Gynecologic Oncology 138 (2015) 238–245

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Women at extreme risk for obesity-related carcinogenesis: Baseline endometrial pathology and impact of bariatric surgery on weight, metabolic profiles and quality of life

Susan C. Modesitt [a,*], Peter T. Hallowell [b], Jill K. Slack-Davis [c], Ryan D. Michalek [d], Kristen A. Atkins [e], Sarah L. Kelley [f], Sanja Arapovic [a], Margaret A. Shupnik [g], Kyle Hoehn [h,i]

[a] Gynecologic Oncology Division, University of Virginia School of Medicine, Charlottesville, VA, United States
[b] Department of Surgery, University of Virginia School of Medicine, Charlottesville, VA, United States
[c] Department of Microbiology, Immunology and Cancer Biology, University of Virginia School of Medicine, Charlottesville, VA, United States
[d] Metabolon, Inc., Durham, NC, United States
[e] Department of Pathology, University of Virginia School of Medicine, Charlottesville, VA, United States
[f] College of Arts and Sciences, University of Virginia, Charlottesville, VA, United States
[g] Endocrinology Division, University of Virginia School of Medicine, Charlottesville, VA, United States
[h] Department of Pharmacology, University of Virginia School of Medicine, Charlottesville, VA, United States
[i] School of BABS, University of New South Wales, Sydney, Australia

HIGHLIGHTS

- Women presenting for bariatric surgery had a 10% baseline endometrial hyperplasia prevalence.
- Bariatric surgery resulted in excellent weight loss (mean 45 kg) and significantly improved physical quality of life.
- Bariatric surgery was accompanied by improved glucose homeostasis, insulin responsiveness, and inflammation to a greater extent than hormonal changes.

ARTICLE INFO

Article history:
Received 18 April 2015
Accepted 19 May 2015
Available online 23 May 2015

Keywords:
Bariatric surgery
Morbid obesity
Metabolomics
Endometrial hyperplasia
QOL

ABSTRACT

Objectives. The study objectives were to determine baseline endometrial histology in morbidly obese women undergoing bariatric surgery and to assess the surgical intervention's impact on serum metabolic parameters, quality of life (QOL), and weight.

Methods. Women undergoing bariatric surgery were enrolled. Demographic and clinicopathologic data, serum, and endometrium (if no prior hysterectomy) were collected preoperatively and serum collected postoperatively. Serum global biochemical data were assessed pre/postoperatively. Welch's two sample *t*-tests and paired *t*-tests were used to identify significant differences.

Results. Mean age of the 71 women enrolled was 44.2 years, mean body mass index (BMI) was 50.9 kg/m^2, and mean weight loss was 45.7 kg. Endometrial biopsy results: proliferative (13/30; 43%), insufficient (8/30; 27%), secretory (6/30; 20%) and hyperplasia (3/30; 10%—1 complex atypical, 2 simple). QOL data showed significant improvement in physical component scores (PCS means 33.9 vs. 47.2 before/after surgery; $p < 0.001$). Twenty women underwent metabolic analysis which demonstrated significantly improved glucose homeostasis, improved insulin responsiveness, and free fatty acid levels. Significant perturbations in tryptophan, phenylalanine and heme metabolism suggested decreased inflammation and alterations in the intestinal microbiome. Most steroid hormones were not significantly impacted with the exception of decreased DHEAS and 4-androsten metabolites.

Conclusions. Bariatric surgery is accompanied by an improved physical quality of life as well as beneficial changes in glucose homeostasis, insulin responsiveness, and inflammation to a greater extent than the hormonal milieu. The potential cancer protective effects of bariatric surgery may be due to other mechanisms other than simply hormonal changes.

* Corresponding author at: Gynecologic Oncology Division, Richard N. and Louise R. Crockett Professor of Obstetrics and Gynecology, Box 800712, University of Virginia Health System, Charlottesville, VA 22908-0712, United States. Fax: +61 434 982 1840.

http://dx.doi.org/10.1016/j.ygyno.2015.05.015
0090-8258/

1. Background

The obesity crisis continues to escalate and impacts all facets of healthcare [1]. According to the Centers for Disease Control, 35.7% of US adults are obese based on body mass index (BMI ≥30 kg/m^2) and 6.3% qualify as morbidly obese (BMI ≥40 kg/m^2) [2]. Obesity markedly increases cancer risk yet whether the main mechanisms are hormonal, inflammatory or metabolic remain uncertain. Despite that, there appears to be a dose-dependent relationship in that the greater the BMI, the greater the relative risk for developing cancer, particularly cancers of the endometrium, gall bladder, breast, and colon [3–5]. Bhaskaran et al. recently calculated that 40.8% of uterine cancer cases in the United Kingdom were attributed to obesity; this far exceeded the attributable risk for all other types of cancer as the next closest was gallbladder (20.3%) followed by kidney (16.6%), liver (15.6%), and colon cancers (11.1%) [6]. A retrospective study conducted at the University of Virginia demonstrated that women undergoing bariatric surgery had a significantly decreased cancer rate compared to obese controls (3.5% vs. 5.8%; $p = 0.002$) [7]. Women contemplating bariatric surgery are at an extremely high risk for endometrial cancer and Argenta et al. confirmed that approximately 7% of women presenting for bariatric surgery were found to have concomitant endometrial hyperplasia [8].

