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Pre-Surgery Leptin Level and Weight Loss in Female Bariatric Surgery Patients

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Pre-Surgery Leptin Level and Weight Loss in Female Bariatric Surgery Patients

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Abstract

Background**:** Improving weight loss after bariatric surgery remains a clinical concern. An unstudied factor is the role of leptin, the appetite control hormone, as a pre-surgical predictor of weight loss. High serum levels, often seen in obesity, are an indicator of leptin resistance as the brain no longer responds to satiety signaling.

Methods**:** Leptin levels were ascertained in female bariatric patients (n=28) prior to surgery at a large tertiary care hospital. We calculated Pearson Correlation Coefficients (r) and unstandardized β coefficients using multivariate linear regression. Primary outcome: Percent Excess Weight Loss (%EWL) evaluated at 6- and 12-months post-surgery. Independent variables were: baseline leptin and, potential confounders, Healthy Eating Behavioral Index (HEBI) and age.

Results: Pearson correlation coefficients showed inverse associations baseline leptin with %EWL at 6-months ($r=-.45$, $p<.01$) and 12 months ($r=-.21$, $p>,05$). In Linear Regression, baseline leptin was inversely associated with %EWL at 6-months (adjusted $\beta=-.12$, $p=.03$) but the relationship was attenuated at 12-months (adjusted $\beta=-.07$. $p=.47$). HEBI was positively associated with %EWL at both 6-months (adjusted $\beta=.19$, $p=.04$) and 12-months (adjusted $\beta=.26$, $p=.07$).

Conclusion and Discussion: Findings suggest that higher baseline leptin might impede weight loss in the first 6 months after surgery possibly due to reduced appetite control, which held when controlling for age and healthy eating. The effect at 6-months (adjusted $\beta = -.12$) denotes that a 75 ng/mL increase in leptin might incur about 10

percentage points less EWL. Longitudinal investigations in larger studies containing an array of potential explanatory variables are recommended.

Competencies Addressed

Foundational Competencies

3. Analyze quantitative and qualitative data using biostatistics, informatics, computer-based programming and software, as appropriate
4. Interpret results of data analysis for public health research, policy, or practice
21. Perform effectively on interprofessional teams

Concentration-specific Competencies

3. Demonstrate high personal and professional ethical conduct in contributing to team-based activities

Systems Thinking Framework

Obesity is one of the most pressing and complex public health issues in the United States today. Understanding the physiological mechanisms of obesity, specifically barriers to weight loss success, are of vital importance. This research focuses on baseline plasma leptin levels as an indicator of successful postoperative outcomes in females undergoing bariatric weight loss surgery. While healthy outcomes cannot be predicted by leptin levels alone, baseline leptin values may play a role in informing patient care by predicting when patients may need additional weight loss guidance and support, and what the nature of that support may entail.

Baseline plasma leptin was identified as an area of interest because of its relation to the relatively novel phenomenon of leptin resistance. In short, this phenomenon is characterized by a malfunction in the metabolic hormone leptin, which normally indicates satiety via high plasma levels after meals. In many individuals with obesity, the brain does not properly respond to plasma leptin levels and the individual will continue to consume food. Individuals with leptin resistance are therefore prone to overeating and often find it extremely difficult to lose weight. Since leptin is secreted by adipocytes, individuals with a higher BMI often secrete more leptin, leading to consistently high plasma leptin levels.

The causes of leptin resistance are unknown, but this can pose a significant barrier to weight loss as well as weight management. Therefore, it is important that physicians understand leptin resistance and consider this condition when treating patients with obesity. This novel research will contribute to the knowledge base

surrounding leptin resistance. In the future, it would be beneficial to develop a clinical definition of leptin resistance and methods to increase leptin sensitivity. Until that point, comprehension and awareness of leptin resistance can help healthcare workers understand barriers to weight loss success and may inform treatment. For instance, a leptin resistant patient may have difficulty adhering to advice regarding portion control, and instead may benefit from advice focusing on the quality of food consumed rather than the quantity.

These findings can be extrapolated to the public health level. Weight loss success is equally psychological as it is physiological, if not more. After investing effort in weight loss, individuals that do not achieve success may be prone to giving up, especially if they are being misdirected or even blamed by healthcare professionals. Many people, including those in the healthcare fields, view obesity as a lack of self-discipline, especially with dietary nonadherence. The stigma surrounding obesity is still very present despite the growing prevalence of obesity, so it is more important now than ever before that healthcare professionals and public health professionals alike understand its complex nature and work alongside the public to combat this issue.

To effectively translate this research into meaningful change, it is important to examine the current systems and framework related to obesity, bariatric surgery, leptin resistance. As one of the fastest-growing public health crises in America, patients grappling with obesity will become increasingly prevalent, and it is important to understand how to care for these patients on a physiological, psychological, and emotional level. As previously mentioned, sensitivity towards the stigma surrounding

obesity and “fat-shaming” should be increased not only by doctors and nurses, but all members of the public. This may pose some challenges in the medical community as many healthcare workers struggle to find a balance between expressing the dangers of obesity and encouraging body acceptance at all sizes. Additionally, a better understanding of leptin resistance may warrant its incorporation into medical school curriculums and physician workshops. It is important for doctors of all ages to understand that scientific understanding is incomplete and to consider the multitude of factors that affect disease. Increasing knowledge of leptin resistance should also inform public health messages regarding weight management from elementary school health classes to the MyPlate USDA recommendations for caloric consumption. Additionally, private stakeholder companies such as Weight Watchers and the like may use this information to help those who struggle particularly with weight loss. Novel research of this kind may potentially inspire many other related studies to continue developing our understanding of leptin resistance, and upon finding a clinical definition and treatment, this research will warrant an updated standard of care.

Background

Obesity:

Obesity proves to be one of the largest public health challenges today, starting in the last decades of the 20th century (Hales et al., 2020). Obesity is defined as having a body mass index (BMI) of 30 kg/m^2 or higher, and its growing prevalence has prompted its acknowledgement as a public health crisis and epidemic by the Center for Disease Control and Prevention (USDHHS, 2001). According to the U.S. National Health and Nutrition Examination Survey (NHANES) data from 2017-2018, obesity affects over 100 million Americans, making up 42.4% of the population. Additionally, 7.6% of Americans are morbidly obese, as defined as having a body mass index (BMI) of 40 kg/m^2 or higher (Hales et al., 2020). The excess adipose tissue is harmful by itself, but it also puts a heavy strain on the body systems leading to other chronic diseases such as type II diabetes, heart failure and heart disease, stroke, certain types of cancers, kidney disease, sleep apnea, COPD, and others (CDC, 2020). Healthcare costs for obesity are estimated to be roughly $147 billion in 2008 dollars (CDC, 2020). Conventional weight loss recommendations include diet control and regular exercise, but many individuals experience challenges to successful weight loss with these methods. Recommendations of diet and exercise can be derailed by poor lifelong habits or unhealthy psychological relationships with food consumption (Luotolahti et al., 2015).

Many factors impact an individual's ability to combat obesity such as age, sex, socioeconomic status, income, education, and environment. In a recent study using data from the U.S. National Health and Nutrition Examination Survey (NHANES) 2017-

2018, women were found to have nearly twice the prevalence of severe, or morbid, obesity than men (11.5% and 6.9%, respectively) (Hales et al., 2020). Obesity is significantly more common in non-Hispanic Blacks (49.6%) and Hispanics (44.8%) than in non-Hispanic whites (42.2%) and non-Hispanic Asians (17.4%) (Hales et al., 2020). Socioeconomic factors influence the prevalence of obesity as well. Overall, individuals with college degrees have lower rates of obesity than those without college degrees. Additionally, higher income is correlated with lower obesity rates. Higher education is often indicative of a privileged lifestyle with access to exercise equipment and healthy foods, and a higher income means that those individuals likely live in neighborhoods with parks, sidewalks, and green spaces, which are all known to increase overall health. Conversely, the underserved population faces barriers to health. Individuals with lower incomes are commonly forced to live in neighborhoods without safe places to exercise or be outside. Many live in “food deserts”, urban locations without grocery stores, meaning that fresh fruits and vegetables are not readily available. Instead, fast food restaurants are prevalent, and people often turn to these convenient options.

Bariatric Surgery:

For patients struggling with traditional weight loss methods, bariatric surgery may be a viable option. In the United States, common bariatric weight loss surgeries include laparoscopic adjustable gastric band, sleeve gastrectomy, and Roux-en-Y gastric bypass. Bariatric procedures have become increasingly common due to low rates of complications, cost effectiveness, and reduction of comorbidities (Buchwald, 2005; Panteliou and Miras, 2017). In 2017, there were 228,000 bariatric procedures performed

in the United States. This is a 5.6% increase from 2015 and a 44.3% increase from 2011 (ASMBS, 2018). Qualifications for bariatric surgery include a BMI over 40 or a BMI of over 35 combined with an obesity-related condition. Because it has been demonstrated that women are at a higher risk of morbid obesity, it follows that women comprised 81% of bariatric surgery patients from 2002-2011 (Young et al., 2016). Due to the increasing use of bariatric surgery coupled with imperfect realization of weight loss after surgery (ASMBS, 2018), it is essential to understand the factors that may influence its success.

Understanding Leptin Resistance:

A relatively novel phenomenon termed “leptin resistance” (Myers et al., 2012), may be a potential barrier to bariatric weight loss success in general. Leptin is a metabolic hormone that signals satiety, while the hormone ghrelin promotes hunger. Normally, when an individual is hungry, leptin levels are low while ghrelin levels are high, and the brain signals the body to seek food. As the body digests food, leptin levels rise and ghrelin levels fall which eventually invokes a feeling of satiety. At that point, the body has ingested a sufficient amount of food and the individual typically stops eating. Obesity appears to dysregulate leptin signaling (Gruzdeva et al., 2019). When an individual become resistant to leptin, the brain does not respond to the satiety signal, even when they have ingested sufficient food. Thus, paradoxically, in obese individuals, high leptin values may indicate leptin resistance and no longer trigger satiety. Leptin is secreted proportionally by adipocytes, or fat cells, which indicate to the brain how much fat is stored. Many researchers point to leptin resistance as a causal factor of continued

obesity. To our knowledge, it remains unknown, however, if leptin resistance persists after bariatric surgery, thereby reducing the chance of successful weight loss after surgery.

Several mechanisms of leptin resistance have been proposed, but it is unclear which one most accurately represents the phenomenon. Some researchers argue that a certain proportion of leptin resistance in populations is hereditary, caused by mutations in the genes for leptin and its receptors (Wabtisch et al., 2015; Friedman, 2014). Others contend that leptin resistance originates at the blood brain barrier, a specialized network of blood vessels that regulates passage of molecules between the body's circulatory system and the interstitial fluid surrounding the brain and spinal cord. Leptin is secreted by adipocytes and affects the hypothalamus, so it is necessary that some amount passes from the body's circulatory system into the midbrain in order for the brain to sense and respond to it; however, this transport is tightly regulated, and disruption of this regulation could contribute to leptin resistance via excess secretion (Chamakova et al., 2015; Mantzoros, 1999). Another increasingly accepted theory of leptin resistance focuses on the role of inflammation given the close functional and anatomical relationship between adipocytes and lymphoid cells. Leptin does indeed serve as a proinflammatory cytokine and plays an active role in immune system function and regulation (Friedman and Halaas, 1998; Landman et al., 2003). Recent nutritional research provides strong evidence for the causal relationship between diets high in fat and increased low-grade inflammation in peripheral tissues, specifically adipose tissue (Mattace et al., 2013). Additionally, acute immunodeficiency is seen in cases of

congenital leptin deficiency. Although the mechanisms and molecular interactions of leptin are yet to be understood, the scientific community has made steady progress in its clinical understanding of leptin resistance (Gruzdeva et al., 2019).

Implications of Leptin Resistance:

While there are currently no established diagnostic criteria for leptin resistance, clinical researchers are beginning to gain a better understanding of the variety of factors that might be indicative of this state (Myers et al., 2012). For instance, serum concentrations of leptin might not be sufficient to diagnose leptin resistance as expression of the OB gene as well as leptin clearance and receptor activity must also be accounted for (Gruzdeva et al., 2019). There are no clinically recommended treatment options due to lack of understanding, although multiple potential mechanisms are being investigated. Reseland et al. demonstrated that a decreased intake of dietary fat combined with increased physical activity resulted in a reduction of plasma leptin levels beyond simply the expected reduction based on loss of adipose tissue (Reseland et al., 2001). Another study by Spiegel et al. demonstrated that nightly sleep duration also affects leptin levels (Spiegel et al., 2004). Although extensive research is needed to determine the mechanisms, causes, clinical definition, and treatment avenues of leptin resistance, researchers worldwide are dedicated to investigating this potentially major factor in combating obesity.

Our study attempts to quantify whether or not baseline leptin levels may be a potential impediment to weight loss after surgery. Although bariatric surgery is now relatively safe and affordable, it is still an invasive surgical intervention that requires

multiple weeks of recovery and can cost upwards of $28,000. Furthermore, there is a lack of consensus regarding which factors predict success after bariatric surgery (Panteliou and Miras, 2017). If excess leptin levels are a significant barrier to successful weight loss after bariatric surgery, it is important to include that component in holistic weight loss guidance. Understanding the predictive value of leptin on weight loss success is the first step to developing methods to cope with it. Although this particular research will investigate factors with implications of potentially improving outcomes in bariatric procedures, our findings may be able to contribute to non-surgical, holistic weight loss methods. This will be beneficial not only to individuals suffering from morbid obesity, but to others as well who may have elevated plasma leptin levels.

Materials and Methods

This research was conducted under the parent grant entitled *Predictors of weight loss success after bariatric surgery: Baseline status and post-surgical alterations in oral sensory phenotype and food preference,* and funded by the Institute for Collaboration on Health, Intervention, and Policy at the University of Connecticut (Helen Swede, PhD; Pavlos Papasavas, MD, Co-Principal Investigators).

Sample:

One hundred females were recruited from the Surgical Weight Loss Center at Hartford Hospital. Patients were scheduled or had already undergone either Roux-en-Y gastric bypass or sleeve gastrectomy surgery in 2016-2017 and agreed to complete a series of diet-related and taste testing questionnaires. Of these, a convenience sample of 41 patients consented to have blood drawn for leptin testing. After exclusion of those who had already received bariatric surgery, our analytic sample consisted of 28 patients with baseline plasma leptin data along with the diet-related information. The parent study recruited only females to enhance external generalizability because the majority (>75%) of patients undergoing bariatric weight loss surgery in the United States are female (ASMBS, 2018). Individuals were excluded if they had current Axis I or Axis II mental health disorders, past medical history of Axis II disorder, Grave's disease or other thyroid problem, breastfeeding, or pregnancy. The study has approval from IRBs at Hartford Hospital and University of Connecticut Health. Patients were recruited at office visits by screening upcoming clinic schedules as well as via phone or provider referral. Patients were evaluated for participation 2 to 4 weeks before surgery. One

patient withdrew during the study and one patient died 6 months after surgery due to an acute Myocardial Infarction. Patients provided informed and written consent and were paid $20 in the form of a gift card for their participation.

Variables:

The key predictive variables included in this study are baseline and post-surgical leptin values. Blood assays of up to 10mL were collected by trained phlebotomists at Clinical Laboratory Partners and serum analyses were conducted at the CRC Laboratories. Leptin assays were taken before (baseline) as well as 6- and 12-months after bariatric surgery.

Weight related variables were also measured for each patient before and after surgery. Pre-Surgery Weight is defined as the patient's baseline weight before undergoing bariatric surgery. A patient's Ideal Weight is defined by the weight corresponding to a BMI of 25 kg/m^2. Excess Weight can then be calculated as the difference between the patient's Pre-Surgery Weight and Ideal Weight. After the surgery, a patient's 6-Month Post-Surgery Weight and 12-Month Post-Surgery Weight were measured at their 6-month and 12-month follow up appointments, respectively. Percent Excess Weight Lost (%EWL) is defined as [(Initial Weight) – (Postop Weight)] / [(Initial Weight) – (Ideal Weight)]. Body Mass Index (BMI) was calculated at baseline as well as 6 and 12 months after bariatric surgery. We also examined the percent of baseline BMI lost at 6- and 12-months post-surgery, defined as [(Preop BMI) – (Postop BMI)] / (Preop BMI).

A liking survey was used to assess taste preferences as well as provide a proxy for dietary healthiness for bariatric surgery patients (Zohgbi et al., 2019; Hubert et al., 2019). Survey reported likes and dislikes correlates with reported intake, biomarkers of dietary intake, as well as measures of cardiometabolic health (Sharafi et al., 2018; Xu et al., 2020). The reported likes and dislikes were theoretically coded into indexes of diet quality (DQI) and healthy behaviors (HBI), combined in the Healthy Eating and Behavior Index (HEBI) (Zoghbi et al., 2019; Hubert et al., 2019; Xu et al., 2020). The liking survey includes foods, beverages, sedentary activities, physical activities, pleasant experiences, and unpleasant experiences. Scores range from -100 to 100 where a negative score indicated a disliking and a positive score indicated a liking for each item.

Statistical Analyses:

Data was analyzed using the Statistical Package for Social Sciences (SPSS) software from IBM. Primary statistical analyses will include the Pearson Correlation Coefficient and the Multivariate Linear Regression. The Multivariate Linear Regression assesses potentially confounding variables. In this analysis, I will use various medical, demographic, and socioeconomic variables to examine other factors that may contribute to the success of bariatric surgery.

Research Results

Table 1: Demographic and Weight-related Characteristics of Female Bariatric Patients (n=28) at Hartford Hospital, 2015-2017

	n	
Age Mean (SD) Range	28	 46.93 (10.96) 25 – 65
Pre-Op BMI Mean (SD) Range	28	 44.89 (7.50) 34 – 71
6 Month BMI Mean (SD) Range	26	 35.31 (6.70) 23.91 – 56.00
1 Year BMI Mean (SD) Range	23	 33.74 (6.41) 21.00 – 50.00
6 Month Percent BMI Lost Mean (SD) Range	26	 21.53 (6.57) 8.89 – 35.37
1 Year Percent BMI Lost Mean (SD) Range	23	 23.86 (10.45) 1.06 – 43.24
6 Month Excess Weight Loss Percent Mean (SD) Range	26	 50.54 (19.29) 17.27 – 109.07
1 Year Excess Weight Loss Percent Mean (SD) Range	23	 55.36 (28.14) 3.10 – 128.10
HEBI Mean (SD) Range	28	 -1.75 (40.95) -88.18 – 88.91
Diet Quality Mean (SD) Range	28	 -4.61 (39.80) -81.40 – 93.80
Baseline Leptin Mean (SD) Range	28	 93.35 (59.05) 21.41 – 246.88

Table 2: Pearson Correlation Coefficients between Baseline Leptin and Weight-related Outcomes after Bariatric Surgery (n=28)

		Leptin	6mo%EWL	1yr%EWL	BMI.pct.lost.6mn	BMI.pct.lost.1yr	BMI.preop	6moBMI	1yrBMI
Leptin	Pearson Correlation	1	−.452*	−.208	−.163	.042	.618**	.632**	.489*
	Sig. (2-tailed)		.021	.342	.425	.849	.000	.001	.018
	N	28	26	23	26	23	28	26	23
6mo%EWL	Pearson Correlation	−.452*	1	.849**	.833**	.689**	−.454*	−.759**	−.810**
	Sig. (2-tailed)	.021		.000	.000	.000	.020	.000	.000
	N	26	26	22	26	22	26	26	22
1yr%EWL	Pearson Correlation	−.208	.849**	1	.736**	.898**	−.224	−.549**	−.798**
	Sig. (2-tailed)	.342	.000		.000	.000	.305	.008	.000
	N	23	22	23	22	23	23	22	23
BMI.pct.lost.6mn	Pearson Correlation	−.163	.833**	.736**	1	.824**	.016	−.407*	−.518*
	Sig. (2-tailed)	.425	.000	.000		.000	.937	.039	.014
	N	26	26	22	26	22	26	26	22
BMI.pct.lost.1yr	Pearson Correlation	.042	.689**	.898**	.824**	1	.166	−.233	−.541**
	Sig. (2-tailed)	.849	.000	.000	.000		.448	.297	.008
	N	23	22	23	22	23	23	22	23
BMI.preop	Pearson Correlation	.618**	−.454*	−.224	.016	.166	1	.906**	.736**
	Sig. (2-tailed)	.000	.020	.305	.937	.448		.000	.000
	N	28	26	23	26	23	28	26	23
6moBMI	Pearson Correlation	.632**	−.759**	−.549**	−.407*	−.233	.906**	1	.917**
	Sig. (2-tailed)	.001	.000	.008	.039	.297	.000		.000
	N	26	26	22	26	22	26	26	22
1yrBMI	Pearson Correlation	.489*	−.810**	−.798**	−.518*	−.541**	.736**	.917**	1
	Sig. (2-tailed)	.018	.000	.000	.014	.008	.000	.000	
	N	23	22	23	22	23	23	22	23

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 3: Unadjusted β and Adjusted Linear Regression of Baseline Leptin as Predictor of Percent Excess Weight Loss at 6 and 12 months after Bariatric Surgery (n=28)

	Unadjusted β[1]	Age-Adjusted β	Adjusted β (Age, HEBI[2])
6 months %EWL			
Leptin (ng/mL)	-.15 (.06) p=.02	-.14 (.06) p=.03	-.12 (.06) p=.04
Age (years)	-.31 (.37) p=.41	-	-.29 (.35) p=.41
HEBI (score)	.19 (.09) p=.04	.19 (.09) p=.04	-
12 months %EWL			
Leptin (ng/mL)	.09 (.10) p=.34	.09 (.10) p=.36	-.07 (.09) p=.47
Age (years)	-.19 (.59) p=.75	-	-.23 (.56) p=.69
HEBI (score)	.26 (.13) p=.06	.26 (.13) p=.07	-

[1] *Beta Coefficient and Standard Error derived from Linear Regression; Results did not appreciably vary when using log-transformed Leptin to account for skewness.*

[2] *HEBI (Healthy Eating and Behavior Index) quantifies consumption of healthy and unhealthy foods as well as healthy behaviors, such as exercise. Scores range from -100 to 150, with higher scores corresponding to better adherence to the 2015 Dietary Guidelines for Americans (USDA).*

Table 3 demonstrates the Pearson Correlation Coefficients for baseline leptin values and percent Excess Weight Loss at both 6 and 12 months after bariatric surgery. At the 6-month point, it appears that the higher the leptin value, the lower the weight loss: for every unit increase in plasma leptin (ng/mL), a patient will lose 0.15% less of their EWL 6 months after surgery. This effect weakens slightly (i.e., 0.14 – 0.12%, p=0.03 – 0.04) in linear regression models that adjust for age and eating behavior survey responses. While this appears to be a modest correlation in absolute terms, it was found to be statistically significant (p=.02). At 12 months after surgery, this correlation weakens further (i.e., 0.09%, p=0.34), suggesting that baseline leptin level is a better predictor of short-term weight loss (6 months) outcomes than long-term (12 months).

Although baseline leptin seems to be a moderate predictor of weight loss outcomes at the six-month post-surgery point (above), age appears to also have an effect. With each additional year of age, patients lost about 0.31% less of their EWL at 6 months after surgery; this correlation drops to 0.19% at 12 months. While the estimated effect size for age is greater than the value calculated for baseline leptin, the p-value is high ($p=.41$, $p=.75$ at 6 and 12 months, respectively). Women go through a variety of hormonal changes at various phases throughout their lives, which can make it more difficult to lose weight with age.

Eating and exercise behaviors appear to be the most significant predictors of EWL at 6 and 12 months after bariatric surgery. Individuals who score just 1 point higher on the Healthy Eating and Behavior Index (HEBI) lose an average of 0.19% of their EWL at 6 months after surgery; the correlation is even stronger (0.26%) at 12 months after surgery. Considering the wide range of these survey values (-100 to 150), these point differences can add up to significantly predict successful weight loss post-surgery. It is plausible that individuals who engage in healthy behaviors and eat more nutritious foods on a regular basis are more successful at losing weight in both the short and long term.

