peripheral fat mass ratio, type 2 diabetes, dyslipidaemia and hypertension [16]. There is however no effect on reproductive function or fertility.

Functional defects within the MC4R gene that are responsible for obesity include: decreased or absent ligand binding, decreased cell surface receptor expression because of intracellular retention of mutant receptors, incorrect protein folding (which results in the receptor never being released from the endoplasmic reticulum) [17] and a reduction in signal transduction [6]. It has been suggested that the most common functional defect is a reduction in the constitutive activity of the receptor [6].

MC4R mutations have a co-dominant pattern of inheritance with modulation of penetrance and expressivity, and thus homozygotes are known to be more obese that heterozygotes [18]. Certain mutations have a recessive pattern of inheritance while others lead to the production of a receptor that is indistinguishable from the wild-type, rendering it non-pathogenic [19]. Two hypotheses have been posed as to why discrepancies exist when considering phenotypic penetrance of MC4 mutations; firstly differing ethnic backgrounds illustrate varying penetrance, as already mentioned, and secondly whether or not the mutation leads to a functional defect [4]. In addition, the variability of expression of obesity in individuals carrying MC4R mutations suggests that MC4R may interact with other genetically-determined elements in the control of appetite and body weight, and may be affected by environmental factors [10].

Children that harbour polymorphisms within the MC4R gene have severe early onset obesity; however adult individuals seem to have a BMI that is more middle of the range than at the upper end of the scale. A compelling study investigated this phenomenon using a pedigree analysis, and found that the proband, who was a fourteen year old boy and had severe early onset obesity, had received the mutation in the MC4R gene from his father [20]. The father, who had a BMI in the range of 30-40 kg/m^2, reported having had severe obesity during childhood, but as he aged he did not progress to a morbidly obese state [20]. This observation illustrates that obesity associated with certain MC4R polymorphisms is most prominent in younger individuals in

the form of severe early onset obesity; however as these individuals age it becomes less pronounced [20]. This suggests that when searching for mutations within this gene in an adult population group, they may be more prevalent in patients that have a BMI that is more "moderate" and not in the so-called morbidly obese end of the spectrum [20]. Male and female adult subjects harbouring MC4R mutations do however have an increased risk of becoming obese, estimated to be an increase in BMI of ~4 and ~9.5 kg/m^2 respectively when compared to wild-type relatives [21].

In 2008 a total of 100 mutations within the MC4R gene had been described [22]. MC4R mutations are not always responsible for causing obesity; this statement is extrapolated from *in vitro* findings which have revealed normal receptor functioning in obese individuals and conversely loss-of-function mutations in the non-obese subjects [4]. Functional characterization of a purportedly defective MC4R is of utmost importance when concluding that the mutation is in fact responsible for the obesity observed [4]. Research in this field is non-existent in South Africa.

Thus the primary aim of this initial pilot project was to perform a retrospective assessment of the relationship between MC4R polymorphisms and BMI within 3 non-randomly selected groups of South African individuals. A secondary aim of the study was to investigate the effect of ethnicity on MC4R frequency within the 3 above-mentioned groups.

4.3 Materials and Methods

4.3.1 Patient Selection and Sampling

A total of 259 adult (age – mean ± SEM: 43.9 ± 0.92) patients were analyzed.

Patient recruitment strategy (Ethics approval – Appendix B):

- Patients were asked whether or not they would like to participate in the study.

- They were informed about what was going to be done with their blood samples.
- They were then asked to sign informed consent forms which had previously been approved by the University of Pretoria Ethics Committee.
- Anthropometric measurements (height, weight and waist circumference) were recorded.
- Blood was drawn by authorised medical personal using EDTA blood collection tubes and transported to the laboratory for further analysis.

Patient Groups:

Group 1:
This group comprised 109 individuals that were not on treatment for obesity or its co-morbidities. The mean BMI for this group was 26.3 ± 0.63 kg/m^2 and the BMI range was from 15.6 to 42.4 kg/m^2.

Group 2:
This group comprised 100 patients, all of whom had been seen at a tertiary hospital for anti-diabetic treatment. The mean BMI for this group was 27.6 ± 1.02 kg/m^2 and the BMI range was from 15.9 to 55.7 kg/m^2. A possible limitation with this group might be that the treatment regimes (insulin, biguinides, sulphonylureas or a combination of these) could have affected weight and thus BMI.

Group 3:
This group comprised 50 overweight (BMI > 25) and obese (BMI > 30) subjects that had been clinically assessed for obesity and its co-morbidities by a registered endocrinologist. This groups' mean BMI was 35.98 ± 2.2 kg/m^2 and BMI ranged from 27.4 to 49.8 kg/m^2. These subjects formed part of another study designed to assess the pharmacological efficacy of Topiramate® on sustained weight loss; the anthropometric parameters for this group were however recorded before the intervention.

The ethnic distribution was as follows: 157 (60.6%) black African (BL), 60 (23.2%) Caucasian (CA), 21 (8.1%) Coloured or mixed race (CL) and 21 (8.1%) Indian (IN) (Figure 4).

4.3.2 DNA Extraction

Whole blood was centrifuged at 2000 rpm in a macrocentrifuge (Beckman Coulter, Inc. Fullerton, California, USA) in order to separate it into serum, buffy coat and red blood cells. 200 µl buffy coat was removed and DNA extracted using the Genomic DNA Purification kit (Fermentas Life Sciences, Inc. Maryland, USA).

4.3.3 PCR Amplification

PCR was performed in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA). Conditions of amplification were as follows: 95ºC / 5 min; 30 cycles of 94ºC / 30s, 58ºC / 30s, 72ºC / 2min; and a final elongation step of 72ºC for 10min. Primers were designed from the MC4R reference sequence (GenBank accession no. NM_005912) and their sequences were: MC4 EF - 5' - GCT CTG GAC TTG TGA CAT TTA C - 3' and MC4 ER - 5' - CCA GTA CCC TAC ACG GAA GAG - 3' (Figure 2). PCR reactions were carried out in a total volume of 50 µl, which included 2µl (~ 100ng/µl) genomic DNA, 1.25U GoTaq® Flexi DNA Polymerase (Promega, Madison WI, USA), 10 µl 5X buffer, 25 pmol of each primer, 200µM of each dNTP, and 1.5mM $MgCl_2$. PCR products were visualized under UV light on 1% agarose gels and purified using the DNA Clean and Concentrator -5 kit (Zymo Research Corporation © 2005-2006).

4.3.4 DNA Sequencing

Sequencing was performed by Inqaba Biotechnical Industries Pty. Ltd., South Africa using the ABI Big Dye Terminator Cycle Sequencing kit version 3.1 (Applied Biosystems Inc., Foster City, California). Cycling conditions were as follows: 94ºC / 2min; 30 cycles of 94ºC / 10s, 50ºC / 10s, 60ºC / 4min. Primers

used included the PCR primers as externals and two newly designed internal primers: MC4 IF 5' – GCA GTG GAC AGG TAC TTT ACT ATC – 3' and MC4 IR 5' – GTC ATA ATG TTA TGG TAC TG – 3' (Figure 2). Reactions were carried out in a total volume of 10µl, which included 0.75µl purified PCR product, 1.5 pmol primer, 2.25µl 5x dilution buffer and 1µl ABI Prism Big Dye Terminator mix, v3.1 (Applied Biosystems). The sequencing products were cleaned using the ZR-96 DNA Sequencing Clean-up kit™ (Zymo Research Corporation © 2005-2006). Products were run on the Applied Biosystems / Hitachi 3130x1 Genetic Analyzer.

Note: The MC4R gene was sequenced once in every patient.