Bariatric surgery and subsequent weight loss may be associated with decreased cancer risk, but the responsible mechanisms also remain unclear due in part to the lack of long-term follow-up and missing outcome data reported in many studies. A recent meta-analysis showed the mean percentage weight loss was greatest for gastric bypass (65.7%), followed by gastric sleeve (64.5%) and then by the gastric band (45%); further the bypass seems to have better outcomes with sustained weight loss and remission of associated comorbidities (e.g. Type 2 diabetes, hypertension, and hyperlipidemia) [9]. The largest study to examine the link to cancer and bariatric surgery to date is the Swedish Obese Subjects (SOS) study which followed surgery patients versus controls over 10 years and found a decreased cancer rate in obese women who underwent bariatric surgery (HR 0.58, 0.44–0.77; $p = 0.0001$) [10].

The objectives of this study were to prospectively enroll morbidly obese women undergoing bariatric surgery in order to evaluate: 1) the baseline prevalence of menstrual irregularities and endometrial pathology, and 2) to interrogate biochemical profiles manifested in serum samples collected prior to and following bariatric surgery with the aim of characterizing metabolic migration and biomarkers associated with surgical intervention. Additional exploratory objectives were to evaluate the impact of surgery on quality of life and to determine how surgery affects biomarkers/pathways thought to be involved in obesity-mediated carcinogenesis.

2. Methods

2.1. Study participants/conduct

The University of Virginia's Institutional Review Board for Health Sciences Research approved this study. Women who were at least 18 years of age and presenting for consideration of bariatric surgery for weight loss were approached about inclusion into the study and signed informed consent to participate. Demographic, quality of life (QOL, SF-36 form utilized), and clinicopathologic data were recorded. Serum was collected preoperatively and postoperatively, and endometrial tissue (if no prior hysterectomy) was collected via endometrial biopsy at time of surgery. These endometrial biopsies were cached and read by one pathologist (KA) at the conclusion of the study. Postoperative serum and QOL questionnaires were scheduled to be collected at 6 and 12 month time points but, due to lack of compliance, the protocol was changed to collect at any time the patient came for evaluation or for the QOL to be performed via telephone. Multiple attempts via telephone and mail were made to contact and encourage non-compliant patients to come in for their follow-up studies. Statistical analysis was performed with SPSS 22.0 (Chicago IL). Frequency data were reported as a percentage of respondents. Mean, standard deviation, and range were determined when applicable; chi-square and one-way analysis of variance (ANOVA) were used for comparison of groups as necessary. QOL data before and after bariatric surgery were compared using paired *t*-tests. A p value of <0.05 was deemed significant.

Serum global biochemical profiles of 732 compounds (see specific methods below; Metabolon Inc, Durham, NC) were analyzed for a subset of 20 patients who had an endometrial biopsy and both pre/post-surgery bloods collected. Welch's two sample *t*-tests and paired *t*-tests were used to identify significant differences ($p < 0.05$).

2.2. Sample preparation for global metabolomics

Samples were stored at −80 °C until processed. Sample preparation was carried out as described previously [11] at Metabolon, Inc. Briefly, recovery standards were added prior to the first step in the extraction process for quality control purposes. To remove protein, dissociate small molecules bound to protein or trapped in the precipitated protein matrix, and to recover chemically diverse metabolites, proteins were precipitated with methanol under vigorous shaking for 2 min (Glen Mills Genogrinder 2000) followed by centrifugation. The resulting extract was divided into five fractions: one for analysis by ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS; positive ionization), one for analysis by UPLC-MS/MS (negative ionization), one for the UPLC-MS/MS polar platform (negative ionization), one for analysis by gas chromatography–mass spectrometry (GC-MS), and one sample was reserved for backup.

Three types of controls were analyzed in concert with the experimental samples: samples generated from a pool of human plasma extensively characterized by Metabolon, Inc. or generated from a small portion of each experimental sample of interest served as technical replicate throughout the data set; extracted water samples served as process blanks; and a cocktail of standards spiked into every analyzed sample allowed instrument performance monitoring. Instrument variability was determined by calculating the median relative standard deviation (RSD) for the standards that were added to each sample prior to injection into the mass spectrometers (median RSD typically = 4–6%; $n \geq 30$ standards). Overall process variability was determined by calculating the median RSD for all endogenous metabolites (i.e., non-instrument standards) present in 100% of the pooled human plasma or client matrix samples (median RSD = 8–12%; n = several hundred metabolites). Experimental samples and controls were randomized across the platform run.

2.3. Mass spectrometry analysis

Non-targeted MS analysis was performed at Metabolon, Inc. Extracts were subjected to either GC-MS [12] or UPLC-MS/MS [11]. The chromatography was standardized and, once the method was validated no further changes were made. As part of Metabolon's general practice, all columns were purchased from a single manufacturer's lot at the outset of experiments. All solvents were similarly purchased in bulk from a single manufacturer's lot in sufficient quantity to complete all related experiments. For each sample, vacuum-dried samples were dissolved in injection solvent containing eight or more injection standards at fixed concentrations, depending on the platform. The internal standards were used both to assure injection and chromatographic consistency. Instruments were tuned and calibrated for mass resolution and mass accuracy daily.

The UPLC-MS/MS platform utilized a Waters Acquity UPLC with Waters UPLC BEH C18-2.1 × 100 mm, 1.7 μm columns and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer