Ethnic-specific associations between abdominal and gluteal fat distribution and the metabolic complications of obesity: Implications for the use of liposuction

by

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28 March 2011

DECLARATION

I, *Philip Hayes* hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Contents of Dissertation

PART A: PROTOCOL

ETHNIC-SPECIFIC ASSOCIATIONS BETWEEN ABDOMINAL FAT DISTRIBUTION AND THE METABOLIC COMPLICATIONS OF OBESITY: IMPLICATIONS FOR THE USE OF LIPOSUCTION

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Please note that this research forms part of a much larger study for which ethics approval was granted in April 2003; reference number 053/2003. Below is a copy of the original proposal:

ADIPOSE TISSUE DISTRIBUTION AND METABOLISM EXPLAIN THE OBESITY-RELATED COMORBIDITIES IN BLACK SOUTH AFRICAN WOMEN

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Introduction

More than three-quarters (77%) of the 40.5 million people living in South Africa are black African, of which more than 40% are urbanised [1]. Black African women living in urban areas have a significantly higher prevalence (62%) of overweight than urban black males (28%) or white females (53%, [2]). It was previously thought that obesity in black South African women was not associated with deleterious metabolic sequelae and was termed "healthy" obesity [3]. However, this is not the case as obese black women have double the diabetes (7.0 vs. 3.6%, [4,5]) and hypertension (30 vs. 15%, [6]) prevalence rates of obese white women, whereas obese white women more commonly present with coronary heart disease and hypercholesterolaemia [7].

Interestingly, in small samples of obese women studied in South Africa, obese black women are more insulin resistant and have greater rates of lipolysis than obese white women [8-10]. This is surprising as black women have significantly less visceral adipose tissue than white women (~72 vs. 140 mm^2 [11,12]). These findings are similar to those in African America women. Lovejoy et al. [13] found that African American women are more insulin resistant than Caucasian women, despite the fact that the African American women also have less visceral adipose tissue. Insulin resistance in these women is associated with hyperinsulinaemia [13], which is in contrast to the findings in obese black South African women who more commonly present with relative insulinopaenia [8,14-16]. However, no direct assessment of insulin secretion in relation to the degree of insulin sensitivity has been performed in these women, which may lead to erroneous conclusions [17]. Determination of this relationship will increase our understanding of the underlying mechanisms contributing to the insulin resistance in these women, leading to more appropriate management or treatment.

Visceral adipose tissue is strongly associated with insulin resistance, hypertension, dyslipidaemias amongst other factors, which contribute to an increased risk of coronary heart disease [18]. However, more recently the importance of subcutaneous adipose tissue as a significant determinant of insulin resistance has been recognised [19-22]. When partitioning subcutaneous adipose tissue into superficial and deep compartments, Kelley et al. [20] found that deep subcutaneous adipose tissue showed as strong a relationship with insulin resistance as visceral adipose tissue ($R=0.64$ and $R=0.61$, respectively, both $P<0.001$), but there was no association between superficial subcutaneous adipose tissue and insulin sensitivity ($R = -0.29$, NS). This finding may be important in explaining the higher prevalence of insulin resistance in obese black South African women who have relatively low levels of visceral adipose tissue, but high levels of subcutaneous adipose tissue compared to obese white women.

One of the factors that may underlie the association between regional fat distribution and morbid sequelae may be exposure to glucocorticoids. A case in point is the pathological condition of cortisol excess, Cushings syndrome, which is characterised by central obesity, hypertension, glucose intolerance and insulin resistance [23]. Studies examining changes in activity of the hypothalamic-pituitary-adrenal (HPA) axis with obesity have found that circulating levels of cortisol are normal, or even low [24-27]. However, obese women, particularly those with visceral fat accumulation have elevated cortisol metabolic clearance rates, partly explained by increased urinary cortisol excretion [28,29], as well as hyperactivity of the HPA axis, characterised by elevated ACTH and cortisol secretion in response to CRF administration [28-31]. The increased sensitivity of the HPA axis in obesity, particularly central obesity measured by the waist:hip ratio, may be due, at least in part, to a reduction in the inhibitory feedback action of glucocorticoids on the HPA axis, as indicated by low-dose dexamethasone suppression tests [25] or 24-hr low-dose hydrocortisone infusions [27]. A single study examining the possible differences in the HPA axis in 18 African American compared to

30 Caucasian age- and weight-matched women, revealed only exaggerated plasma ACTH responses to CRH stimulation in the African America women [32].

Recent studies suggest that glucocorticoid action on target tissues depends not only on the circulating concentrations, but also on the tissue sensitivity to glucocorticoids, which is determined by the levels of expression of glucocorticoid receptor isoforms (Grα and GRß - a dominant negative splicing variant present in variable amounts in some tissues) and 11ß-hydroxysteroid dehydrogenase type 1 (11 ß-HSD1). 11ß-HSD1 is a NADP(H)-dependent reductase enzyme that reactivates cortisone to active cortisol in specific cells [33-38]. Obesity is associated with reduced activity of 11ß-HSD1 in liver, which may also explain the increased metabolic clearance rate of cortisone in patients with obesity [35,39]. In contrast, 11ß-HSD1 activity in adipose tissue is positively correlated with BMI [39,40]. It therefore appears that obesity is associated with tissue-specific amplification of cortisol action, determined in part by increased expression of 11ß-HSD1, in combination with increased glucocorticoid secretion. These effects may be further exacerbated under stress, for example, urbanisation.

Increased exposure of adipocytes to glucocorticoids may play a crucial role in adipocyte differentiation and the development of hypertension via its effects on the local renin-angiotensin system [41]. Expression of angiotensinogen and angiotensin II is increased in adipose tissue, especially visceral adipose tissue, in obese [42] and obese hypertensive subjects [43]. Angiotensin II plays a dual role in regulating blood pressure and stimulating the production and release of prostacyclin, which promotes differentiation of precursor cells into adipocytes [44]. Recently, Masuzaki et al. [45] found that angiotensinogen expression is increased in transgenic mice that over-express 11ßHSD1 selectively in adipose tissue and present with visceral obesity, hypertension and insulin-resistant diabetes (personal communication, JR Seckl).

11ßHSD1 expression in human adipose tissue is up-regulated by TNFα [46], a proinflammatory cytokine that is expressed in adipose tissue in proportion to the degree of adiposity [47]. TNFα has been implicated as a causative factor in obesity-associated insulin resistance via its proposed effects on lipolysis, insulin-mediated signalling pathways, GLUT4 and PPARγ [48]. More recently, other proteins released from adipose tissue have also been implicated as potential mediators of obesity-associated insulin resistance. Adiponectin, which is exclusively expressed in adipose tissue and is reduced in obesity [49], is positively associated with whole-body insulin sensitivity, independent of adiposity [50,51]. Conversely, the adipocyte-derived protein, resistin, may impair insulin action and glucose tolerance [52]. PPARγ plays a key facilitatory role in altering glucose metabolism by up-regulating adiponectin expression [53] and down-regulating resistin expression [52]. Leptin appears to exert a hypoglycaemic action, which is partly independent of its effects on body composition [54]. Although these adipocyte-derived proteins have been implicated as causative factors in obesity-associated insulin resistance in murine models, their relative importance in human models is less clear. However, it does appear that each factor has distinct properties that may contribute differentially to obesity-associated insulin resistance.

Although numerous studies have documented differences in body fat distribution between black and white South African women, there are no studies that have explored the possibility that the specific body fat distribution of black women, which was once considered to be “healthy”, is in fact the factor that predisposes them to hypertension and diabetes. We therefore propose that it is indeed the differences in body fat distribution, which are associated with alterations in the regulation of the HPA axis, local cortisol metabolism and the expression of adipocyte-derived proteins, that

predispose these women to diabetes and hypertension, despite relatively low levels of visceral adipose tissue.

Hypothesis:

Urbanised black South African women display a specific obesity phenotype, characterised by increased volume of deep subcutaneous adipose tissue. This adipose tissue has increased expression of 11ß-HSD1 and/or glucocorticoid receptors and secondarily, altered local expression of key adipose receptors/mediators such as PPARγ, angiotensinogen, leptin, TNFα, adiponectin and resistin. These mediators act to exacerbate the local and systemic obesity phenotype, also perhaps altering hypothalamic-pituitary-adrenal (HPA) axis feedback and activation, and thereby, predisposing these women to diabetes and hypertension.

Objectives:

The objectives of the study are to investigate differences between lean and obese black and white South African women, with specific reference to:

i) The magnitude of deep vs. superficial subcutaneous vs. visceral adipose tissue;
ii) The degree of insulin secretion in relation to insulin resistance;
iii) The activity and regulation of the HPA axis;
iv) The expression of 11ßHSD-1, glucocorticoid receptor isoforms, PPARγ, angiotensinogen, leptin, TNFα, adiponectin and resistin in visceral adipose tissue vs. deep vs. superficial subcutaneous abdominal adipose tissue vs. gluteal adipose tissue;
v) The lipolytic activity of adipocytes from the different adipose tissue compartments to various physiological stimuli.

The findings of this research can be used to highlight specific areas for intervention in terms of pharmacotherapy for the treatment of the co-morbid conditions associated with obesity in black South African women.

Methods:

Methods for Phase 1:

Subjects:

Eighty urbanised, English-speaking black and white South African women between the ages of 21 and 45 years, matched for level of education, will be recruited for the study. Subjects will be excluded from the trial on the basis of the following criteria: i) receiving treatment for HIV/AIDS; ii) having any overt endocrine abnormality (e.g. diabetes mellitus); iii) undergoing endocrine therapy (e.g. oral corticosteroids, combined contraceptive pill); iv) currently pregnant or lactating; v) having hypertension or taking anti-hypertensive medications; vi) having polycystic ovarian syndrome; vii) having irregular menstrual cycles, viii) having less than a standard 8 (grade 10) education. All testing will be undertaken in the follicular phase of the subjects' menstrual cycles.

Subjects will be selected according to the following criteria:

	Black	**White**
Normal weight – BMI < 25 kg/m^2	20	20
Obese - BMI > 30 kg/m^2	20	20
Total subjects	**40**	**40**

Refer to Addendum for sample size determinations.

Preliminary testing:

Prior to participating in the trial, subjects will undergo preliminary testing to verify that they meet the inclusion criteria. Blood pressure will be measured and glucose tolerance determined. If subjects are found to be hypertensive (140/90 mmHg) and/or diabetic (according to the WHO criteria), they will be excluded from participating in the trial. In addition, HIV screening will be performed and those who test positive for HIV infection will be excluded from the trial as HIV-1 infection is associated with abnormalities in HPA axis activity [55], lipodystrophy and insulin resistance, particularly in patients treated with HIV-1 protease inhibitors [56]. .

i) Blood pressure measurements:

Blood pressure will be measured 3 times at 1-min intervals in each subject using an appropriately sized cuff and an Omron automated blood pressure monitor. These measurements were taken after at least 20 min of seated rest. An average of the last 2 readings will be used in the analyses. If the subjects' blood pressure is found to be over 140/90 mmHg on three separate occasions, they will be excluded from taking part in the study.

i) Oral glucose tolerance tests:

In the morning, after an overnight fast (10-12 hr), subjects will report to the laboratory at the Sports Science Institute. An indwelling cannula will be inserted into an antecubital vein. Venous blood samples will be drawn 15 min prior to and 30, 60, 90 and 120 min after the subjects have ingested 75 g glucose in 250 ml water. One aliquot will be placed into a tube containing potassium oxalate and sodium fluoride for the determination of plasma glucose concentrations. The remaining aliquot will be placed into a tube containing lithium heparin for the subsequent determination of plasma insulin concentrations. All samples will be kept on ice until centrifuged at 3200 g at 4° C for 10 min upon completion of the test. Plasma samples will then be stored at –20° C for subsequent analyses. Plasma glucose concentrations will be determined using the Glucose Oxidase method (Glucose Analyser 2; Beckman Instruments, Fullerton, CA). Plasma insulin concentrations will be determined using a non-specific insulin radioimmunoassay (Count-A-Coat Insulin, Diagnostic Products, Los Angeles, CA).

ii) HIV screening:

On the day of the oral glucose tolerance test, subjects will donate a 5 ml blood sample for HIV testing. Subjects will receive counselling by a trained counsellor prior to the test and after the results are obtained, unless the subject chooses not to know the result. Referral will be made to appropriate HIV clinics for those subjects found to be HIV positive. Strict confidentiality will be maintained throughout the trial.

Measurements:

a. Anthropometry:

a. *Basic anthropometric measurements* will be completed, including weight, height, waist and hip circumference.

b. *Dual-photon x-ray absorptiometry* (DEXA) will be used to accurately measure body composition, including body fatness and muscle mass [57].

c. *Computerised tomography* (CT) will be used to assess regional body fat distribution, including, visceral and deep and superficial subcutaneous adipose tissue area. Deep vs. superficial subcutaneous fat will be separated at the level of the fascia superficialis [20,58]. Each CT image will be taken at 120kV with a scanning time of 2 seconds at 200mA, and a slice thickness of 5mm. Subjects will be scanned in the supine position with legs slightly flexed and arms raised above the head. Scans will be performed in arrested expiration at the L4/L5 level of the vertebra.

b. ***Insulin secretion and insulin sensitivity*:**

Insulin secretion and insulin sensitivity will be measured using the intravenous glucose tolerance test and the euglycaemic hyperinsulinaemic clamp technique[59], respectively. In order to estimate insulin secretion in relation to insulin sensitivity, the two tests will be performed in a sequential manner on the same day (Botnia clamp), as described previously [60,61]. Lehto et al. [60] compared the Botnia clamp to the euglycaemic clamp without prior intravenous glucose infusion and found the two tests to correlate strongly (r=0.94, P=0.0001).

After an overnight fast (10-12 hr), an indwelling cannula will be inserted into an antecubital vein of both arms. Ten minutes after a fasting blood sample is drawn, 0.3 g/kg body weight of a 50 % glucose solution will be administered intravenously (time 0). After 60 min, an infusion (infusion rate of 45 mU/m^2) of short-acting human insulin (Actrapid, Novo Nordisk) will be started and continued until 180 min. A 20 % glucose solution will be infused at a variable rate to maintain blood glucose concentrations unchanged at 5.5 mmol/l. Blood samples (5 ml) will be drawn from the opposite arm at –10, 0, 2, 4, 6, 8, 10, 20, 30, 40, 50, 60 min for the determination of blood glucose and insulin concentrations. Thereafter, blood samples will be obtained at 5-min intervals throughout the euglycaemic clamp for the determination of blood glucose concentrations and at 120, 170 and 180 min for the subsequent determination of plasma insulin concentrations.

Blood will be placed into tubes containing lithium heparin and potassium oxalate and sodium fluoride for the determination of plasma insulin and blood glucose concentrations, respectively. Blood glucose concentrations will be determined immediately using the Glucose Oxidase method (Glucose Analyser 2; Beckman Instruments, Fullerton, CA). The remaining blood sample will be kept on ice until centrifuged at 3200 g at 4° C for 10 min, upon completion of the clamp. The plasma will be stored at –20° C for later analysis as previously described.

The incremental trapezoidal area during the first 10 min of the test will be defined as first phase insulin secretion and the 10-60 min period will be defined as late phase insulin secretion [60]. Insulin sensitivity will be calculated from the rate of glucose infusion during the last 60 min of the euglycaemic clamp, and will be

expressed per kilogram of lean body mass (determined previously by DEXA). The function of the HPA axis in relation to insulin sensitivity will be examined.

Measures of the HPA axis:

The following tests will be performed in random order, separated by at least 2 days:

a. *IV CRH test*:

A cannula will be inserted into an antecubital vein at 12h30, at least 3 hr after the last meal. At 13h00, 1μg CRH/kg body weight will be infused into the antecubital vein. Blood samples (8 ml) will be drawn every 15 min for 2 hrs for the determination of serum ACTH and cortisol concentrations [39].

Blood samples for cortisol determination will be placed into tubes containing lithium heparin and stored on ice until centrifuged at 3200 g for 10 min at 4° C. Plasma samples will be stored at –80° C until assayed with a commercial radioimmunoassay (RIA) (Orion Diagnostica, Espoo, Finland).

Blood samples for ACTH determination will be placed into pre-chilled EDTA tubes containing aprotinin (500 U/ml) and centrifuged immediately at 3200 g for 10 min at 4° C. Plasma samples will be stored at –80° C until assayed with a commercial RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA).

b. *Low dose ACTH test:*

After an overnight fast (10-12 hr), an indwelling cannula will be inserted into an antecubital vein. Fifteen minutes later (between 08h30 to 09h00), 2 basal blood samples will drawn at 15 min intervals for the subsequent determination of plasma cortisol concentrations. Thereafter a 1μg bolus intravenous injection of synthetic ACTH (Cortrosyn) will be administered [62]. Further blood samples for cortisol determination will be drawn 15, 20, 30, 60 and 120 minutes after the injection. Blood samples will be stored and assayed as described above.

c. *Low dose dexamethasone suppression test*:

3.5 μg/kg body weight dexamethasone will be administered orally at 23h00. Subjects will report to the laboratory at 07h45, after an overnight fast (10-12 hr). A blood sample will be drawn from the antecubital vein at 08h00, after the subjects have rested quietly for 15 min [39]. The blood sample will be placed in tubes containing lithium heparin and EDTA for the subsequent determinations of plasma cortisol and dexamethasone concentrations, respectively. Blood samples will be centrifuged immediately at 3200 g for 10 min at 4° C and stored at –80° C. Plasma cortisol samples will be analysed as described above. Plasma dexamethasone samples will be assayed using a RIA using a specific antibody [63].

d. *Urinary cortisol metabolites*:

A timed 24 hr urine sample will be collected for the measurement of urinary cortisol metabolites. Urine aliquots will be stored at -20^0C for subsequent determination of cortisol and its metabolites (listed below) by gas chromatography and electron impact mass spectrometry following Sep-Pak C18 extraction, as previously described [64,65].

i. Free cortisol concentrations
ii. Free cortisone concentrations
iii. 5α-tetrahydrocortisol (5α-THF) concentrations
iv. 5β-THF concentrations
v. Tetrahydrocortisone (THE) concentrations
vi. Creatinine concentrations (to assess the adequacy of the 24 hr urine specimen)

The ratio of 5α-THF + 5β-THF/THE reflects the balance of whole body 11β-HSD activity [39].

c. Blood pressure

Blood pressure will be measured 3 times at 1-min intervals in each subject using an appropriately sized cuff and an Omron automated blood pressure monitor. These measurements were taken after at least 20 min of seated rest. An average of the last 2 readings will be used in the analyses.

During the trial, the subjects will be required to visit the laboratory on 6 occasions, including one day for the preliminary testing.

Methods for Phase 2:

Subjects:

A sub-sample of subjects from Phase 1 of the study will be recruited to undergo percutaneous adipose tissue biopsies while undergoing computerised tomography for Phase 1 of the study.

	Black	**White**
Normal weight – BMI < 25 kg/m^2	10	10
Obese - BMI > 30 kg/m^2	10	10
Total subjects	**20**	**20**

Refer to Addendum for sample size determinations.

As it is not possible to obtain visceral adipose tissue using the percutaneous needle biopsy technique, a separate sample of 10 obese black and 10 obese white women who are undergoing abdominal surgery to correct benign conditions that do not alter endocrine function, such as peptic ulcers, gallstones, hernia repair, urinary tract disorders and certain gynaecological procedures, will be recruited for Phase 2 of the study.

Adipose tissue biopsies:

After local anaesthesia with Lignocaine hydrochloride (2 % Xylotox S.E. Plain, Adcock Ingram, Bryanston, South Africa), adipose tissue samples of deep and superficial subcutaneous adipose tissue from the abdominal (right of the umbilicus) and gluteal areas (upper right quadrant) will be obtained using the percutaneous needle biopsy technique [66]. The deep and superficial adipose tissue will be separated at the level of the fascia superficialis, as described previously [19]. The biopsies will be performed under the direction of computerised tomography by a radiologist. Approximately 1.5 cm^3 fat will be excised from each site through a 0.5-1 cm incision. Adipose tissue will be frozen immediately in liquid nitrogen and stored at –80° C for subsequent analyses.

In the sample of women undergoing benign abdominal surgery, adipose tissue samples (~ 2 g) from visceral and deep subcutaneous adipose tissue compartments will be obtained during the surgical procedure. Samples will be snap-frozen in liquid nitrogen and be stored at –80° C for subsequent *in vitro* analyses as described below.

Adipose tissue analyses:

The adipose tissue samples from the different compartments will be used for the following *in vitro* analyses:

a. *Adipose 11β-HSD1 activity*:

   Homogenised fat samples will be incubated with 1,2,6,7-$^3H_4$-cortisol to measure the 11ß -HSD1 activity in the dehydrogenase direction (i.e. cortisol to cortisone) as described by Bujalska et al. [33] and Rask et al. [24]. Aliquots of the reaction will be separated on an HPLC with on-line liquid scintillation detection to measure the rate of cortisone production over a 30-hr period.

b. *Adipose gene expression and protein content:*

   Total mRNA will be extracted from the adipose tissue according to standard methods. The expression of 11β-HSD1 [33], angiotensinogen [67], TNFα [68], PPARγ [69], adiponectin [70], resistin [71], leptin [72] and the glucocorticoid receptor (GR) isoforms, GRα and GRβ [73] mRNA [74] will be determined using RT-PCR and/or Northern blot analysis. The results will be normalised to the signal generated from β-actin mRNA. Where applicable, the levels of these proteins will be determined by Western blot analysis in a total protein extract from a separate portion of the adipose tissue sample.

c. *In vitro adipose lipolytic activity*:

   Adipose tissue will be treated with collagenase, and the released adipocytes will be isolated and purified, as previously described [9]. The lipolytic activity of the purified adipocytes in response to cortisol, isoproterenol and insulin will be measured as described by Buthelezi et al. [9].

d. RNA will be stored for microarray analysis once future funding has been procured.

Ethics:

No subjects will be enrolled into the study until the Research Ethics Committee of the University of Cape Town has approved the protocol and Patient Information Sheet, in writing. The investigators maintain responsibility for informing the Research Ethics Committee in writing of any amendment to the protocol, or of any adverse events resulting from participation in the study. The study will be performed in accordance with the principles of the Declaration of Helsinki, ICH Good Clinical Practice (GCP) and the laws of South Africa. No subject will enter the study without signing informed consent, after and the investigator has provided a full and adequate oral and written explanation of the study, including possible risks. Subjects have the right to withdraw from the trial at any stage without stating a reason, and trial personnel may also withdraw a subject from the study at any time. Patients who violate the trial protocol should be withdrawn. Data generated from the trial will be stored in a computer database in a secure facility, and in a manner that maintains patients confidentiality. For data verification and quality control purposes regulatory authorities and/or members of the Research Ethics Committee may be allowed access to patient data under conditions of strict confidentiality. This is stated in the Patient Information Sheet. The anonymity of participants is ensured in any publication of the data.

ADDENDUM A: SAMPLE SIZE DETERMINATION

Sample size determination using a power of 80% (1-β) and an α-level of 0.01. A Bonferroni correction factor was used to account for the 3 factors being investigated, namely, ethnicity, level of obesity and body fat distribution (α-level = 0.05/3 = 0.017)

Parameter	Smallest mean difference	SD of score	N per group	Reference
Comparisons reflect differences between black and white overweight women.				
Visceral fat area (cm^2)	30	14	6	van der Merwe et al. [14]
Fasting [insulin] (pM/ml)	65	32	6	van der Merwe et al. [8,15]
Fasting [C-peptide] (pM)	1200	297	2	van der Merwe et al. [14]
M ($mM/kg.min^{-1}x100$)	6.6	2.9	5	van der Merwe et al. [14]
M/I ($mM/kg.min^{-1}x100/pMx1000$)	0.12	0.047	4	van der Merwe et al. [14]
Fasting [FFA] (pM)	450	70	4	van der Merwe et al. [14]
Fasting [cholesterol] (mM)	1.36	0.25	1	van der Merwe et al. [14]
CRH-stimulated plasma [ACTH] (AUC, pM)	1278	183	12	Yanovski et al. [32]
Inhibition of isoproterenol-stimulated lipolysis by insulin (%)	28	22	15	Buthelezi et al. [9]
Comparisons reflect differences between lean and obese women.				
M/I ($mM/kg.min^{-1}x100/pMx1000$)	6.7	2.2	3	Rask et al. [24]
Urinary (5aTHF +5bTHF)/THE (mg/d)	0.31	0.33	23	Rask et al. [24], Stewart et al. [35]
Plasma [cortisol] after DEX (nM)	170	110	10	Rask et al. [24]
Serum [cortisol] after oral cortisone (AUC, nM.min)	57 276	20 000	3	Rask et al. [39]
11βHSD1 activity (% conversion at 3 hr)	34	17	6	Rask et al. [24]
Comparisons reflect differences between overweight and obese women.				
M/I ($mmol/kg.min^{-1}x100/pMx1000$)	4	2.7	11	Rask et al. [24]
Urinary (5aTHF +5bTHF)/THE (mg/d)	0.42	0.48	27	Rask et al. [24]
Plasma [cortisol] after DEX (nM)	85	110	34	Rask et al. [24]
Serum [cortisol] after oral cortisone (AUC, nM.min)	25 443	22 000	18	Rask et al. [39]
11βHSD1 activity (% conversion at 3 hr)	12	17	30	Rask et al. [24]
Comparisons reflect differences between abdominally obese and peripherally obese women				
Plasma [cortisol] after CRH (AUC, nM.min)	5788	3151	7	Pasquali et al. [29]
Plasma [ACTH] after CRH (AUC, nM.min)	192	105	7	Pasquali et al. [29]
Urinary free [cortisol] (nM/m^2)	15	4	2	Vicennati et al. [30]
Homa index	1.8	2.0	29	Vicennati et al. [30]

SD, standard deviation; M, mean glucose disposal rate; M/I, mean glucose disposal rate, expressed as glucose sensitivity index; FFA, free fatty acids; AUC, area under the curve; CI, confidence interval

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Part B: Structured Literature Review

Ethnic-specific associations between abdominal and gluteal fat distribution and the Metabolic complications of obesity: Implications for the use of Liposuction

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List of Contents of Literature Review

a) Objectives of Literature review

1. To gain information regarding the demographics of obesity globally as well as in South Africa
2. To understand the impact obesity is having on health
3. To gain information on fat physiology (fat is now considered to be an endocrine organ) as well as information on the adipokines and inflammatory mediators that are produced in fat and their functions in healthy individuals
4. To learn about the mechanisms underlying the associations between obesity and certain diseases, especially insulin resistance and diabetes, hypertension, dyslipidaemia and atherosclerosis
5. To examine the available literature regarding the relative contribution of abdominal and gluteal fat depots to metabolic risk factors including insulin resistance, hypertension, dyslipidaemia
6. To examine published data investigating the metabolic effects of liposuction and identifying factors that may account for any disparate findings with respect to the effects of liposuction on the metabolic complications of obesity

b) Literature search strategy

- Pubmed search engine was used to locate journal articles. Examples of search words and phrases used include: obesity, adipokines, adipocytokines, names of specific adipokines and inflammatory mediators, diabetes and obesity, liposuction and obesity, metabolic effects of liposuction, liposuction and insulin resistance, ethnic differences in fat distribution
- Advanced search function and Author searches were used
- Related citations suggested by the search engine were also occasionally used
- Some journal articles were obtained from the references of the journal articles already obtained
- Some journal articles were obtained from my supervisor
- Some journal articles (those not available on the UCT journal portal) were obtained directly from the author
- The only limit used was for publications in English

c) Glossary

Adipokine: is a loosely applied term referring to any substance/ peptide hormone released by adipose tissue.

Adipocytokine: is a loosely applied term referring to any substance/ peptide hormone released by adipose tissue but showing specific inclusion of inflammatory cytokines.

Adiposopathy: is a term coined by Bays (18) and refers to the development of pathogenic adipose tissue in the presence of a positive caloric balance and

sedentary lifestyle in genetically prone individuals. Pathophysiologically it results in diverse metabolic and immune consequences leading to clinically evident metabolic disease.

Large volume liposuction is defined as the removal of more than 5000mL of lipoaspirate (as defined by Liposuction task force of the American society of plastic surgeons)(87).

Metabolic complications of obesity (MCO): Hypertrophic adipose tissue secretes a range of substances including abnormal amounts of various adipokines, pro-inflammatory mediators and free fatty acids. This ultimately results in increased insulin resistance (impaired glucose tolerance and diabetes mellitus), dyslipidaemia, essential hypertension and accelerated atherosclerosis.

Obesity is defined by a body mass index (BMI) greater than 30kg/m^2 or more reliably (with regard to cardiovascular risk) by waist circumference of >102cm/ 40 inches in a male and >88cm/ 35 inches in a female (40).

d) Abbreviations

BMI- body mass index (kg/m^2)
CV risk- cardiovascular risk
CRP – C-reactive protein
DM – Diabetes mellitus
FFA- free fatty acid
IL-6 - interleukin 6
IL-10 - interleukin 10
IS- insulin sensitivity
IR- insulin resistance
LVL- large volume liposuction
MCO- metabolic complications of obesity
PAI – Plasminogen activator inhibitor
PPAR-g - peroxisome proliferator-activated receptor gamma
SAT- subcutaneous abdominal adipose tissue
SSAT- superficial subcutaneous abdominal adipose tissue
DSAT- deep subcutaneous abdominal adipose tissue
TNF-$\propto$ - tumour necrosis factor alpha
VAT- visceral abdominal adipose tissue

e) Review of the Literature:

1. Introduction

Obesity is an epidemic of the 21st century. In 2005 over a billion adults worldwide were overweight (Body mass index (BMI) >25kg/m^2), with more than 300 million being obese (BMI >30kg/m^2) (1). Obesity is aetiologically associated with a host of medical conditions and even certain cancers (see table 1 in addendum) (2, 3).
In South Africa 87% of type 2 Diabetes, 68% of hypertensive disease, 45% of ischaemic strokes and 38% of ischaemic heart disease is attributable to obesity (4). Obesity was responsible for 7% of all South African deaths in 2000 (4) and has been declared the 5th leading independent cause of death in South African adults (5). It is as a result of these alarming statistics that obesity has been classified as a disease in its own right by the World Health organization (4).

2. Fat Physiology and the Metabolic Complications of Obesity

Although the association between obesity and these conditions is well established, the mechanisms via which obesity results in disease are less well understood. Of paramount importance is the fundamental shift in our understanding that adipose tissue is not a simple energy storage devise but an endocrine organ. Over 50 adipokines are produced in adipose tissue (6). These adipokines are hormone-like communication molecules that play an integral role in normal physiology (7). In the obese state however, the abnormally hypertrophied adipocytes recruit macrophages and promote inflammation, as well as secreting of a range of substances including excessive amounts of free fatty acids (FFA) and abnormal amounts of adipokines (figure 1). This ultimately results in increased insulin resistance (IR), dyslipidaemia and essential hypertension (6). The term adiposopathy was coined by Bays to denote this pathological process (8).

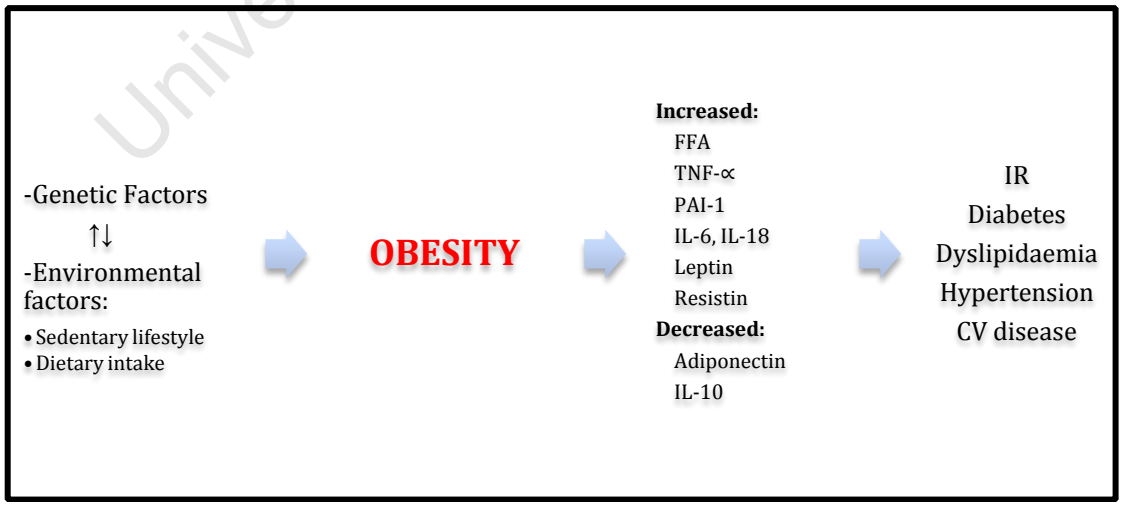

Figure 1) The metabolic complications of obesity
FFA, free fatty acids; TNF-∝, tumour necrosis factor alpha; PAI-1, plasminogen activator inhibitor-1; IL, interleukin; CV disease, cardiovascular disease.

2.1 Adipokines

Leptin was the first adipokine to be discovered (in 1994). In the non-obese, leptin plays an important role in lipid metabolism as well as improving insulin sensitivity (9). In low energy states, leptin acts via the hypothalamus to stimulate an increased appetite as well as via the thyroid axis to slow the basal metabolic rate (10).
Leptin concentrations increase in proportion to increasing obesity (adiposity). Excessively elevated leptin levels however, result in self induced leptin resistance (6), decreased insulin sensitivity (IS) and hypertension (via an induced hypercatecholaminaemia) (11).

Adiponectin, another well-known adipokine, is produced by all adipocytes. Its secretion however, decreases with increasing adiposity (12). It plays important roles in increasing IS (13), improving free fatty acid metabolism, promoting a healthy endothelial cell function and decreasing atherosclerosis (partly by inhibition of tumour necrosis factor alpha (TNF-∝)) (14). Adiponectin levels are favorably increased by exercise, loss of weight (15) and certain anti-diabetic medications (Thiazolidinediones) (10). Adiponectin production is inhibited by pro-inflammatory mediators like interleukin-6 (IL-6) and TNF-∝ (16). The low adiponectin levels in obesity are associated with insulin resistance and hyperinsulinaemia (ultimately type 2 diabetes mellitus), dyslipidaemia and increased atherosclerosis (6, 10, 14-15).

Peroxisome proliferator-activated receptor gamma (PPAR-g) is a ligand-activated nuclear transcription factor involved in the regulation of lipid and glucose metabolism, including adipocyte function and differentiation (17). No factor has been discovered that promotes adipogenesis in the absence of PPAR-g. Most pro-adipogenic factors seem to function, at least in part, by activating PPAR-g expression or activity (9). Thiazolidinediones, a group of antidiabetic agents, are activators of both adiponectin as well as PPAR-g (18). In addition, the PPAR ligands suppress the production of proinflammatory cytokines mainly through suppression of nuclear transcription factor kappa-beta (19).

Numerous other adipokines have been described but a detailed explanation of each adipokine is beyond the scope of this text. The role of several inflammatory mediators and FFA will however be briefly discussed below.

2.2 Inflammatory mediators in obesity

TNF-∝ plays important roles in obesity-related insulin resistance (19), increased FFA release into the circulation, hypertension and a reduction in adiponectin synthesis (20). TNF-∝ activates nuclear transcription factor kappa-beta, which orchestrates a series of inflammatory changes (especially in vascular tissue) that promotes atherosclerosis. (16).
There is also a strong association between obesity and platelet-activation, with more than a three-fold increase in the excretion of platelet-derived thromboxanes in the urine of obese women (21). This, together with increased plasminogen

activator inhibitor-1 in obesity, further promotes atherosclerosis and intravascular thromboses (22, 23).

IL-6 is a circulating cytokine that is secreted by many cell types. Approximately one third of the circulating IL-6 is secreted from adipose tissue (24). Elevated plasma IL-6 levels positively correlate with human obesity and insulin resistance and predict the development of type 2 diabetes (25) and future myocardial infarction (26).

Although C-reactive protein (CRP) is not an adipokine, its circulating concentrations are under the control of adipokines (especially IL-6) (16). Cross sectional analyses have shown strong and independent associations of CRP levels with measures of body fat (27). High levels of CRP in obesity also predict later development of diabetes (25, 28) and have become an important marker of vascular inflammation and a predictor of atherosclerosis (29).

Interleukin-10 (IL-10) is a cytokine produced by macrophages and lymphocytes. It possesses multifaceted anti-inflammatory (16) and insulin sensitizing properties, partly through its inhibition of IL-6 and TNF-∝ (30). A low production capacity of IL-10 has been found to be associated with the metabolic syndrome and type 2 diabetes (31).

Interleukin 18 (IL18) is a pleotrophic pro-inflammatory cytokine that is secreted in adipose tissue and is a strong risk factor for the development of cardiovascular disease (32). Not only is IL-18 pro-atherogenic, but elevated IL-18 levels are also thought to cause myocardial dysfunction (33). Further, elevated IL-18 levels are associated with the metabolic syndrome independent of obesity and insulin resistance (34).

2.3 Free Fatty Acids

In addition to deranged expression of adipokines and inflammatory markers, perlipins (which play a role in preventing triacylglycerol breakdown in adipocytes) are deficient in obese adipocytes (6). This results in excessive amounts of FFA being released into the circulation. These circulating FFA are taken up by a variety of cells. Intracellular accumulation of FFA and their metabolites (especially in liver and muscle) hinder insulin's ability to promote glucose uptake resulting in increased IR (35). In addition, the free fatty acids affect endothelial function (resulting in hypercoagulability) as well as promoting atherosclerosis (18). Raised FFA levels are also associated with the development of essential hypertension (36).

3. Adipose Tissue Depots

Not all adipose tissue is associated with this metabolic deterioration in the presence of obesity.

Peripheral obesity (e.g. gluteofemoral) has not been shown to be causally related to the metabolic complications of obesity (MCO) (11). Gluteofemoral fat exerts specific functional properties that are associated with an improved metabolic and cardiovascular risk profile (20). These protective properties of gluteofemoral fat

are thought to be as a result of a beneficial adipokine profile as well as improved FFA metabolism (37).

Central obesity on the other hand, is causally related to the MCO (38, 39). In this regard central obesity, measured by an increased waist circumference (male >102cm/ 40 inches, females >88cm/ 35 inches)(40), has consistently been associated with an increased risk of diabetes and cardiovascular disease independently of total adiposity, as measured by BMI (41-43).
The central fat depot is however made up of several histologically and functionally distinct adipose tissue compartments including intra-abdominal and subcutaneous compartments.

3.1 Abdominal Adipose Tissue Depots and Metabolic risk

The abdominal adipose tissue compartment has contributions from both the intra-abdominal and subcutaneous adipose tissue compartments.

3.1.1 Visceral Adipose Tissue

The intra-abdominal adipose compartment or visceral adipose tissue (VAT) is made up of both intra-peritoneal fat (mesenteric and omental fat depots) and retroperitoneal fat. Numerous studies have confirmed the direct association between VAT volume, inflammatory marker expression (44), cardiovascular risk (CV risk) and increasing IR (45-47).

3.1.2 Subcutaneous Abdominal Adipose Tissue

It was initially thought that the increased volume of VAT, which is closely related to the liver and pancreas, was wholly responsible for MCO. Matarasso et al, in 1998, suggested that by performing large volume liposuction (LVL) the relative proportion of VAT to total body fat increased (as a result of decreasing the volume of subcutaneous abdominal adipose tissue (SAT)). They suggested that as the VAT volume is a risk factor for the metabolic complications of obesity (MCO), the metabolic effects of LVL may be deleterious and needed to be evaluated (48).
In addition, it has been hypothesized ('lipotoxicity theory') (20) that excess VAT is more harmful than excess subcutaneous fat because lipolysis of VAT triglycerides, releases FFA into the portal vein, which are then taken to the liver (22, 40). Nielsen et al, have however found that only approximately 5% and 20% of portal vein FFA originated from VAT in lean and obese subjects respectively i.e. SAT supplies more FFA to the portal circulation, and hence the liver, than VAT (36, 49). In keeping with this, Abate found that SAT was more closely correlated to IR than VAT in his studies (50, 51).

To complicate matters further, Scarpa's fascia separates SAT into the superficial subcutaneous adipose tissue (SSAT) and the deep subcutaneous adipose tissue (DSAT). The fat in these two subcutaneous compartments are histologically distinct; the DSAT contains more loosely distributed fascial septae with larger more irregularly distributed fat lobules versus the SSAT with its compact fascial septae and small tightly packed fat lobules. The volume of adipose tissue is almost equally distributed between the DSAT and SSAT (52), but 76% of the DSAT is located in the posterior half of the abdomen (39, 53). Thus SAT in the anterior half

of the abdomen, which is the area where most abdominal liposuction is performed, consists largely of SSAT.
It is therefore important to clarify the risk profile of each of these subcutaneous fat depots independently e.g. DSAT may be associated with the MCO but SSAT may be protective (and should therefore be preserved).

Numerous studies have shown that an increased volume of DSAT is equally, if not more closely correlated with the development of essential hypertension, dyslipidaemia, impaired fibrinolysis, raised IR and increased CV risk than VAT volume (50, 54-57).

The relationship between SSAT volume and this metabolic risk are however less clear. Several authors believe that SSAT accumulation is not associated with the MCO, and may even be protective (39, 58). Other authors, using similar study techniques but larger population samples, have found that SSAT behaves similarly to DSAT and VAT with respect to metabolic risk (50, 51, 54, 57).

So it would seem that by reducing the volume of either VAT or DSAT the metabolic profile should improve. As the association of SSAT with the MCO is less clearly established, lipectomy of the SSAT (which occurs invariably during liposuction) could either improve or worsen the metabolic profile. Further research is therefore needed to clarify SSAT role in adiposopathy.

In summary; adipose tissue is the largest endocrine organ in the body and in the obese state, the central portion seems to 'malfunction' resulting in the secretion of abnormal amounts and types of adipokines and inflammatory mediators that ultimately result in increased insulin resistance, essential hypertension and dyslipidaemia (2, 6, 20). The triad of increased IR, dyslipidaemia and essential hypertension in the presence of abdominal obesity is known as the metabolic syndrome that promotes atherosclerosis, cardiovascular disease and ultimately premature death (18, 59).

4. Ethnic Variations

Libovitz and Banerji point out that there "seems to be large ethnic variations of metabolic activity of regional fat masses e.g. Asian Indians with the same fat mass and distribution as Caucasians have increased IR, FFA, CRP, plasminogen activator inhibitor-1 (PAI), and decreased adiponectin" (22).
In the South African context, obese black women are phenotypically different to obese white women. Caucasian women accumulate more fat centrally compared to their African/ black counterparts, who accumulate more gluteofemoral and subcutaneous adipose tissue (SAT) (60) and develop prominent steatopygia. Further, black women have significantly less VAT than white women (~72 vs. 140 cm^2) (61, 62). Despite this, obese black Southern African women have double the prevalence of diabetes (7.0% vs. 3.6%) (63, 64) and hypertension (30% vs. 15%) (65) compared to obese white women who more commonly present with coronary heart disease and hypercholesterolaemia (66, 67).
These findings would be supportive of our phenotypical observations. As white women tend to collect more central adiposity (largely due to more VAT), we would expect them to have a higher cardiovascular risk profile than the black women (as the waist circumference is the best anthropometric determinant of CV risk), however we would also expect them to have a stronger association with IR than obese black women.

But obese black women, who collect more SAT and less VAT than obese white women, have a higher prevalence of type 2 diabetes mellitus and hypertension. This suggests that SAT may be more closely correlated with IR in obese black women than obese white women. Thus obese black women may potentially benefit more metabolically from a reduction in SAT volume (by for example liposuction) than obese white women.

One of our aims therefore was to confirm the ethnic variation in fat distributions in South African women. In addition we aimed to determine the correlation between each of these fat depots (specifically SSAT vs. DSAT) and IR as well as their association with markers of cardiovascular risk. From these results we aimed to draw hypotheses about the possible usefulness of liposuction to prevent MCO.

5. Lipectomy and the Metabolic Complications of Obesity

Current public health initiatives have failed to reverse the obesity epidemic (68-70). It would therefore be desirable to intervene and remove the 'sick truncal fat' (18) to curb the pathophysiological deterioration and potentially preventing the development of type 2 diabetes and the cardiovascular complications of obesity.

In accordance with this Hansen et al found that reducing VAT volume (by omentectomy) doubled the IS in a dog model (35). Similarly Barzilai et al showed that the onset of diabetes, in Zucker Diabetic Fatty rats, could be delayed by removing visceral fat (71). Arner et al observed a significant long term improvement in IS in patients who had removal of less than 1kg of visceral fat (omentectomy) during bariatric surgery (gastric band), compared to those who did not have any visceral fat removed during bariatric surgery (72, 73). These studies suggest that omentectomy may be a useful modality to improve IS and possibly prevent the onset of diabetes in the obese. Unfortunately VAT is not easily accessible for surgical removal. SAT can however be easily removed via liposuction with minimal morbidity and risk (74-77).

5.1 Liposuction and the Metabolic Complications of Obesity

Ever since the realization that adipose tissue is metabolically active, there has been much debate on the effectiveness of liposuction as a means of improving insulin sensitivity and the metabolic profile (22, 78). Several prospective trials (7, 23, 78-85) have been performed examining the effects of large volume liposuction (lipoaspirate volume of greater than 5000mL (86)) on insulin resistance, dyslipidaemia, inflammatory mediators and hypertension. The results of these trials were however conflicting and it is by no means clear what the metabolic consequences of large volume liposuction (LVL) are (see table 2 in addendum).

5.1.1 Summary of the studies of the Metabolic Effects of Liposuction

All of the above trials (table 2) examining the metabolic effects of liposuction were performed on obese or overweight premenopausal women. None of the studies commented on ethnicity of their patients. Liposuction was performed predominantly on the lower abdomen, but most patients also had some liposuction of their buttocks and thighs. None of the studies specified which layer

of SAT was predominantly removed from the lower abdomen (although the DSAT is generally the target area) or the volume of abdominal compared to peripheral fat removed.

75% of the studies showed a statistically significant improvement in IS, but these positive studies represent 88.6% (233/263 patients) of the total number of patients studied. 71% of the studies showed a statistically significant improvement in the adipokine levels. Only 50% and 57% respectively, showed improved inflammatory markers and dyslipidaemia post liposuction. Of the 4 studies that monitored blood pressure, only 2 found a significant improvement post liposuction.

All of the studies that showed a significant improvement in IS, were performed in healthy (non-diabetic) women. The techniques used to measure the IS varied, but the largest trial published (123 patients by D'Andrea et al) (78) used the euglycaemic hyperinsulinaemic clamp technique (considered to be the gold standard) and found a statistically significant improvement in IS ($R = 0.53$, $p < 0.01$). All of the trials that found an improvement in IS and also measured the adipokines and inflammatory mediators, found an improvement in these profiles too.

The study by Bassetto et al (79) is an exception to the above. They performed megalipoplasty (lipoaspirate of >10L) in 15 obese women (the health status was not documented) and found a statistically significant improvement in IS at 6 months of follow-up. There was however no significant improvement in the inflammatory marker or adipokine profile of their patients during the same period.

The follow-up periods, of the positive outcome trials, varied from 21 days to 6 months although the majority (83%) conducted patient follow-up for at least 3 months. Interestingly, D'Andrea et al (78) found that no further significant improvement in the metabolic profile occurred beyond 21 days of follow-up.

Only two studies found no significant improvement in IS (80, 82).
Klein et al performed megalipoplasty on 8 healthy and 7 diabetic obese women (80). Only leptin showed a significant improvement in their follow-up period of 10 to 12 weeks. This study was criticized for the small patient numbers and short follow-up duration. As a result, Klein et al performed a long-term follow-up (range 1.5-4 years) on the same group of patients (84). However, only 7 women agreed to participate in the follow-up study (3 diabetics and 4 healthy). Although the women's post liposuction weight remained stable, 4 of the 7 women had changes in their medication (the 3 diabetics had changes in their anti-diabetic medication and one healthy patient was started on lipid modulating therapy). The long-term follow-up did not demonstrate any improvement in any of the metabolic parameters that were measured in the first trial.

Robles-Cervantes et al performed liposuction in 15 healthy women with a BMI range from 25-27 kg/m^2 (82). They measured IS as well as the lipid profile, but only found a significant improvement in the dyslipidaemia during their follow-up period of 3 weeks.

Two studies were excluded from the liposuction table. The first, by
Rizzo et al (87), examined the metabolic effects of lower abdominal dermolipectomy during abdominoplasty (as opposed to liposuction) in 20 healthy

obese women. Notably, they report a significant improvement in the IS ($r = 0.53$, $p < 0.01$), the adipokine levels ($p < 0.03$), inflammatory marker profiles ($p < 0.001$), the dyslipidaemia (including FFA levels) and the blood pressure by lower abdominal dermolipectomy.
In contrast, Samdal et al (85) studied the effect of LVL on sex hormones, and glucose and lipid metabolism in 9 women. Their patients varied from lean (BMI 23.5kg/m^2) to morbidly obese women (BMI 39kg/m^2). Four of their patients only had fat removed from extra-abdominal regions, but these results were grouped together with those having abdominal liposuction. An oral glucose tolerance test was performed and the serum insulin concentration measured. Extrapolations regarding the IS were drawn from these measurements without directly calculating the IS. In addition the patients diets, exercise regimes and weights were not regulated during the study period. As a result of these findings, this study was also excluded from the table assessing the metabolic effects of liposuction.

5.1.2 Possible Explanations for the Conflicting results of these studies

There are several explanations that could account for the variable outcomes of these trials:

1. Small number of patients in most of the trials
2. Clinical condition (healthy vs. diabetic) of the patients
3. Ethnicity of the patients
4. Technique of IS measurement (euglycaemic hyperinsulinaemic clamp vs. HOMA)
5. Type and site of fat removal (e.g. gluteofemoral vs. SAT, DSAT vs. SSAT)
6. Variable lipectomy volume (lipoaspirate volumes varied from 3.5L (7) to 26.3L (79))
7. Duration of follow-up
8. Failure to regulate amount of exercise and dietary intake preoperatively and for the duration of the follow-up

The conflicting results of these clinical trials on the metabolic effects of large volume liposuction stimulated our interest in the ethnic variations of fat distribution and the metabolic activity of regional fat depots.

6. Aims and Objectives of the current study

1. To determine the ethnic specific variations (black vs. white) in fat distribution of lean compared to obese South African women.
2. To determine the ethnic specific associations of subcutaneous fat depots and insulin resistance (especially SSAT which is the least investigated fat depot with regard to metabolic risk).
3. To determine the ethnic variations in the expression of adipokines and inflammatory markers in each of the fat depots and their associations with IS.
4. To draw hypotheses from our results that may account for the conflicting results of previous studies examining the effects of liposuction on IR, hypertension and dyslipidaemia.

f) Addendum

Insulin resistance	Type 2 diabetes
Cardiovascular:	–Hypertension –Atherosclerosis –Ischaemic heart disease
Neurological:	–Strokes/ CVAs –Neurodegenerative disorders e.g. dementia
Airway:	–Asthma –Sleep apnoea
Other:	–Some cancers (endometrial, colonic, renal and postmenopausal breast cancer) –Fatty liver –Gall bladder disease –Osteoarthritis

Table 1) Obesity related diseases.
Obesity is considered to be a central feature that increases the risk for a vast array of diseases, with significant morbidity and mortality.

Table 2) Studies of the metabolic effects of liposuction
Please see attached Excel spread sheet

LIPOSUCTION TABLE INSERT HERE!!!!

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Part C: Results of the study in the form of an article

Ethnic-specific associations between abdominal and gluteal fat distribution and the Metabolic complications of obesity: Implications for the use of Liposuction

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List of Contents of Literature Review

a) Abstract

Ethnic-specific associations between abdominal and gluteal fat distribution and the metabolic complications of obesity: Implications for the use of Liposuction

Philip Hayes[1], Kevin Adams[1] and Julia Goedecke[2,3]

Background: Adipose tissue is no longer considered to simply be an energy storage depot but is now considered to be an endocrine organ. Enlarged adipocytes of obese individuals release a range of adipokines and inflammatory mediators that promote insulin resistance and ultimately the development of type 2 diabetes and cardiovascular disease. Ever since the realization that adipose tissue is metabolically active, there has been much debate on the effectiveness of liposuction as a means of improving insulin sensitivity and the metabolic profile.

Aim: To examine the ethnic and physiological differences of abdominal and gluteal fat compartments and their relationship to insulin sensitivity.
This study will provide information on the potential metabolic benefits of liposuction in women of different ethnic origin.

Methods: Fifty-four lean and obese, black and white premenopausal women with no known disease were recruited for the study. Body composition, fat distribution (DXA and CT), insulin sensitivity (frequently sampled intravenous glucose tolerance test with minimal model analysis) and serum levels of lipids, adipokines (leptin and adiponectin) and inflammatory mediators (IL-18) were measured. Biopsies were taken from the deep and superficial subcutaneous abdominal adipose tissue (SAT) and gluteal depots for the measurement of leptin, adiponectin and IL-18 mRNA levels.

Results: When matched for body fatness, obese black women had less visceral adipose tissue (VAT) (98.4±11.3 vs. 147.2±11.8 $p<0.05$), but more superficial SAT (325.1±14.8 vs. 247.3 ± 15.4 $p<0.01$) and gluteal fat (4.09±0.18 vs. 3.54±0.2 $p=0.043$) than their white counterparts. There were no ethnic differences in deep SAT ($P=0.92$). Nonetheless, both the lean and the obese black women were less insulin sensitive (IS) than white women (IS lean: 2.8±0.5 vs. 6.1±0.5 and IS obese: 1.8±0.5 vs. 3.8±0.5 $x10^{-4}min^{-1}/mU/l$, $P<0.01$). IS correlated with VAT in white, but not black women ($R=-0.53$, $P<0.01$ and $R=-0.31$, $P=0.21$, respectively). In contrast, IS correlated with deep SAT (whites $R=-0.54$, $p<0.01$, blacks $R=-0.59$, $p<0.01$) and superficial SAT (whites $R=-0.55$, $p<0.01$, blacks $R=-0.50$, $p=0.01$) in both ethnic groups. In addition, gynoid (gluteal) fat mass was found to be inversely associated IS ($R= -0.46$, $p=0.033$) in black but not white women ($r= 0.28$, $p=0.22$), after adjusting for body fatness. Within the adipose tissue depots, we found that the gluteal depot had higher inflammatory marker (IL-18 mRNA: $p<0.001$) and leptin mRNA levels ($p<0.001$), than the DSAT and SSAT abdominal depots, in both ethnic groups. Despite this, only gluteal adiponectin mRNA levels ($r=0.44$, $p=0.03$) in black women and SSAT leptin mRNA levels ($r =-0.54$, $p=0.01$) in white women were associated with IS.

Conclusion: We found that both lean and obese black South African women were more insulin resistant than their white counterparts. SSAT and DSAT volumes correlated inversely with IS in both races, but obese black women had larger volumes of SSAT (and therefore SAT) than obese white women. VAT was only associated with decreasing IS in white women, whereas the gluteal depot was only associated with decreasing IS in black women. Based on these findings, we hypothesise that a reduction in the SAT volume may improve IS and the metabolic profile in both black and white women, but may be more beneficial in black compared to white women.
Further, a reduction in gluteal fat volume by liposuction may be beneficial for black, but not white women. These hypotheses need to be tested in prospective studies.

b) Journal Article

Ethnic-specific associations between abdominal and gluteal fat distribution and the metabolic complications of obesity: Implications for the use of Liposuction

Philip Hayes[1], Kevin Adams[1] and Julia Goedecke[2, 3]

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Abbreviations

BMI- body mass index (kg/m^2)
CV risk- cardiovascular risk
CRP – C-reactive protein
DM – Diabetes mellitus
DXA- Dual-energy x-ray absorptiometry
FFA- free fatty acid
IL-18 - interleukin 18
IS- insulin sensitivity
IR- insulin resistance
LVL- large volume liposuction
MCO- metabolic complications of obesity
PAI – Plasminogen activator inhibitor-1
SAT- subcutaneous abdominal adipose tissue
SSAT- superficial subcutaneous abdominal adipose tissue
DSAT- deep subcutaneous abdominal adipose tissue
VAT- visceral abdominal adipose tissue

1. Introduction

Obesity is an epidemic of the 21st century (1) and is aetiologically associated with a host of medical conditions (including type 2 diabetes mellitus and cardiovascular disease) and even certain cancers (2, 3). Although the association between obesity and these diseases is well established, the mechanisms via which obesity results in disease are less well understood. Of paramount importance is the fundamental shift in our understanding that adipose tissue is not a simple energy storage devise but an endocrine organ (4).

In obesity however, hypertrophic truncal adipose tissue has deranged function and secretes a variety of substances including abnormal amounts of adipokines, free fatty acids and chronic inflammatory mediators (5). This results in increased insulin resistance (IR), dyslipidaemia and essential hypertension (2) and promotes the development of type 2 diabetes mellitus (6), atherosclerosis, cardiovascular disease and ultimately premature death (7).

Following the realization that adipose tissue is metabolically active, there has been much debate regarding the effectiveness of liposuction as a means of improving insulin sensitivity (IS) and the metabolic profile (8, 9). Several prospective clinical trials (8, 10-16) have been performed examining the effects of large volume liposuction (LVL) on IR, dyslipidaemia, inflammatory mediators and hypertension. The results of these trials are however conflicting (see table 1 in addendum).

Approximately 75% of the quoted studies (8, 10, 13, 14, 16), which represent 88.6% (233/263 patients) of the total number of patients studied, showed a statistically significant improvement in IS and the metabolic profile in both overweight (body mass index (BMI) >25kg/m^2) and obese (BMI >30kg/m^2) non-diabetic women. In contrast, other studies (12, 15) showed no significant improvement in the metabolic profile and suggested that liposuction was purely cosmetic and that loss of weight, by dieting and exercise, was needed to improve the metabolic profile (17).

There are several reasons that could account for these conflicting results. For example, the majority of the studies had very small patient population sizes (less than 15) with the clinical status of the patients varying from healthy to diabetic. In addition, the technique used to measure IS and the duration of follow-up varied significantly between trials. Additional factors that could influence the outcome of a trial examining the metabolic effects of LVL are the site and volume of fat removal as the metabolic activity and profile of regional fat depots varies.

In this regard central obesity, measured by an increased waist circumference (male >102cm/ 40 inches, females >88cm/ 35 inches) (18), has consistently been associated with an increased risk of diabetes and cardiovascular disease independently of total adiposity, as measured by BMI (19-21). The central fat depot however, includes contributions from the retroperitoneal and intra-abdominal fat (or visceral adipose tissue (VAT)) as well as the subcutaneous abdominal adipose tissue (SAT). However, only the volume of SAT, and not VAT, can be easily reduced surgically via liposuction.
SAT is further divided into the superficial subcutaneous abdominal adipose tissue (SSAT) and the deep subcutaneous abdominal adipose tissue (DSAT) by Scarpa's fascia. The fat in these two subcutaneous compartments are histologically distinct; the DSAT contains more loosely distributed fascial septae with larger, more irregularly distributed fat lobules, versus the SSAT with its compact fascial septae and small tightly-packed fat lobules (22).

It was initially thought that the increased volume of VAT, which is closely related to the liver and pancreas, was responsible for the MCO (21, 23, 24). Numerous studies have however shown that DSAT is equally, if not more closely correlated with the development of essential hypertension, dyslipidaemia, impaired fibrinolysis, raised IR and increased cardiovascular risk (CV risk) than VAT volume (25-28).

The relationship between SSAT and this metabolic risk is however less clear. Several authors believe that SSAT accumulation is not associated with the MCO, and may even be protective (29, 30). Other authors, using similar study techniques but larger population samples, have found that SSAT behaves similarly to DSAT and VAT with respect to metabolic risk (25, 26, 28, 31). However, as the association of SSAT with the MCO is not clearly established, lipectomy of SSAT (which occurs invariably during liposuction) could either improve or worsen the

metabolic profile. Further research is therefore needed to clarify SSAT role in obesity.

One possible explanation for the conflicting results of these SSAT studies, as well as for the conflicting results of the studies examining the metabolic effects of LVL, is the influence that ethnicity has on the metabolic activity of the regional fat depots (32, 33).
In this regard Libovitz and Banerji showed that Asian Indians with the same body fat and distribution as Caucasians have increased: IR, free fatty acids (FFA), C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI); and decreased adiponectin (9). In the South African context, obese black women are phenotypically different to obese white women. Caucasian women accumulate more fat centrally compared to their African/ black counterparts, who accumulate more gluteofemoral and subcutaneous adipose tissue (SAT) (32) and develop prominent steatopygia. Further, black women have significantly less VAT than white women (~72 vs. 140 cm^2) (34, 35). Despite this, obese black Southern African women have double the prevalence of diabetes (7.0% vs. 3.6%) (36, 37) and hypertension (30% vs. 15%) (38) compared to obese white women who more commonly present with coronary heart disease and hypercholesterolaemia (39, 40).
This suggests that SAT volume in black women may be more strongly correlated with the development of IR than in white women. Thus, obese black women may benefit more metabolically from a reduction in SAT volume (by for example liposuction) than obese white women.

Therefore the aims of our study are:

1. To determine the ethnic specific (black vs. white) variations in fat distribution of lean compared to obese South African women
2. To determine the ethnic specific associations of these fat depots with insulin resistance (especially SSAT which is the least investigated fat depot with regard to metabolic risk)
3. To determine the ethnic variations in the expression of adipokines and inflammatory markers in each of the fat depots and their associations with IS

2. Methods

2.1 Subjects

Fifty-four South African women were recruited by advertisement in local newspapers and from local church groups, community centers, and universities. The study population consisted of 14 normal weight (body mass index [BMI] 18-25 kg/m^2) black, 13 normal weight white, 14 obese (BMI >30 kg/m^2) black, and 13 obese white South African women.
Inclusion criteria were as follows:
(1) age from 18 to 45 years; (2) no known diseases or taking medication for dyslipidemia, diabetes, hypertension, HIV/ AIDS, or any other metabolic disorders; (3) not currently pregnant, lactating, or postmenopausal; and (4) of South African ancestry. The study was approved by the Human Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town. Before participating in the study, procedures and risks were explained to the subjects, all of whom gave written informed consent to participate in the study.

2.2 Study design

2.2.1 Body composition and blood pressure measurement

Basic anthropometric measurements including weight, height, and waist (at the level of the umbilicus) and hip (largest gluteal area) circumference were taken. Body fat percentage and the gynoid region of interest (41) were measured using dual-energy x-ray absorptiometry (Discovery-W, Software version 4.40; Hologic, Bedford, MA) the measurement of which has a coefficient of variation of 1.7%. Abdominal visceral (VAT) and subcutaneous adipose tissue (SAT) area was measured at the level of L4-L5 lumbar vertebrae using computed tomography (Toshiba X-press Helical Scanner, Tokyo, Japan). The fascia superficialis was used to distinguish between the deep (DSAT) and superficial (SSAT) SAT depots. Blood pressure was measured in a seated position after 5-10 minutes of rest using an automated blood pressure monitor (Omron 711; Omron health Care, Hamburg, Germany). The average of three measurements taken at 1 minute intervals was used in the analysis.

2.2.2 Measurement of glucose tolerance, insulin sensitivity and insulin release

After an overnight fast, a blood sample was taken for the determination of fasting plasma glucose, serum insulin, lipids, adiponectin, leptin and high-sensitivity C-reactive protein (CRP) levels and interleukin-18 (IL-18). Subjects then underwent an insulin-modified frequently sampled intravenous glucose tolerance test (FSIGT) to quantify insulin sensitivity. Baseline samples were drawn at −15, −5, and −1 minute before the infusion of glucose (50% dextrose; 11.4 g/m2 body surface area) over 60 seconds at time 0. At 20 minutes, human insulin (0.02 U/kg; Actrapid, Novo Nordisk, Sandton, South Africa) was infused over 5 minutes at a constant rate (i.e. a slow bolus of insulin). Plasma glucose and serum insulin concentrations were measured in the 3 baseline samples and the 32 samples drawn over 240 minutes after commencement of the glucose infusion. Glucose and insulin data from the FSIGT were used to calculate the insulin sensitivity index (IS) using the minimal model of glucose kinetics of Bergman et al (42). The samples were centrifuged at 3000 rpm for 10 minutes at 4^0C, the plasma was stored at -20^0C for subsequent analysis of glucose concentrations, and the serum was stored at -80^0C for the subsequent analysis of insulin concentrations.

2.3 Biochemical analysis

Plasma glucose concentrations were determined using the glucose oxidase method (YSI 2300 STAT PLUS; YSI Life Sciences, Yellow Springs, OH). Serum insulin concentrations were determined by immunochemiluminometric assays using the ADVIA Centaur (Bayer Diagnostics, Tarrytown, NJ). The intra- and interassay coefficients of variation for plasma glucose and serum insulin concentrations were 0.6 and 2.5%, and 4.5 and 12.2%, respectively.

Blood lipids were analyzed using the Roche Modular autoanalyzer (Penzberg, Germany). Enzymatic colorimetric assays were used to analyze total cholesterol, triglyceride, and HDL cholesterol concentrations. The LDL cholesterol concentrations were determined using the Friedewald formula.

Serum concentrations of leptin (Linco Research, St Charles, Missouri, USA), high molecular weight (HMW) adiponectin (Linco Research, St Charles, Missouri, USA), high sensitive C-reactive protein (hsCRP, Immun Diagnostik AG, Bensheim, Germany) and IL-18 (BioSource Europe S.A., Nivelles, Belgium) were all analyzed using commercially available ELISA kits according to the manufacturer's protocols.

2.4 Fat harvest technique

On a separate day and after a 4 hour fast, fat biopsies were obtained from the abdominal DSAT, SSAT and gluteal areas, using a mini-liposuction technique. After local anaesthesia with Lignocaine hydrochloride (2%, Intramed, Port Elizabeth, South Africa), a small incision was made directly above the umbilicus. 200-300 ml of normal saline with adrenaline (0.1%, Intramed, Port Elizabeth, South Africa) and Lignocaine (0.75%, Intramed, Port Elizabeth, South Africa) was infused using an infiltration cannula (Lamis 14ga x 15cm, Byron Medical Inc., Tucson, AZ). An aspiration cannula (Coleman, 12ga x 15cm, Byron Medical Inc, Tucson, AZ) attached to a 10ml syringe was used to aspirate fat from above (SSAT) and below (DSAT) the fascia superficialis, under ultrasound guidance by a radiologist. Gluteal samples were obtained from the right upper quadrant using the same procedure. Approximately 2 ml of fat was extracted from each site and washed three times with normal saline or until no blood was visible. AT was then placed into vials and frozen immediately in liquid nitrogen and stored at –80° C for subsequent analyses.

2.5 Quantification of mRNA by real-time quantitative PCR

Total RNA was extracted from snap-frozen tissue samples using the Qiagen RNeasy system, and 500ng reverse transcribed into cDNA with random primers using the QuantiTect DNase/reverse transcription kit (Qiagen Ltd, Crawley, UK). cDNA (equivalent to 1ng total RNA) was incubated in triplicate in 1x Roche LightCycler®480 Probes master mix (Roche Diagnostics Ltd, Burgess Hill, UK) with gene specific primers (Invitrogen Ltd, Paisley, UK). A standard curve was constructed for each primer probe set using a serial dilution of cDNA pooled from all samples. The NormFinder algorithm identified a combination of PPIA, 18S and RPLPO as the best normalization gene set, with expression levels not being altered by obesity, ethnicity or depot. Results are presented as the ratio of abundance of gene of interest: mean of abundance of PPIA, 18S and RPLO.

3. Statistics

Results are presented as means ± standard error. Two-way analysis of covariance adjusting for age, was used to compare anthropometric and metabolic measures between normal-weight and obese, black and white women, with Bonferroni posthoc analyses. Ethnic differences in abdominal adipose tissue depots were also adjusted for total fat mass. Data were normalized by log transformation, if required. Pearson correlation coefficients were used to explore the relationships between body composition and metabolic outcomes.

Differences in mRNA levels between ethnicity and BMI groups within each SAT depot were analysed using two-way ANOVA, adjusting for age, with Bonferroni posthoc analysis. Repeated measures ANOVA, with Tukey posthoc analysis, was used to determine depot differences in gene expression. Partial correlations, adjusting for age and fat mass, were used to explore the associations between gene expression and measures of insulin sensitivity in both black and white women. Data were analyzed using STATISTICA version 10 (Statsoft Inc., Tulsa, OK).

4. Results

The obese women were significantly older than the normal weight women (mean of 29 years vs. 25 years) (Table 2). Consequently, all subsequent analyses were adjusted for age.

4.1 Body composition

By design, all measures of body composition and regional fat deposition were significantly greater in the obese than the normal-weight women (Table 2).
The lean and the obese black and white groups were well matched for BMI and total adiposity (dual-energy X-ray absorptiometry derived fat mass and % body fat).
In normal-weight subjects, there were no significant ethnic differences in VAT or total SAT areas. However, obese black women had significantly greater SSAT (325.1 ± 14.8 vs. 247.3 ± 15.4 cm^2, $p < 0.01$) and significantly less VAT (98.4 ±11.3 vs. 147.2 ± 11.8, cm^2, $p < 0.05$) compared to obese white women. No significant ethnic differences in deep SAT were observed (269.9 ± 14.3 vs. 247.9 ± 14.3, $p = 0.92$). Obese black women also had significantly greater gynoid fat than obese white women when adjusted for age and total fat mass (4.09 ± 0.18 vs. 3.54 ± 0.2 kg, respectively, $p = 0.043$).

4.2 Blood pressure

None of the women were hypertensive, but obese women had significantly higher diastolic blood pressures compared to the lean group ($p < 0.01$) (table 2). There were no ethnic differences in either systolic or diastolic pressures.

4.3 Insulin sensitivity and associations abdominal fat depots

Fasting glucose levels did not differ between ethnic and BMI groups (Table 3) and all subjects had normal glucose tolerance according to American Diabetes Association Criteria (43).
The obese groups had a significantly greater fasting serum insulin concentration ($p < 0.01$) and correspondingly lower IS ($p < 0.01$) than the lean groups (table 3). Interestingly, both the lean and the obese black women had significantly lower IS ($p < 0.01$) than their white counterparts (Table 3).

In both black and white women (figure 1), DSAT (whites $R = -0.55$, $p < 0.01$, blacks $R = -0.59$, $p < 0.01$) and SSAT (whites $R = -0.55$, $p < 0.01$, blacks $R = -0.50$, $p = 0.01$) were significantly negatively correlated with IS. In contrast, VAT was significantly negatively correlated with IS in white but not black women (white $R = -0.53$, $p < 0.01$, black $R = -0.31$, $p = 0.13$). In addition, gynoid (gluteal) fat mass was negatively associated IS ($R = -0.46$, $p = 0.03$) in black but not white women ($R =$

0.28, p = 0.22) (table 4). In contrast, hip circumference was positively associated with IS in white women (R = 0.45, p = 0.044) and not black women (R = -0.01, p = 0.96). There were no significant associations between leg fat mass and IS in either ethnic groups.

4.4 Lipids

Obese white women had higher total cholesterol (p < 0.01), HDL cholesterol (p < 0.01) and TAG (p < 0.01) compared to obese black women. There was no significant ethnic difference in LDL (table 3).

4.5 Adipocytokines

4.5.1 Circulating levels

There were no significant ethnic differences in the levels of circulating adiponectin, leptin, hsCRP or IL-18, after adjusting for age (table 3). However, adiponectin levels were lower (p < 0.01), while leptin (p < 0.01) and hsCRP (p < 0.01) levels were higher in the obese compared to lean subjects. Plasma IL-18 did not differ in lean vs. obese groups after correction for age (table 3).

Circulating adiponectin (r = 0.49, p = 0.045), leptin (r = -0.62, p = 0.007) and hsCRP (r = -0.59, p = 0.002) levels were significantly associated with IS in white but not black women (adiponectin: r = 0.03, p = 0.86; leptin: r = -0.15, p = 0.44; CRP: r = -0.17, p = 0.35).

4.5.2. Tissue mRNA levels

Leptin mRNA levels correlated significantly with circulating leptin levels in all fat depots (DSAT: r = 0.72, p < 0.001; SSAT: r = 0.73, p < 0.001; Gluteal: r = 0.61, p < 0.001). Similarly, adiponectin mRNA levels correlated significantly with circulating adiponectin levels in all fat depots *(DSAT: r = 0.56, p < 0.001; SSAT: r = 0.63, p < 0.001; Gluteal depot in blacks: r = 0.72, p < 0.001)* except the gluteal depot of whites (r = 0.26, p = 0.196).
In contrast, IL-18 mRNA levels did not correlate with circulating levels (data not shown).

With obesity, adiponectin mRNA levels decreased significantly while leptin and IL-18 mRNA levels increased significantly in all fat depots (SSAT, DSAT and gluteal; p < 0.001). Only leptin mRNA levels showed a significant ethnic difference (after being adjusted for age) being higher in black women in all three depots (p < 0.001) (figure 3).

Leptin and IL-18 mRNA levels, were higher in the gluteal than in either DSAT or SSAT depots (p < 0.001), whereas gluteal adiponectin mRNA levels were lower in the gluteal than the DSAT or SSAT depots (p < 0.001) in both ethnic groups.

4.5.3 Tissue mRNA levels and associations with IS

After adjusting for age and fat mass, only gluteal adiponectin mRNA levels (r = 0.44, p = 0.03) in black women were associated with IS. In white women, IS was associated with SSAT leptin mRNA levels (r = -0.54, p = 0.01) and IL-18 mRNA levels in all fat depots (p < 0.001) (data not shown).

For all tables and graphs of results please see (c) Addendum

5. Discussion

With the realization that adipose tissue is metabolically active, the hypothesis that the benefits of liposuction may go beyond aesthetics and the patient's self-esteem began to be tested (44). Several prospective clinical trials (table 1) have examined the effects of LVL on IS, dyslipidaemia, and the inflammatory and adipokine profiles, but the results of these trials are conflicting. Although the clinical status of the patients, technique of IS measurement, volume of fat aspirated, changes in diet or exercise regimes, and duration of follow-up may account for the variable outcomes of these trials, our study has highlighted several other important factors.

We have examined the ethnic specific variations in the distribution of adipose tissue and the associations between these fat depots and IR and CV risk factors. In this regard, we have found that obese black women were more IR, but accumulated more SSAT compared to obese white women who accumulated more VAT. Further, VAT in white women was associated with decreasing IS but not in black women. Rather, SAT, both deep and superficial SAT depots, were associated with decreasing IS in both black and white women. These findings are supported by Lovejoy et al (32) who found that African American women collect more SAT and less VAT than Caucasian women, and that SAT and not VAT was associated with decreased IS in African American women.

In addition, we found that obese black women accumulate significantly more gynoid or gluteal fat than white women. Several cross sectional population studies have described gluteofemoral fat (as measured by thigh circumference, hip circumference and DXA determined thigh adipose tissue mass) as exerting specific functional properties that are associated with an improved metabolic and CV risk profile (45-48). Lemieux suggests that this protective effect may be as a result of peripheral fat having high lipoprotein lipase activity and low fatty acid turnover, thereby acting as a "metabolic sink" for the accumulation of excessive plasma lipids (49).
Many of these cross sectional studies were however, performed in European countries were the relative proportion of black patients may have been small. In contrast, a recent study by Bays et al, in a mixed ethnicity patient cohort, has shown an increased risk of type 2 diabetes with an increase in the hip circumference (50).

To the best of my knowledge, no studies have compared ethnic differences in the association of gluteofemoral fat and IR. Notably, a novel finding of our study was that the gluteal fat depot was associated with decreasing IS in black women, but not white women. On the contrary, an increase in the hip circumference in white women was associated with improved IS (i.e. a protective profile).
This may be explained by their different adipocytokine expression profiles, with higher IL-18 and leptin mRNA levels in black compared to white women, particularly in the gluteal depot. The increased secretion of leptin in obesity is associated with decreased insulin sensitivity (4, 51) and hypertension (50, 52). Elevated IL-18 on the other hand, is pro-atherogenic (53) and is also associated with myocardial dysfunction (54) and the development of IR, independent of obesity (55). Despite this, only the gluteal adiponectin mRNA levels in black

women, and IL-18 mRNA levels and SSAT leptin mRNA levels in white women, were associated with IS.

There are several limitations to our study. Firstly, the number of participants, in each ethnic group and weight category, was relatively small. Also, they were not randomly selected and our cohort may therefore not be representative of the entire population. Similarly, we have only measured a few adipocytokines although numerous other important adipocytokines have been described. In addition, the gynoid area of interest was measured using the standard DXA technique (41), although a CT/ MRI scan may be a more accurate measure of fat in this depot.

6. Conclusion

In conclusion, we have found that black women are more IR than their white counterparts. In addition, obese black women accumulate more SSAT and less VAT than obese white women. VAT in white women was associated with decreasing IS but not in black women. However, SSAT and DSAT were negatively associated with IS in both ethnic groups. Based on these findings, we propose that a reduction in the volume of abdominal SAT (either SSAT or DSAT) by LVL, may improve IS in both ethnic groups. As obese black women accumulate more SSAT (and therefore SAT) however, they may benefit more than obese white women. Further, we have found that the gluteal area has the highest levels of adipocytokine mRNA in both white and black women, but it has variable ethnic associations with IS. In this regard, liposuction of the gluteal area may improve the metabolic profile in black women, but may worsen the IR in white women.

We recommend that future prospective trials examining the metabolic effects of LVL in obesity be stratified according to ethnicity as well as gluteal vs. abdominal depots.

c) Addendum

Table 1) Metabolic effects of large volume liposuction (see Excel spread sheet next page

Table 2. Body composition and distribution

	Lean Black (n=14)	Lean White (n=13)	Obese Black (n=14)	Obese White (n=13)
Age (yrs)	23 ± 2	25 ± 2	28 ± 2	32 ± 2
BMI (kg/m^2)	22.9 ± 1.0^C	22.6 ± 1.0^D	37.9 ± 1.0^C	36.5 ± 1.0^D
Fat mass (kg)	17.2 ± 1.9^C	18.9 ± 2.0^D	43.6 ± 1.9^C	45.6 ± 2.0^D
Body fat (%)	30.4 ± 1.3^C	29.4 ± 1.4^D	47.2 ± 1.3^C	45.3 ± 1.4^D
Regional body fat distribution				
Waist (cm)	76.2 ± 2.8^C	79.7 ± 2.9^D	113.0 ± 2.8^C	108.3 ± 2.9^D
Hip (cm)	98.4 ± 2.4^C	102.0 ± 2.4^D	126.1 ± 2.4^C	125.3 ± 2.4^D
WHR	0.78 ± 0.02^C	0.78 ± 0.02^D	0.90 ± .02^C	0.86 ± 0.02^D
VAT (cm^2)	60.4 ± 10.9^C	56.7 ± 10.9^D	98.4 ± 11.3^{b,C}	147.2 ±11.8^{b,D}
Deep SAT (cm^2)	68.0 ± 14.5^C	83.3 ± 14.5^D	264.6 ± 15.1^C	257.6 ± 15.8^D
Superficial SAT (cm^2)	98.0 ± 14.2^C	95.2 ± 14.2^D	325.1 ±14.8^{B,C}	247.3 ±15.4^{B,D}
Total SAT (cm^2)	176.7 ± 22.3^C	175.5 ± 20.7^D	591.3 ± 21.4^{b,c}	492.2 ±21.4^{b,d}
Gynoid fat (kg)	1.76 ± 0.18^C	1.70 ± 0.18^D	4.09 ± 0.18^C	3.54 ± 0.20^D
Blood Pressure				
Systolic (mmHg)	104 ± 4	110 ± 4	110 ± 4	112 ± 4
Diastolic (mmHg)	69 ± 2^C	70 ± 2^D	77 ± 2^C	76 ± 2^D

Values are unadjusted mean ± SEM. All P values adjusted for age.
a P < 0.05 and A P < 0.01: Lean black vs. Lean white.
b P < 0.05 and B P < 0.01: Obese black vs. obese white.
c P < 0.05 and C P < 0.01: Lean vs. obese black.
d P < 0.05 and D P < 0.01: Lean vs. obese white.

Table 3. Metabolic parameters

	Lean Black (n=14)	Lean White (n=13)	Obese Black (n=14)	Obese White (n=13)
Glucose tolerance and insulin sensitivity				
Fasting glucose (mmol/l)	4.4 ± 0.1	4.3 ± 0.1	4.6 ± 0.1	4.6 ± 0.1
Fasting insulin (mU/l)	8.2 ± 1.3^{C}	4.2 ± 1.3	14.9 ± 1.3^{B,C}	8.7 ± 1.3^{B}
IS (x10^{-4} min/μU/ml)	2.8 ± 0.5^{A,C}	6.1 ± 0.5^{A,D}	1.8 ± 0.5^{B,C}	3.8 ± 0.5^{B,D}
Lipid profile				
FFA (mmol/l)	0.36 ± 0.04	0.35 ± 0.04	0.40 ± 0.04	0.46 ± 0.04
Triglycerides (mmol/l)	0.61 ± 0.08 ^{a}	0.82 ± 0.09^{a}	0.82 ± 0.08^{B}	0.90 ± 0.09^{B}
Total cholesterol (mmol/l)	3.6 ± 0.3	4.1 ± 0.3 ^{d}	3.8 ± 0.3^{B}	5.0 ± 0.3$^{b, d}$
HDL-cholesterol (mmol/l)	1.3 ± 0.1^{A}	1.8 ± 0.1^{A}	1.1 ± 0.1^{b}	1.6 ± 0.1^{b}
LDL-cholesterol (mmol/l)	2.0 ± 0.2	1.9 ± 0.3^{d}	2.3 ± 0.2	2.9 ± 0.3^{d}
Circulating Adipokines				
Adiponectin (ng/ml)	6.6 ± 0.7^{c}	6.8 ± 0.7^{d}	3.8 ± 0.7^{C}	4.1 ± 0.7^{D}
Leptin (ng/ml)	15.3 ± 3.3^{C}	18.8 ± 3.4^{D}	57.7 ± 3.3^{C}	50.9 ± 3.4^{D}
hsCRP (μg/ml)	2.8 ± 0.8^{c}	1.4 ± 0.8^{D}	6.1 ± 0.8^{c}	6.4 ± 0.8^{D}
IL-18 (pg/ml)	167.3 ± 44.8	170.7 ± 20.0	174.5 ± 37.8	279.6 ± 28.6

Values are unadjusted mean ± SEM. All P values adjusted for age.
a P < 0.05 and A P < 0.01: Lean black vs. Lean white.
b P < 0.05 and B P < 0.01: Obese black vs. obese white.
c P < 0.05 and C P < 0.01: Lean vs. obese black.
d P < 0.05 and D P < 0.01: Lean vs. obese white.

IS, insulin sensitivity index; hs-CRP, high-sensitivity C-reactive protein

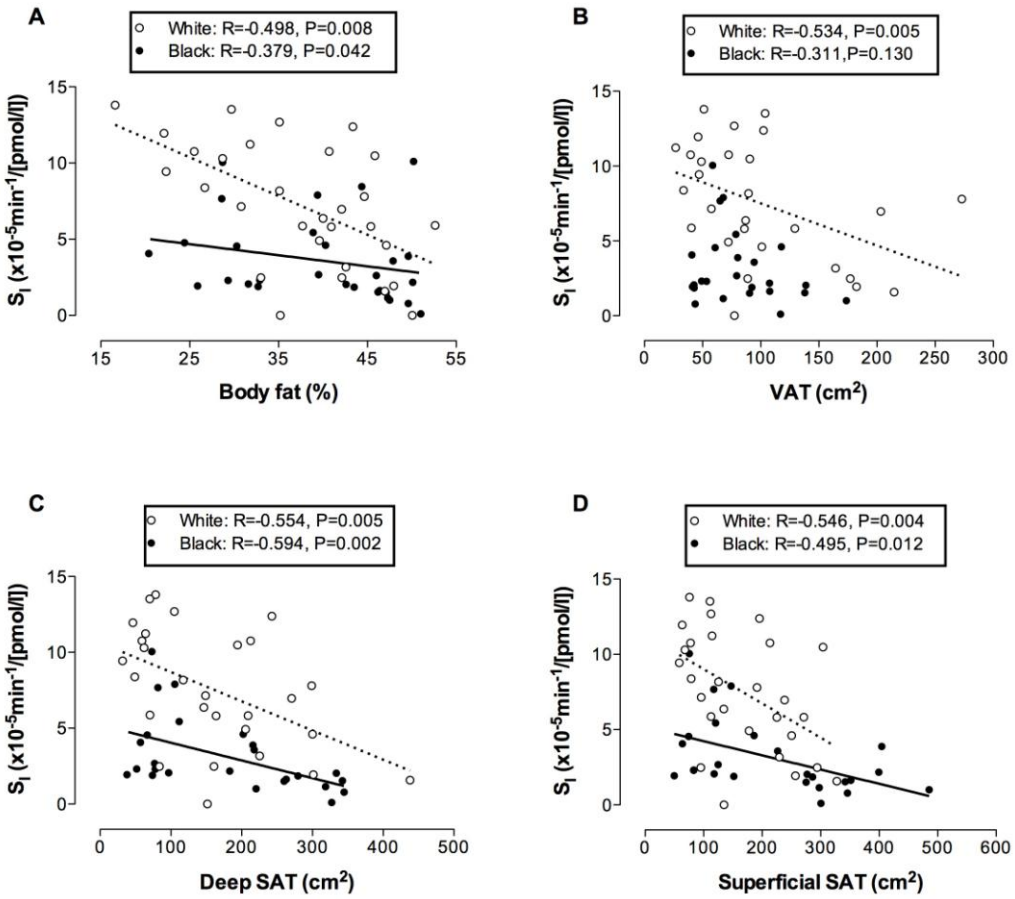

Figure 1. Insulin sensitivity vs. volume in fat depots

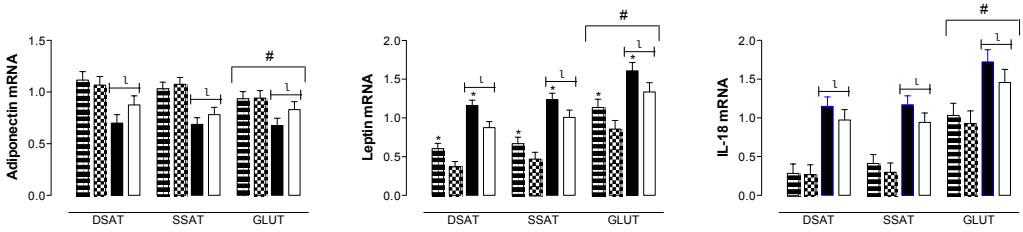

Figure 3. Expression of adiponectin, leptin and interleukin 18 in abdominal deep (DSAT) and superficial subcutaneous (SSAT) and gluteal (GLUT) adipose tissue depots in normal weight and obese black and white women. normal weight black; normal weight white; obese black; obese white. Bars represent means ± SE. * P<0.01, black vs. white; $^{\lambda}$ P<0.05, obese vs. normal weight; $^{\#}$ P<0.05, Gluteal vs. DSAT and SSAT depots.

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f) Disclosure

The authors declare no conflict of interest.

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FSIGT and Biopsy information Sheet and Informed Consent:

INSULIN SECRETION IN RELATION TO INSULIN SENSITIVITY AND THE ASSOCIATION WITH DEPOT-SPECIFIC ADIPOKINE EXPRESSION IN SOUTH AFRICAN WOMEN

Purpose:

More than three-quarters (77%) of the 40.5 million people living in South Africa are black African, of which more than 40% are urbanised. Black African women living in urban areas are more often overweight or obese than urban black males or white females. It was previously thought that obesity in black South African women was "healthy" and not associated with any diseases. However, although black South African women rarely develop heart disease, they often develop diabetes (sugar disease) or high blood pressure.

Previous studies done by Wits University have shown that some overweight black South African women don't produce enough insulin, which is the hormone (natural chemical messenger) released from the body that helps to move glucose into the cells of the body. In addition, they showed that even if these women do produce insulin, the cells in their body are not able to make use of it easily. In this study we will investigate these findings more thoroughly.

The research group at Wits University also found that black women have less fat around their stomachs, but more fat around their thighs and the lower part of their bodies than white women. Fat situated around the stomach is strongly associated with changes in stress hormones and diseases such as sugar disease, high blood pressure and heart disease. Therefore, it is surprising that black women suffer from these diseases. We would therefore like to investigate whether the fat that is situated deep in the lower part of the body may be as "unhealthy" as the fat situated around the stomach and may increase the chance of overweight or obese black South African women developing sugar disease and high blood pressure.

It was always thought that fat situated around the body was just used for storage purposes. However, researchers have recently discovered that fat cells also produce natural chemical messengers (hormones) and other proteins that affect the functioning of the body. In this study, we will investigate whether the hormones and proteins are different in the different fat stores (upper and lower body) of black and white women and whether these differences may explain the diseases that they develop.

Therefore, the aim of the study is to test whether the body fat distribution of black women is in fact the factor that increases their chances of developing sugar disease and high blood pressure.

Who can participate?

Sixty black and white South African women between the ages of ~20 and 45 years, who are living in urban areas, will be recruited for the study. Only women that are classified as lean (BMI > 19 and < 25 kg/m^2) or obese (BMI > 30 kg/m^2) will be included in the study. Subjects will be excluded from the trial on the basis of the following criteria: i) receiving therapy for HIV/AIDS; ii) taking medication for

any endocrine abnormality (e.g. sugar disease, thyroid problems) or taking other medications, such as oral corticosteroids, and anti-hypertensive medications; iii) currently pregnant or breastfeeding; iv) having polycystic ovarian syndrome and, and vii) having irregular menstrual cycles.

Benefits:

You will receive the final results of the study as well as your individual results, including, body composition (weight, height, body fatness, muscle mass, body fat distribution), dietary intake, risk of diabetes, an estimate of your insulin sensitivity and secretion, blood lipids and blood pressure. The results obtained from the fat biopsies will also be provided. In addition, you will be paid a nominal sum of money for transport and the inconvenience to you.

The findings of this research can be used to highlight specific areas for intervention in terms of drug therapy, for the treatment and management of the diseases associated with obesity in South African women.

Procedures and potential risks:

You will be required to complete approximately 3-4 sessions of testing, which will include the following tests:

Transport (or money for transport) to and from testing will be provided.

Session 1: Early morning before breakfast for approximately 4 hrs (this testing session can be divided in 2 parts)

You will be requested to come to the laboratory at the Sports Science Institute in the morning after an overnight fast (10-12 hrs). In other words, you must not eat or drink anything, except water for at least 10 hours. You will be required to undergo the following tests/measurements:

1) *Information and consent:*
The purpose and procedures, as well as the risks and benefits of the trial will be explained to you. You will be given the opportunity to ask any questions, which will be answered by the investigator or the doctor involved in the study. If you agree to participate in the study, you will sign the informed consent form and be entered into the trial.

2) *Demographic and lifestyle questionnaire:*
A demographic questionnaire will be administered and will include measures of socio-economic status (on the basis of factors such as; asset index, education, housing and housing density, occupation), as well as questions related to family history, personal health, reproductive history, parity, dietary intake and physical activity and health habits.

3) *Blood pressure measurement:*
Blood pressure will be measured 3 times after you have rested for at least 20 min.

4) *Fasting blood sample and oral glucose tolerance test:*
A small plastic tube will be placed into a vein in your arm and a small tap attached from which blood samples (1 teaspoon – 5 ml) will be drawn at 30 min intervals during the 2 hr test. First, a fasting blood sample will be drawn from your arm for the measurement of blood lipids, glucose and various hormones. You will then be asked to drink a cup of water containing 75 g of glucose. Blood glucose, insulin and free fatty acid concentrations will be measured from the blood samples taken before and 30, 60, 90 and 120 min after ingesting the glucose solution. There are no appreciable risks for this test, other than those associated with routine blood sampling. All procedures will be supervised and carried out by appropriately trained medical personnel using sterile techniques to minimise any risks of infection. This test is used routinely for both research and medical purposes.

5) *Body composition:*
Basic body composition measurements will be taken, including weight, height, waist and hip circumference. In addition, you will undergo a dual-photon x-ray absorptiometry (DEXA) scan, which will accurately measure your fat and muscle mass, as well as your bone density. The radiation exposure with a DEXA scan is 11.3 microSieverts, which is significantly less than the radiation absorbed by a passenger on a return transcontinetnal flight (60 microSieverts). The scan will take approximately 20 minutes to perform.

6) *Body fat distribution and liver fat content: - N1 City Medi-Clinic*

You will be required to travel to N1-City Medi-Clinic where you will undergo magnetic resonance imaging (MRI scan) and computerised tomography (CT scan) in the radiology department. The CT scan will be used to measure your body fat distribution, in other words, where the fat is situated around your belly. The exposure to ionising radiation with the CT scan is less than 10 % of the total allowable occupational exposure per year (about 500 millirems out of 5000 millirems). The MRI scan will be used to measure the fat content of your liver. MRI uses magnetism and radio waves, and will be used to generate a picture of your abdomen and your liver. You will be required to lie on a bed, which is moved into a wide-bore tubular structure. This is open at both ends. You will be required to lie still for 15 minutes while being in constant voice contact with the Radiographer. You will not experience any pain or discomfort and there are no risks associated with the scan. However, you may experience slight claustrophobia during the scan.

Session 2: Early morning before breakfast for approximately 4.5 hrs

Insulin secretion and insulin sensitivity:

You will be requested to come to the laboratory at the Sports Science Institute in the morning after an overnight fast (10-12 hrs). In other words, you must not eat or drink anything, except water for at least 10 hours. A small plastic tube will be placed into a vein in each arm. You will then be required to undergo a test that will measure how much insulin your body produces and how sensitive your body is to insulin. We will inject a concentrated glucose solution (~ 30-100 ml, depending on your weight) into one vein over a 1-minute period. Small amounts of blood (1 teaspoon) will be withdrawn from the other arm at regular intervals (1-2 minutes) for 20 min. After 20 min, insulin will be infused into your arm, which will assist your body take up the glucose into the cells. Further blood samples (1 teaspoon each) will be drawn from your other arm for a further 3.5 hrs. During this test, a maximum of 200 ml of blood will be drawn (1/3rd of the amount drawn when you donate blood). During the tests, you will be required to sit or lie quietly and videos will be provided for entertainment. There are no appreciable risks for this test, other than those associated with routine blood sampling. All procedures will be supervised and carried out by a medical doctor and appropriately trained medical personnel using sterile techniques to minimise any risks of infection. These tests are used routinely in research to accurately determine insulin secretion and insulin sensitivity. This test should be undertaken at the follicular phase of your menstrual cycle i.e. within 10 days of starting menstruation.

Session 3: Early morning before breakfast for approximately 1 hr

Fat biopsies:

You will be requested to undergo fat biopsies from the stomach and buttocks areas. The fat samples will be used to analyse the proteins and hormones produced by the fat cells, which may explain why black and white South African women get certain diseases. You will be required to come to the laboratory the Sports Science institute in the morning after an overnight fast (10-12 hrs). In other words, you must not eat or drink anything, except water for at least 10 hours. A medical doctor will perform the fat biopsies with the assistance of a radiographer at the Sports Science Institute of South Africa. A local anaesthetic will be administered prior to the procedure. Please

inform us if you have had any previous reactions to any other anaesthetics, for example at the dentist. After the anaesthetic has taken effect, small incision (0.5-1 cm) will then be made in the skin and fat samples (1.5 cm^3) will be removed using a needle connected to a syringe. After this procedure, a waterproof sterile dressing will be applied. You may develop bruising and some discomfort from the procedure. This procedure is used frequently for research purposes.

Assurances:

Participation in this research study is absolutely voluntary. You do not have to take part if you do not want to. You may withdraw at any time without stating a reason and without prejudice. The doctor or researcher can also withdraw you from the study. You will be provided with all your individual results, as well as a summary of the trial results on completion of the study.

Strict confidentiality of results will be maintained. All records will be kept in a locked room and in a secure computer database in the research unit. Your name will not be used in any publication of the results.

The University of Cape Town and its team of researchers, who are working under the mandate of the University, will be responsible for treating any adverse or untoward events arising from participation in this research study. Trained medical professionals will perform all medical or invasive procedures, under sterile conditions.

Thank you for your participation. Please contact me immediately if you would like to ask any questions or you experience any problems during or after the tests.

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Subject code:

INSULIN SECRETION IN RELATION TO INSULIN SENSITIVITY AND THE ASSOCIATION WITH DEPOT-SPECIFIC ADIPOKINE EXPRESSION IN SOUTH AFRICAN WOMEN

Consent:

"I, _____, hereby give consent to participate in this research trial to be conducted by the UCT/MRC Research Unit for Exercise Science and Sports Medicine, within the Department of Human Biology at the University of Cape Town. I understand that I will undergo testing at the Sports Science Institute, which will include completion of a demographic and lifestyle questionnaire, the measurement of blood pressure, body composition including DEXA, CT and MRI scans, blood lipid and hormone levels, oral glucose tolerance, as well as insulin secretion and sensitivity. I also understand that fat samples (~1.5 cm^3) will be taken from the fat stores in my abdominal (stomach) and gluteal (bottom) areas. I have read and have had explained to me the procedures described above. I have had an opportunity to ask questions. My questions have been answered in a satisfactory way. I understand the nature of the trial and the risks and benefits associated with my participation. I understand that I am free to withdraw from this study at any time.

Name: _____ Signature: _____ Date: _____

Witness: _____ Signature _____