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1       aaagcaacgc tcaggctgga aacagaagct tccgagaggc agccgatgtg agcatgtgcg
61      cacagattcg tctcccaatg gcatggcagc ttcaaggaaa attattttga acagacttga
121     atgcataaga ttaaagttaa agcagaagtg agaacaagaa agcaaagagc agactctttc
181     aactgagaat gaatattttg aagcccaaga ttttaaagtg atgatgatta gagtcgtacc
241     taaaagagac taaaaactcc atgtcaagct ctggacttgt gacatttact cacagcaggc
301     atggcaattt tagcctcaca actttcagac agataaagac ttggaggaaa taactgagac
361     gactccctga cccaggaggt taaatcaatt caggggggaca ctggaattct cctgccagca
421     tggtgaactc cacccaccgt gggatgcaca cttctctgca cctctggaac cgcagcagtt
481     acagactgca cagcaatgcc agtgagtccc ttggaaaagg ctactctgat ggagggtgct
541     acgagcaact ttttgtctct cctgaggtgt ttgtgactct gggtgtcatc agcttgttgg
601     agaatatctt agtgattgtg gcaatagcca agaacaagaa tctgcattca cccatgtact
661     ttttcatctg cagcttggct gtggctgata tgctggtgag cgtttcaaat ggatcagaaa
721     ccattgtcat caccctatta aacagtacag atacggatgc acagagtttc acagtgaata
781     ttgataatgt cattgactcg gtgatctgta gctccttgct tgcatccatt tgcagcctgc
841     tttcaattgc agtggacagg tactttacta tcttctatgc tctccagtac cataacatta
901     tgacagttaa gcgggttggg atcatcataa gttgtatctg ggcagcttgc acggtttcag
961     gcattttgtt catcatttac tcagatagta gtgctgtcat catctgcctc atcaccatgt
1021    tcttcaccat gctggctctc atggcttctc tctatgtcca catgttcctg atggccaggc
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1141    tgaagggagc gattaccttg accatcctga ttggcgtctt tgttgtctgc tgggccccat
1201    tcttcctcca cttaatattc tacatctctt gtcctcagaa tccatattgt gtgtgcttca
1261    tgtctcactt taacttgtat ctcatactga tcatgtgtaa ttcaatcatc gatcctctga
1321    tttatgcact ccggagtcaa gaactgagga aaaccttcaa agagatcatc tgttgctatc
1381    ccctgggagg cctttgtgac ttgtctagca gatattaaat ggggacagag cacgcaatat
1441    aggaacatgc ataagagact ttttcactct taccctacct gaatattgta cttctgcaac
1501    agctttctct tccgtgtagg gtactggttg agatatccat tgtgtaaatt taagcctatg
1561    atttttaatg agaaaaaatg cccagtctct gtattatttc caatgtcatg ctacttttt
1621    ggccataaaa tatgaatcta tgttataggt tgtaggcact gtggatttac aaaaagaaaa
1681    gtccttatta aaagctt
```

Figure 2: Map of the MC4R gene highlighting the four primer sequences (EF, ER, IF, IR) used in PCR and sequencing reactions as well as the start and termination codons.

4.3.5 Sequence Analysis

Sequence result electropherograms were edited using FinchTV Version 1.4.0 (Copyright © 2004-2006, Geospiza Inc.). Alignments were carried out using CLC Free Workbench Version 4.0.1 (CLC bio A/S 2005) with gap settings as follows: gap open cost: 10; gap extension cost: 1; end gap cost: as any other. The alignment was set at the "Slow (very accurate)" setting.

4.4 Results

4.4.1 PCR Amplification

Following PCR amplification, a strong amplicon product was visualized in 1% agarose gels. The product was approximately 1200 base pairs (bp) in length as expected (Figure 3).

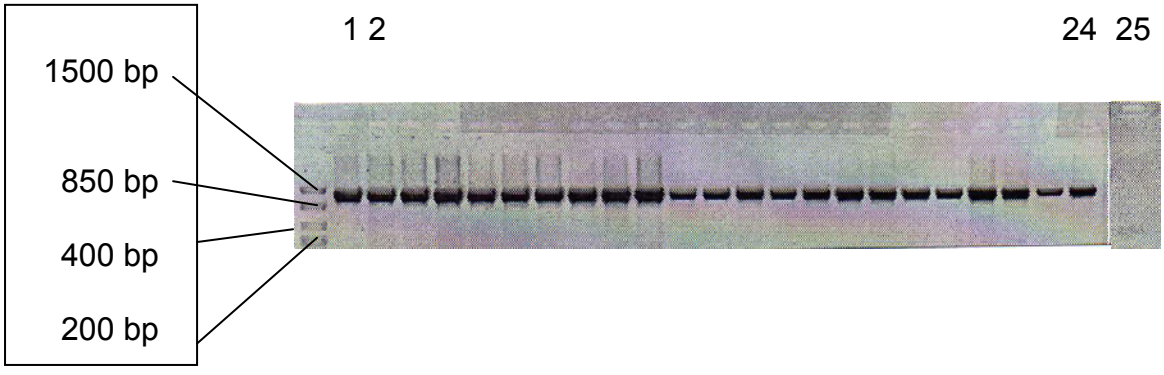

Figure 3: PCR amplification products of the MC4R gene. Lane 1: FastRuler™ DNA Ladder, Low Range (Fermentas Life Sciences). Lanes 2-24: MC4R gene amplicons. Lane 25: negative control.

4.4.2 DNA Sequencing

35 of the 259 (13.51%) subjects analyzed presented with MC4R single nucleotide polymorphisms (SNPs), in heterozygotic form (Tables 1, 2 & 3; Figures 4, 5, 6, 7 & 8). Of these 35 subjects, 23 (8.89%) had common non-pathogenic polymorphisms that have been found with high frequency in other study populations (Table 1; Figures 5, 7 & 8) while 12 (4.63%) had either pathogenic or rare mutations (Tables 2 & 3; Figures 6, 7 & 8). In this latter group, 10 subjects had previously described mutations while 2 had novel mutations.

Table 1 provides a comparison of the number of individuals within each ethnic group, as well as those with pathogenic/rare and non-pathogenic/common polymorphisms. The average age of the carriers was 45.9 ± 2.71 years; 31.4% were men and 68.6% were women.

Table 1: Number of individuals from the various ethnic groups including pathogenic/rare and non-pathogenic/common polymorphism carriers.

CATEGORIES	ETHNIC GROUPS			
	Black African	**Caucasian**	**Indian**	**Mixed Race**
Sample size	157	60	21	21
Carriers	29	2	0	4
Pathogenic / rare	10	0	0	2
Non-pathogenic / common	19	2	0	2

Most carriers presented with one SNP, however four patients had two SNPs in the form of a haplotype (Table 1 & 2). The non-pathogenic/common V103I [21, 23, 24, 25, 26, 27] sequence variant was most commonly identified within a total of 20 individuals (4 from group 1, 12 from group 2 and 4 from group 3), two of whom possessed an additional variant (I251L and I170V) forming a haplotype.

V103I was also identified in an individual that was super obese (BMI = 126.0 kg/m^2). Within the non-pathogenic/common mutation carrier group there was a large BMI range (19.6 – 126.0 kg/m^2) (Table 1 & Figure 5).

The next most common mutation was the pathogenic/rare I170V [28, 29] (Table 2 & Figure 6) variant which was found in 7 subjects; 2 from group 2 (1 in haplotype with V103I) and 5 from group 1; 4 of the 5 obese individuals within the pathogenic/rare mutation carrier group were carriers of I170V (Table 2 & Figure 6).

The pathogenic/rare R165Q mutation [23, 30] was identified in an individual from group 2 who was slightly underweight (BMI – 24.1 kg/m^2) (Table 2 & Figure 6). I251L, another non-pathogenic/common polymorphism that has been detected previously [10, 20, 23, 25] was found in haplotype form with the V103I variant in an individual from group 3 who fell into the overweight category (BMI – 29.3 kg/m^2) (Table 1 & Figure 5). The pathogenic/rare F202L mutation identified by Tao and Segaloff in 2005 was found in two individuals, one being overweight (BMI – 27.2) from group 1 and the other being morbidly obese with a BMI of 49.5 from group 2 (Table 2 & Figure 6). The F202L mutation in the context of the current study was only ever found in haplotype form with the same sense I198I mutation. Sequence variants that had not been identified in previous studies included the same sense mutation I198I, R7H and S36T (Tables 3; Figure 7 & 8).

Table 2: Non-pathogenic/common MC4R polymorphisms and their corresponding measurements (group 1 individuals represented in black; group 2 individuals in red and group 3 individuals in grey).

Patient Code	Age	Ethnic Group	Gender	BMI (kg/m^2)	Waist (cm)	Nt change (position)	AA change
1) DJM	48	CA	M	34.8	114	G-A (726) A-C (1170)	V103I I251L
2) NCM	48	BL	F	49.1	128	G-A (726)	V103I
3) OMJ	42	CA	F	32.4	89	G-A (726)	V103I
4) HER	19	BL	F	126.0	X	G-A (726)	V103I
5) HK	75	BL	F	32.0	89	C-T (1013)	I198I
6) A-BL-12	39	BL	F	25.1	82	G-A (726)	V103I
7) A-BL-16	30	BL	F	34.9	91	C-T (1013)	I198I
8) A-BL-40	28	BL	M	19.6	73.8	G-A (726)	V103I
9) A-BL-42	30	BL	M	20.3	80	G-A (726)	V103I
10) A-BL-45	30	BL	M	20.2	72	G-A (726)	V103I
11) 5	68	BL	F	29.5	X	G-A (726)	V103I
12) 20	64	BL	F	36.9	X	G-A (726)	V103I
13) 32	63	BL	F	30.8	X	G-A (726)	V103I
14) 51	58	BL	M	22.0	X	G-A (726)	V103I
15) 52	60	BL	M	39.2	X	G-A (726)	V103I
16) 76	34	BL	M	28.9	X	G-A (726)	V103I
17) 77	54	BL	F	29.8	X	G-A (726)	V103I
18) 80	24	BL	F	27.4	X	G-A (726)	V103I
19) 82	56	BL	F	34.0	X	G-A (726)	V103I
20) 88	52	CL	F	32.7	X	C-T (1013)	I198I
21) 90	74	CL	F	24.4	X	G-A (726)	V103I

22) 92	71	BL	M	26.2	X	G-A (726)	V103I
23) 96	59	BL	M	37.1	X	G-A (726)	V103I

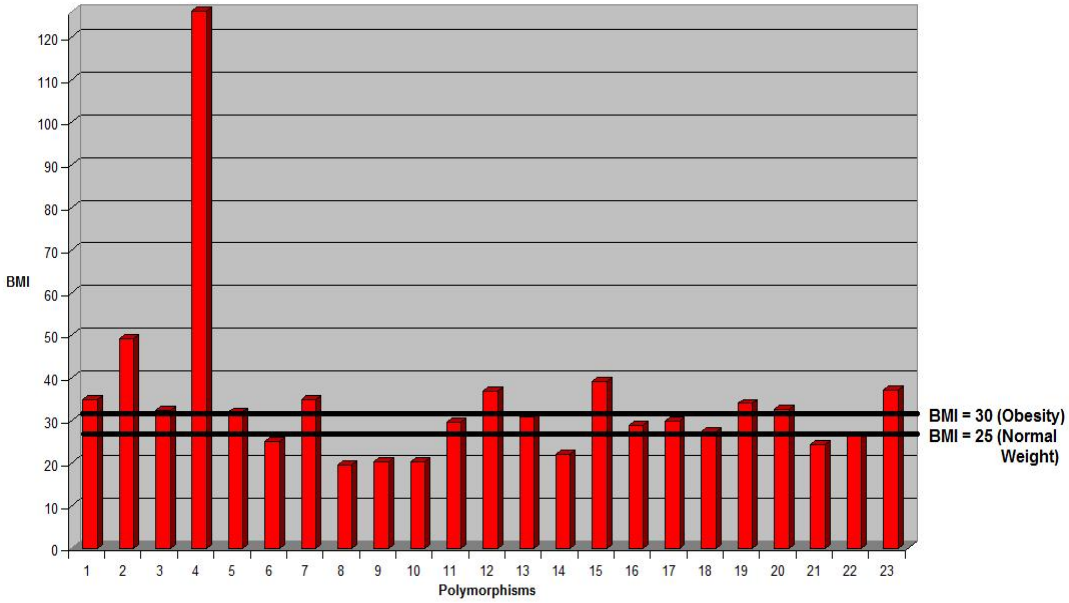

Figure 4: Relationship between non-pathogenic/common MC4R polymorphisms and BMI (numbers on the x-axis correspond to the highlighted numbers in Table 1).

Table 3: Pathogenic/rare MC4R polymorphisms and their corresponding measurements (group 1 individuals represented in black; group 2 in red and group 3 in grey).

Patient Code	Age	Ethnic Group	Gender	BMI (kg/m^2)	Waist (cm)	Nt change (position)	AA change
1) 43	71	CL	F	37	X	G-A (726) A-G (927)	V103I I170V
2) 55	33	CL	M	49.5	X	C-T (1013) C-A (1025)	I198I F202L
3) 57	42	BL	F	25.6	X	A-G (927)	I170V
4) 86	32	BL	F	24.1	X	G-A (913)	R165Q
5) A-BL-10	42	BL	F	42.4	118	A-G (927)	I170V
6) A-BL-13	58	BL	M	31.2	107	A-G (927)	I170V
7) A-BL-52	43	BL	F	28.9	95	A-G (927)	I170V
8) A-BL-55	29	BL	F	25.2	78	A-G (927)	I170V
9) A-BL-70	24	BL	F	27.2	82.5	C-T (1013) C-A (1025)	I198I F202L
10) EM	44	BL	F	53.1	107.5	A-G (927)	I170V

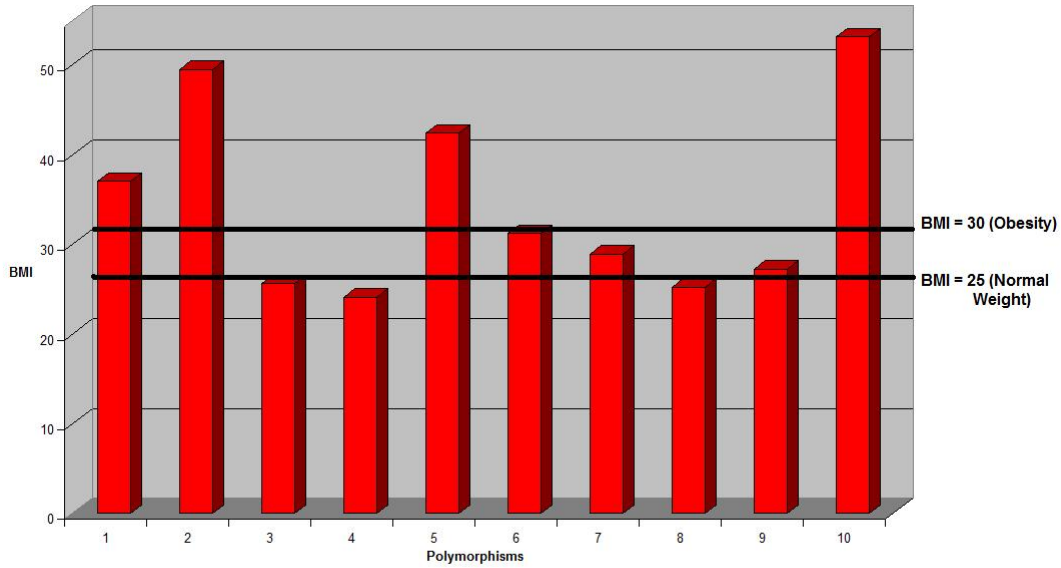

Figure 5: Relationship between pathogenic/rare MC4R polymorphisms and BMI (numbers on the x-axis correspond to highlighted numbers in table 2).

Table 4: Rare/unknown MC4R polymorphisms and their corresponding measurements (group 1 individual represented in black and group 2 in red).

Patient Code	Age	Ethnic Group	Gender	BMI (kg/m^2)	Waist (cm)	Nt change (position)	AA change
1) 26	28	BL	M	22.6	X	G-A (439)	R7H
2) A-BL-38	38	BL	F	28.2	91	T-A (525)	S36T

To sum up the results; 22 individuals were carriers of non-pathogenic/common polymorphisms: V103I (19), I198I (3); 11 obese, 6 overweight and 5 lean. Nine individuals were pathogenic/rare polymorphism carriers: I170V (6), R165Q (1), R7H (1), S36T (1); 3 obese, 4 overweight and 2 lean. The 4 individuals that harboured haplotypes (1 [V103I + I251L], 1 [V103I + I170V], 2 [I198I + F202L]) were all overweight, and 2 of them presented with morbid obesity (Tables 1 & 2; Figures 4 & 5).

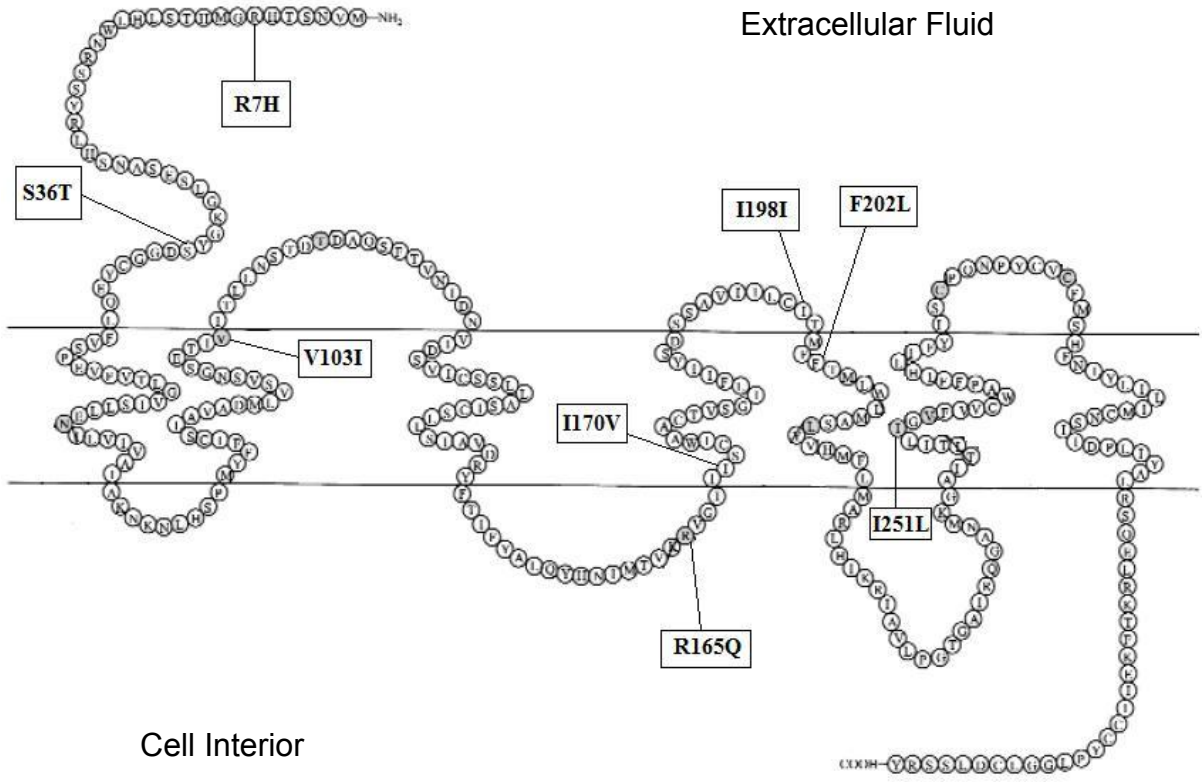

Figure 6: Amino acid sequence of the MC4 receptor depicting sequence variants identified and their respective positions. (Original unedited diagram - Farooqi *et al.* 2000 [19]).

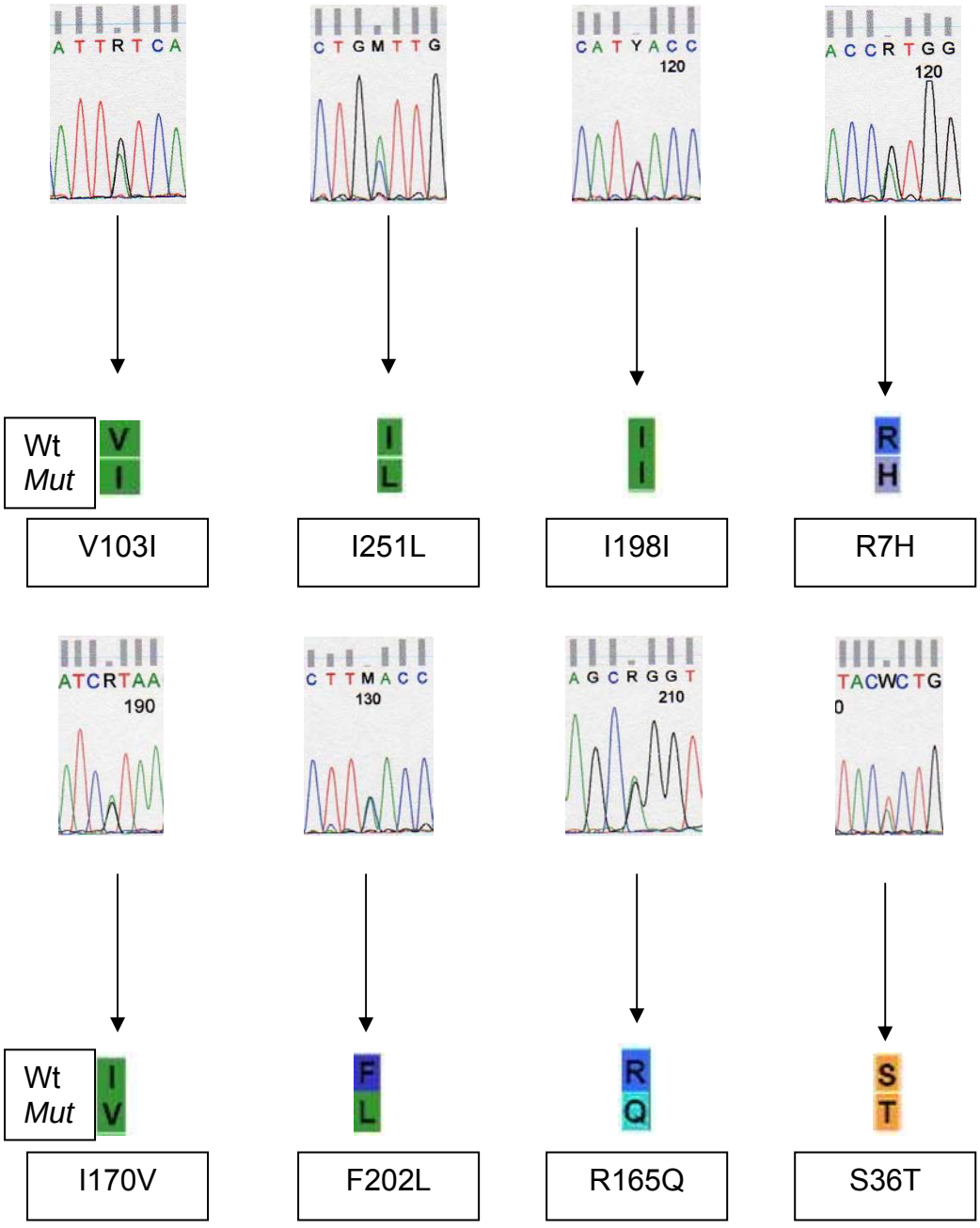

Figure 7: MC4R gene polymorphisms (electropherograms) and the resultant amino acid alterations in the receptors' polypeptide sequence. Amino acid sequences show reference sequence amino acid on the top row (Wt) and mutant amino acid on the bottom (*Mut)*.

4.5 Discussion

The 35 occurrences of MC4R polymorphisms found in this study need to be separated on the basis of common, non-pathogenic polymorphisms vs rare, missense mutations that are more likely to cause disease. A total of 12 out of 259 (4.63%) individuals harboured polymorphisms from the latter group.

4.5.1 Haplotypes

A total of four haplotypes were identified in this study; two of the individuals were overweight and the other two were morbidly obese. Two of the four haplotypes were formed with the V103I allele; V103I + I251L was identified in an individual from group 3 who had a BMI of 29.3 kg/m^2, both of these mutations when previously functionally characterised caused the receptor to function normally [10, 11, 23, 24, 26, 27, 28]. V103I + I170V was identified in a group 2 individual who was morbidly obese (BMI 37.0 kg/m^2). The V103I allele has appeared in previous haplotype analyses and has illustrated consistency in forming haplotypes [24]. The V103I + S127L haplotype was previously identified in a morbidly obese Finnish child and resulted in a defective receptor in which ligand binding and/or signal transduction were affected [20]. Although the V103I mutation has been described as having no effect on the function of the receptor [10, 23, 24, 27] and possibly even as having a negative association with obesity [26], it has been proposed that additional alleles that are in linkage disequilibrium with the mutant allele could be contributing to obesity [26]. Taken together, these findings highlight the possibility that the presence of the V103I allele in haplotype formation may well be associated with a defect in MC4R function.

The other haplotype was I198I + F202L that was identified in two individuals, one being overweight (BMI – 27.2 kg/m^2) from group 1 and the other morbidly obese (BMI – 49.5 kg/m^2) from group 2. The I198I mutation has no effect on receptor function. The F202L mutation, which was only identified within this haplotype in this study, is located in the 5th transmembrane domain of the receptor. Although it conveys an amino acid change that does not alter amino

acid properties (non-polar, hydrophobic), its carriers were overweight and obese. The F202L polymorphism has previously been identified in non-obese individuals [17]. When functionally characterized *in vitro* it demonstrated a decrease in basal receptor activities [17]; it is therefore possible that it could result in obesity due to sub-optimal receptor function.

4.5.2 Non-pathogenic MC4R Polymorphisms: V103I and I251L

The V103I polymorphism is controversial. It was found to have no functional effect [23], it has a similar frequency in obese and non-obese subjects [10, 24, 27] and even a negative association to obesity [24, 25, 31]. When functionally characterized *in vitro* it is similar to the wild-type receptor [24]; it is also implicated in a gain-of-function phenotype that results in protection of the carrier against obesity [24]. As already discussed, it commonly forms haplotypes, which could have effects on receptor expression and thus phenotype. Association of the V103I polymorphism with decreased triglyceride levels as well as a trend towards decreased LDL and increased HDL cholesterol levels [26] again shows that it may be negatively associated with obesity and its co-morbidities. Finally Xiang and colleagues found that the V103I receptor had a 2-fold reduced potency when assayed for AGRP binding, which therefore increasing satiety due to reduced inverse agonist interaction.

The findings of this study identified the V103I polymorphism within individuals from the underweight (BMI – 20.2 kg/m^2) to the super-obese (BMI – 126.0 kg/m^2) i.e. at a similar in frequency across BMI ranges. It was the most commonly identified mutant allele with a total of 20 carriers, 10 of them being black African women. Within this particular ethnic group the prevalence of combined overweight and obesity (BMI > 25) has reached a figure of 75% in South Africa [1]. The V103I polymorphism is non-pathogenic and even negatively associated with obesity, and is therefore unlikely to affect the phenotype of its carriers.

The V103I and I251L polymorphisms identified within this study have previously been identified as being high in frequency, common and non-pathogenic within several populations [10, 11, 23, 24, 26, 27, 28]. The variance in BMI across the individuals within this sub-group is therefore most likely due to other factors including polygenic interactions and/or diet and lifestyle. These mutations should be considered separately from rare or pathogenic polymorphisms; however their total disregard is inappropriate as they may be useful in other settings such as in linkage disequilibrium studies.

4.5.3 Pathogenic or Rare Polymorphisms

The role of the I170V mutation in MC4R function is controversial. Tao and Segaloff (2003) [28] stated that this mutation did not affect function with respect to cell surface expression, agonist binding and cAMP production, and therefore not significantly different from the wild-type receptor, and have thus questioned its role in obesity. However in 2006 Lubrano-Berthelier and colleagues [15] stated that it does result in a loss-of-function phenotype due to decreased cell surface expression.

In this study the I170V mutation was identified across a broad spectrum of BMI's from normal to obese in all 3 of the study groups. A mixed race, obese (BMI – 37.0 kg/m^2), diabetic woman harboured the I170V polymorphism in a haplotype with the V103I polymorphism. There were 5 black African women carriers; 2 normal weight, 1 overweight and 2 with morbid obesity. An obese black African man also presented as an I170V carrier. Although there was thus no consistent finding with regard to the I170V polymorphism, it was observed in 4 out of 5 obese individuals in the pathogenic mutation group. It is possible therefore based on these and previous findings, that it causes decreased cell surface expression and thus produces a loss-of-function phenotype, which would explain its prevalence within the obese patients. Genotype-environment interactions could however also be contributing to specific phenotypic traits, and carriers of the I170V polymorphism might be predisposed to obesity in the wrong lifestyle. This would explain why it is present within overweight and normal weight individuals.

Tao and Segaloff in 2003 [28], following *in vitro* functional studies of the I170V and other MC4 polymorphisms, stated that "Whether and how these variants cause energy imbalance and therefore obesity is unclear." Unfortunately this is still the case as carriers of the I170V polymorphism do not present with a specific phenotype. The co-dominant pattern of inheritance of MC4R mutation carriers suggests that there is modulation of penentrance and expressivity of mutant phenotypes [18]. This theory might explain why there is such wide variance of phenotype among carriers of the same polymorphism and a defiance of the expected monogenic trend. Aside from functional defects of mutant receptors, Tao *et al.* 2005 suggest that differing ethnic backgrounds illustrate varying penetrance. This again is not confirmed in this study as black African carriers of the same polymorphism (I170V) showed wide phenotypic variance.

The I170V and F202L polymorphisms have been reported previously [15, 17, 28] and are associated with obesity. These mutations were seen more frequently within the black African group of this study with 7 individuals from this ethnic group having these mutations. The black African group is of particular concern in South Africa, specifically black South African females, who are becoming more obese due to factors such as urbanization, education level and cultural perceptions of being overweight [32]. A more urbanized diet, lack of education about dietary intake and false perceptions of being overweight (overweight representing prosperity, happiness and health in this culture [32]), on a backdrop of an increased prevalence of rare pathogenic mutations and/or predisposition to polygenic obesity, could be indicative as to why individuals from this ethnic group are experiencing a rapid rise in overweight and obesity and warrants further investigation.

In earlier studies, the R165Q polymorphism was associated with considerably reduced receptor activity when in the presence of endogenous agonists (15-90 fold reduction) [23, 30], although activity with synthetic ligands was only slightly reduced (2-9 fold) [23]. The R165Q polymorphism was identified in 10 obese Pima Indians and showed a strong correlation with the development of early onset obesity (Ma and colleagues [30]). It also effects cell surface

expression of the receptor and has been described as “rare” within populations [30]. In this study the R165Q polymorphism was identified within a normal weight (BMI – 24.1), diabetic, black African woman. This is in contrast to previous observations that link it to obesity. This patient reported that she was of normal weight as a child, and thus the R165Q polymorphism was not associated in an early onset form of the disease. Thus the expected phenotype based on previous findings was not seen in this carrier. Perhaps this polymorphism does not confer obesity on individuals within this specific ethnic group, suggesting that different ethnic backgrounds illustrate varying phenotypic penetrance as suggested by Tao and colleagues [4].

In this study, two mutations were found that have not been identified previously. The first, R7H was discovered in an underweight (BMI – 22.6) black African, diabetic male. This mutation is located in the N-terminus of the MC4R which has been associated with the receptors’ constitutive activity [6, 9, 33]. The N-terminus is also responsible for the binding of AGRP and thus its’ inverse agonistic effect on the receptors’ constitutive activity. The R7H mutation could compromise this interaction and thus cause an increase in the receptors’ basal or constitutive activity. This would ultimately result in an elevated satiety signal within the carrier and could even cause an anorexigenic milieu leading to, in extreme cases, cachexia. This rationale would explain why the carrier in this study is underweight as he may harbour a receptor that is constantly constitutively active and is never subject to the control of the inverse agonist AGRP.

The S36T polymorphism is also located within the N-terminus of MC4R. It was identified within an overweight (BMI – 28.2) black African woman from group 1. Due to its position it too may have an effect on the constitutive activity of the receptor. The phenotype of the individual suggests that the patient may have a tendency to develop obesity. A hypothesis could be that the mutant receptor could produce compromised basal activity and thus have an effect on long-term weight regulation. This however can only be proven through receptor functional studies.

The pathogenic or rare polymorphisms identified in this study are likely to be disease causing. Further study will be needed to investigate whether or not MC4R pathogenic or rare polymorphisms are in fact higher in frequency in South Africa, particularly within the black South African sub-group.

Table 4 provides a summary of all polymorphisms found in this study and classifies them under the headings of: Type (non-pathogenic/common or pathogenic/rare), Designation (position and amino acid change), Haplotype (how many and which polymorphisms), Functional Consequence (what effect the polymorphism has on the MC4 receptor) and Prevalence in this Study (total number of times the polymorphism was identified and a breakdown of its frequency within the four ethnic groups).

Table 5: Summary of mutations/polymorphisms found in this study.

Type	Designation	Haplotype	Functional Consequence	Prevalence in this Study
Non-pathogenic / common	V103I	2: (V103I+I251L) (V103I+I170V)	None; possibly gain-of-function; protection against obesity	**Total:** 20 **BL:** 17 **CA:** 2 **CL:** 1 **IN:** 0
	I251L	1: (I251L+V103I	None	**Total:** 1 **BL:** 0 **CA:** 1 **CL:** 0 **IN:** 0

	I198I	2: both (I198I+F202L)	None	**Total:** 5 **BL:** 3 **CA:** 0 **CL:** 2 **IN:** 0
Pathogenic / rare	I170V	1: (I170V+V103I)	Decreased cell surface expression	**Total:** 7 **BL:** 6 **CA:** 0 **CL:** 1 **IN:** 0
	F202L	2: both (F202L+I198I)	Decreased basal/constitutive receptor activities	**Total:** 2 **BL:** 1 **CA:** 0 **CL:** 1 **IN:** 0
	R165Q	0	Reduced agonist binding; decreased cell surface expression	**Total:** 1 **BL:** 1 **CA:** 0 **CL:** 0 **IN:** 0
	R7H	0	Unknown	**Total:** 1 **BL:** 1 **CA:** 0 **CL:** 0 **IN:** 0
	S36T	0	Unknown	**Total:** 1 **BL:** 1 **CA:** 0 **CL:** 0 **IN:** 0

4.5.4 Concluding Remarks

The BMI ranges associated with pathogenic/rare (22.6 – 53.1 kg/m^2) vs non-pathogenic/common (19.6 – 126.0 kg/m^2) polymorphisms do not correspond to current trend of pathogenic/rare MC4R polymorphisms being associated with obesity. Although polymorphisms that result in defective receptor function will add to an individuals' susceptibility to developing obesity, additional mechanisms working in tandem with the mutant receptor may lead to the final phenotype. This hypothesis is supported by Dempfle and co-workers [21] who indicated that men and woman adult subjects harbouring MC4R mutations do have an elevated risk of obesity, leading to an increase of ~4 and ~9.5 kg/m^2 respectively when compared to BMI of wild-type relatives. Thus the risk of obesity in MC4R mutation carriers is elevated; however the extent to which the mutant receptor is responsible for this phenotype requires further clarification. The MC4R trend defiance in this study is not aligned with the monogenic model of MC4R gene polymorphisms and indicates that further investigation needs to be done to exactly define their pathophysiology.

As a secondary outcome and in agreement with Mackenzie and colleagues, ethnicity is clearly a factor in MC4R mutation frequency investigation; 10 out of 12 pathogenic/rare mutation carriers were from the black African group in this study. This may provide a clue as to why more than half of this population group fall into the overweight and obese categories, however it must be said that the sample was bias toward this particular ethnic group, thus further investigation is needed into this initial finding.

In agreement with Lubrano-Berthelier *et al.* 2006 [15], it was found that no specific clinical phenotype can be utilised to predict whether or not a severely obese adult is an MC4R mutation carrier. However their statement that there is a positive relationship between the presence of any functionally relevant MC4R mutation and the severity and onset of obesity must be challenged in light of this studies' results. The presence of pathogenic/rare MC4R polymorphisms did not always cause obesity and specifically in the case of the R165Q mutation, the individual did not have early-onset or adulthood

obesity. In contrast to this, this study found that non-pathogenic/common MC4R polymorphism carriers have obesity and in particular a V103I carrier presented with super-obesity (BMI – 126.0 kg/m^2). Lubrano-Berthelier *et al.* 2006 [15] also stated that the inclusion of common non-pathogenic polymorphisms in MC4R mutation-obesity studies could lead to complications and improper clinical decisions and thus they should be omitted. The results of this study agree with this statement.

Therefore in light of the aim of this study the presence of pathogenic/rare MC4R polymorphisms is not necessarily correlated with BMI. As a secondary outcome, ethnicity did seem to affect pathogenic/rare mutational frequency. However further study is required in order to confirm this.

The significance of MC4R mutations in the causation of severe human obesity within both early and late onset forms of the disease must be questioned. There is no doubt that they have an effect on an individuals' predisposition to obesity, but the extent to which they contribute to the disease needs further clarification. The use of more complex *in vitro* systems and *in vivo* animal models to investigate the pathophysiological mechanisms of mutant MC4Rs will hopefully provide this.

4.6 Summary of Results

- PCR amplification of the MC4 receptor gene gave a product that was approximately 1200 bp in length.

- 35 out of 259 (13.51%) subjects analyzed presented with MC4R gene SNPs and all were heterozygous.

- 12 (4.63%) individuals carried either pathogenic or rare MC4R mutations, and 23 (8.89%) were non-pathogenic, common polymorphisms carriers; the latter are usually high in frequency within study populations.

- 4 haplotypes were identified.

- The V103I non-pathogenic/common polymorphism was observed in 20 subjects.

- The pathogenic/rare I170Vpolymorphism was observed in 7 subjects, 4 of whom were obese.

- The pathogenic/rare R165Q polymorphism was observed in a diabetic individual that was underweight (BMI – 24.1 kg/m^2).

- The pathogenic/rare F202L mutation was observed in 2 individuals (1 from group 1: BMI – 27.2 kg/m^2 and 1 from group 2; BMI – 49.5 kg/m^2). It was only ever found in a haplotype with the same sense I198I polymorphism.

- Newly identified polymorphisms: I198I, R7H, S36T.

- Non-pathogenic/common MC4R polymorphism carriers ranged in BMI from 19.6 kg/m^2 – 126.0 kg/m^2.

- Pathogenic/rare MC4R polymorphism carriers ranged in BMI from 24.1 kg/m^2 – 53.1 kg/m^2.

4.7 Conclusions

- MC4R polymorphisms need to be classified on the basis of common, non-pathogenic polymorphisms vs rare, missense mutations that are more likely to be disease causing.

- There was a wide variance of phenotypes among carriers of the same pathogenic polymorphism and a defiance of the expected monogenic

trend. The co-dominant pattern of inheritance of MC4R mutations with modulation of penetrance and expressivity may explain these observations.

- The theory of differing ethnic backgrounds illustrating varying penetrance did not hold true in light of this studies' results.

- The current associated trend of MC4R polymorphisms and their phenotype was defied by the BMI ranges observed, and by the fact that pathogenic/rare polymorphisms did not lead to obesity, but non-pathogenic/common polymorphisms were identified in obese carriers.

- Ethnicity was a factor when investigating MC4R pathogenic/rare mutation *frequency* as a secondary outcome; 10 out of the 12 pathogenic/rare polymorphism carriers were from the black African ethnic group.

- No specific clinical phenotype can be utilized to predict whether a severely obese adult is an MC4R mutation carrier or not. The theory that there is a positive relationship between the presence of any functionally relevant MC4R mutation and the severity and onset of obesity was not observed.

- In agreement with Lubrano-Berthelier *et al.* 2006 [15] this study confirmed that the inclusion of common, non-pathogenic polymorphisms in MC4R mutation-obesity studies could lead to complications and improper clinical decisions and thus they should be omitted.

- The presence of pathogenic/rare MC4R polymorphisms is not necessarily correlated with BMI.

- MC4R mutations definitely have an effect on an individuals' predisposition to obesity, however the extent to which they contribute to disease pathogenesis needs clarification.

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Chapter 5: Final Conclusions and Perspectives

I have had the opportunity to study obesity from two distinct perspectives; clinically in the form of bariatric surgery and its post-operative improvements and molecularly from the MC4R standpoint. Although a broad perspective of the pathophysiology / pathogenesis of the disease was not studied, the following conclusions can be drawn from my results:

- Bariatric surgery is a powerful tool in the resolution of obesity and its associated co-morbidities.

- Anthropometric and biochemical assessment of an obese bariatric surgical candidate looking at all associated co-morbid condition measurements pre-operatively and on a continual post-operative basis is of utmost importance to assess improvement / recovery and to adjust pharmacological and dietary interventions.

- Using only BMI for the clinical staging of obesity appears to be insufficient and the bariatric surgery model may well be a very useful future tool to investigate pathophysiology in the obese patient and re-determine the clinical staging of the disease.

- Pathogenic/rare MC4R polymorphism frequency was elevated within this sample group, specifically within black Africans. Further investigation is needed to clarify this.

- The relevance of MC4R polymorphisms to phenotypic manifestations was not clear.

- No specific clinical phenotype can be used to predict whether a severely obese adult is an MC4R mutation carrier or not.

- Clear establishment of the fact that MC4R mutations have an effect on an individuals' predisposition to obesity has been made; however the extent to which they contribute to disease pathogenesis needs clarification.

In light of this new information on obesity in South Africa, the following comments can be made.

Bariatric surgery improves an obese individuals' quality of life. It also reduces the persons' mortality risk from associated co-morbid diseases and therefore increases their longevity.

Genetically, obesity is complex and is most commonly polygenic. Monogenic mechanisms that cause obesity are rare, and in the case of MC4R gene polymorphisms there was no specific trend observed in terms of pathogenicity in this study.

The treatment of obesity has undergone revolutionary advances since the recognition of obesity as a disease. Bariatric surgery induces gut hormone alterations that lead to significant improvements in the patients' condition. Future research perspectives would be to try and mimic these hormonal alterations using pharmacological means thus eliminating invasive surgical methods. To what extent and exactly how genetic mechanisms contribute to the development of obesity is not clear. What is certain is that specific individuals do have an elevated predisposition to obesity development that is genetically determined. The challenge now is to focus molecular research on isolating and investigating those elements and pathways that will lend themselves to the development of therapeutic strategies. The global obesity research community has made much progress in understanding obesity and its associated pathology and molecular components; however there is still much to be uncovered and clarified in the battle against this disease.

Chapter 6: Appendices of additional information

Appendix A:

6.1 MC4R in linkage disequilibrium

In a recent large scale, genome-wide, meta-analysis investigating BMI association to polymorphisms and linkage-disequilibrium, it was ascertained that conventional variants have an impact on fat mass, weight and the risk of obesity [1]. The position of the SNPs (Single Nucleotide Polymorphisms) focused on, as well as their associated phenotypes, illustrates consistency with the effects arbitrated by alteration in the functioning of the MC4 receptor [1]. The SNP whose variants showed the strongest association with BMI, illustrated low pair-wise linkage disequilibrium to the MC4 gene, and were mapped to be 109-188kb from the coding region of MC4 [1]. The SNP was designated rs17782313 and showed positive associations with adult height as well as an even stronger association with weight than with BMI ($P = 1.3 \times 10^{-9}$ vs. $P = 2.8 \times 10^{-21}$), thus indicating that it impacts total adult body size [1]. The effect of the SNP on paediatric weight regulation showed that in children aged 7-11 years each extra copy of rs17782313 correlated with a BMI variation that was twice that detected in the adult cohort [1]. Therefore these common variants near the MC4 gene appear to have additive effects on BMI [1]. The authors speculate that disruption of MC4 transcriptional control is the likely means by which the identified variant executes its effect even though there is such a large distance between itself and the MC4 coding region [1]. When looking at phenotypic characteristics of individuals carrying the rs17782313 SNP in comparison to MC4 variant carriers, the latter had increases in linear growth, children had moderate increases in lean and bone mass and early-onset obesity was also observed [1], all of which are seen in MC4 variant carriers. Importantly this study demonstrates that genetic mechanisms of obesity either monogenic or polygenic coincide at different levels of gene expression. Additionally, variants that are mapped many kilobases from

the candidate gene can effect the phenotype through expressional or translational means [1].

6.2 Nonsense mediated mRNA decay and MC4R

Nonsense mediated mRNA decay (NMD) is a surveillance pathway that causes the removal of transcripts that have premature termination codons (PTCs) that are caused by nonsense or frameshift mutations [2]. NMD performs a quality control function within the cell, in that it prevents the production of dominant negative or any other form of deleterious truncated proteins [2]. The signal for NMD is hypothesized to be the distance between the termination codon and the last exon-exon junction [2]. When focusing on MC4R to investigate the functioning of NMD within an intronless gene, it was found that MC4 was unresponsive to NMD [2]. Thus it showed that NMD is a splicing-dependent process and that cells that express intronless genes with premature termination codons are at risk with regard to the effects of the expression of these genes [2]. Thus although MC4-mediated obesity is essentially classified as being monogenic, other genetic mechanisms could also have substantial effects on the expression of obesity within MC4 mutation carriers.

6.3 Therapeutic Strategies, Agonists and Antagonists of MC4R

As well as being a vital constituent in the control of energy homeostasis, the melanocortin system also exerts its effects on regulation of the reproductive system, sexual and autonomic functions [3]. Thus the possibilities of utilizing it in the production of novel pharmacological agents to treat obesity, sexual dysfunction, anorexia and cachexia have generated significant awareness within this industry [3].

The main focus of melanocortin 4 receptor targeting for therapeutic obesity intervention is based on the hypothesis that an elevation in melanocortinergic

tone will cause an increase in energy expenditure and a subsequent decline in appetite, which would ultimately combat obesity [4]. Polymorphic MC4 receptors have differing responses to the plethora of endogenous as well as synthetic ligands [5, 6] therefore in future studies where the output is therapeutic targeting of mutant receptors; specific pharmacological dynamics must be thoroughly investigated preceding intervention.

The effects of MC4R mutations seem to be less prevalent with age, however controlling the childhood aspect of obesity could also be of great importance and thus warrant the development of agonist anti-obesity MC4R drugs to combat this [2]. Drug specificity is of course a factor that has to be taken into account, and structure-activity relationship (SAR) studies are being carried out to assess the relationship between synthetic agonists and mutant receptors [2]. Another factor to take into account when developing an anti-obesity pharmacological agent is downstream signalling, which in this case is in the form of cAMP, and thus the targeting of the specific cells that are responsible for this [2]. This will thus bypass the mutant MC4 receptor and stimulate downstream signalling, therefore constituting another viable treatment option [2].

Synthetic melanocortin peptide investigation began as early as the 1980's, where conserved sequences were modified and resulted in an improved ability to activate respective receptors [3]. More recently alternative highly active MC4R agonists were discovered during modification of natural sequences [3]. Analogues of natural peptides thus do show promise in the field of drug development as they are simple to prepare and despite the fact that they do not lend themselves to oral administration, methods of delivery are advancing in the form of intranasal and pulmonary inhalation [3]. Focus has however shifted towards non-peptide drug development [3] and these MC4R selective agonists are seen to be the most therapeutically successful [4]. Tetrahydroisoquinoline (THIQ) was the first chief success story in this field and its design was based on elements from the MSH core sequence [3]. THIQ derivatives showed efficacious effects *in vivo* during

animal trials however certain analogues illustrated aspects of toxicity [3]. Many additional non-peptide derivatives have come to the fore, their design being based on either leads discovered during high-throughput screening or from alteration of MSH's core sequence [3]. Many melanocortin-targeted agents do not specifically effect only one receptor subtype; this does in some circumstance have certain advantageous effects, such as MC3 and MC1 receptor agonists causing a decline in inflammation; a MC1,3-5 agonist decreasing myocardial damage and MC 3-5 receptor organic agonists having neuroprotective properties [3].

Results from functional studies using MC4R agonists and cAMP accumulation measurement *in vitro* showed that non-peptide agonists have a lowered intrinsic response when compared to α-MSH, MTII and ACTH [4]. Thus it has been proposed that the current understanding of MC4R signalling is somewhat elementary and the optimal way to investigate this would most definitely be *in vivo* studies using animal models [4].

NDP-MSH is a synthetic melanocortin agonist, it is highly potent and chemically stable [5]. It can also be easily radioactively iodinated, thus it has become very popular when functionally characterizing mutant MC4 receptors [5]. When comparing polymorphic MC4R receptors' NDP-MSH stimulation, 18 illustrated equivalent stimulatory effects, 11 had reduced agonist stimulation and 4 showed an increased stimulatory effect in comparison to the wild-type [5]. Mutant receptor cell lines that did not bind iodinated NDP-MSH above the average binding count included Y35Stop, N62S, P78L, V95I, I102S, I125K, Y287Stop, P299H, TM5Del and TM6Ins [5]. The proposed reasoning for this is that the receptors are not adequately expressed by the HEK293 cells at the cell surface or alternately that the amino acid changes caused by the SNPs result in altered or absent binding affinity for the synthetic ligand by the receptor [5].When assessing AGRP potency 3 mutant receptors had reduced effectiveness and one receptor (Y287Stop) illustrated an increase in AGRP antagonist activity [5].

Side effects of MC4R agonist administration include enhanced erectile responses within animal models, possible retardation of growth patterns as well as a reduction in bone mineral density and tachyphylaxis [4]. The association of the melanocortin system with adiposity and features of autonomic and cardiovascular control which show linkage to ailments of the metabolic syndrome pose certain difficulties to the focus of drug discovery, in that increased activation of the melanocortin system despite its positive effect on food intake and weight gain could lead to marked hypertension [3]. This is most likely due to the fact that the MC4 receptor possesses the ability to activate the sympathetic nervous system which could lead to increased arterial pressure and heart rate [3].

The main focus of drug discovery in the combat of obesity has been to utilize the central melanocortin system by identification of non-peptide MC4R selective agonists [4]. Advancement has been made in recognizing high-affinity MC4R agonists that have increased efficacy with regard to therapeutic intervention in animal models [4], however these agents must still be tested within the human sector and their side effects must be stringently evaluated before this occurs. Contemporary protein modelling techniques such as proteochemometrics which cumulatively builds on data for a variety of ligands and targets are being used to investigate pharmacological properties of receptors and newly developed pharmacological agents [3]. This enables continuous assessment and improvement of the performance of these compounds [3] and provides a non-invasive means for drug development and kinetic investigation. These methods have been implemented when looking at the MC4 receptor as a potential drug target and hopefully will lead to the development of high-impacting pharmaceutical agents to combat obesity and other melanocortin-associated diseases. Individualized therapy is becoming more prominent in the medical world and pharmaceuticals tailored to combat certain mutant variants of the melanocortin receptors could prove useful in the future [3]. However there is still an abundance of work to be done into the investigation of potential side effects, drug

variants and pharmacodynamics of the melanocortin receptors and their effectors.

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Appendix B:

Ethics Approval:

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Faculty of Health Sciences Research Ethics Committee
Fakulteit van Gesondheidswetenskappe Navorsingsetiekkomitee

The Research Ethics Committee, Faculty Health Sciences, University of Pretoria comply with ICH-GCP guidelines and has US Federalwide Assurance. FWA 00002567, Approved dd 22 May 2002 and Expires 24 Jan 2009. IRB 0000 2235 IORG0001762 Approved dd Jan 2006 and Expires 21 Nov 2008.

PROTOCOL NO.	**Student 142/2007**
PROTOCOL TITLE	Investigation of MC4 Receptor Polymorphisms and the effect of Bariatric Surgery on a selected group of obese South African Patients.
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SPONSOR	None.

This **Protocol** and **Informed Consent** and **all the attatchements** have been considered by the Faculty of Health Sciences Research Ethics Committee, University of Pretoria on 26/03/2008 and found to be acceptable

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