Table 4: Baseline Leptin as Predictor of Percent of BMI Lost at 6 and 12 months after Bariatric Surgery (n=28)

	Unadjusted β[1]	Age-Adjusted β	Adjusted β (Age, HEBI[2])
6 months			
Leptin (ng/mL)	-.02 (.02) p=.43	-.02 (.02) p=.48	-.01 (.02) p=.64
Age (years)	-.18 (.12) p=.15	-	-.18 (.12) p=.15
HEBI (score)	.05 (.03) p=.13	.05 (.03) p=.13	-
12 months			
Leptin (ng/mL)	.01 (.04) p=.85	.01 (.04) p=.84	.02 (.04) p=.68
Age (years)	-.18 (.22) p=.42	-	-.19 (.21) p=.38
HEBI (score)	.07 (.05) p=.18	.07 (.05) p=.17	-

[1] *Beta Coefficient and Standard Error derived from Linear Regression; Results did not appreciably vary when using log-transformed Leptin to account for skewness.*

[2] *HEBI (Healthy Eating and Behavioral Index) quantifies consumption of healthy and unhealthy foods as well as healthy behaviors, such as exercise. Scores range from -100 to 150, with higher scores corresponding to better adherence to the 2015 Dietary Guidelines for Americans (USDA).*

Table 4 displays the Pearson Correlation Coefficients for baseline leptin values and Percent Body Mass Index (BMI) lost at both 6 and 12 months after bariatric surgery. BMI is defined as a person's weight (kg) divided by the square of their height (m) and is used to define normal weight, overweight, and obesity. At 6 months post-surgery, it appears that leptin has an almost negligible correlation with percent BMI lost: for every unit increase in plasma leptin (ng/mL), a patient's BMI will decrease 0.02% less from their baseline BMI (p=.43). This correlation remains relatively constant (i.e., 0.01 – 0.02%, p = .48 – .64) in linear regression models that adjust for age and eating behavior survey responses. At 12 months post-surgery, the correlation is reversed: for every unit increase in plasma leptin (ng/mL), a patient's

BMI will decrease by 0.01% more from their baseline BMI (p=.85). Again, this correlation remains constant (i.e., 0.01 – 0.02%, p= .68 – .84) in linear regression models adjusting for age and eating behavior survey responses. However, at 12 months post-surgery, the correlation remains very small and has less statistical significance.

Age appears to be a more significant predictor of percent BMI lost at 6- and 12-months post-surgery. With each additional year of age, a patient's BMI at 6 months post-surgery will decrease by 0.18% less from their baseline BMI (p=.15). This correlation remains relatively constant in linear regression models that adjust for eating behavior survey responses (i.e., 0.18 – 0.20%, p = .11 – .15). Similar correlations are seen at 12 months post-surgery (i.e., 0.18 – 0.22%, p = .32 – .42), although these correlations are not statistically significant. This data reinforces the notion that decreasing BMI becomes more difficult with age, which seems to have a stronger effect in short-term weight loss (6 months) outcomes than long-term (12 months).

Factors that positively influence percent of baseline BMI lost are behavioral; namely, the Healthy Eating and Behavior Index (HEBI). At 6 months post-surgery, individuals who score 1 point higher on the HEBI lose 0.05% more of their baseline BMI (p=.13); this correlation is even stronger at 12 months post-surgery (0.08%, p=.18). This data agrees with previous findings that indicate healthy behaviors and dietary preferences have a positive impact on both short-term (6 months) and long-term (12 months) weight loss outcomes.

Table 5: Baseline Leptin as Predictor of Baseline BMI and BMI at 6 and 12 months after Bariatric Surgery (n=28)

	Unadjusted β[1]	Age-Adjusted β	Adjusted β (Age, HEBI[2])
Baseline BMI			
Leptin (ng/mL)	.08 (.02) p=.00	.08 (.02) p=.00	.08 (.02) p=.00
Age (years)	-.03 (.13) p=.81	-	-.01 (.13) p=.92
HEBI (score)	-.06 (.03) p=.07	-.06 (.03) p=.08	-
6 month BMI			
Leptin (ng/mL)	.07 (.02) p=.00	.07 (.02) p=.00	.06 (.02) p=.00
Age (years)	.05 (.13) p=.70	-	.07 (.12) p=.60
HEBI (score)	-.07 (.03) p=.02	-.07 (.03) p=.03	-
12 month BMI			
Leptin (ng/mL)	.05 (.02) p=.02	.05 (.02) p=.02	.05 (.02) p=.03
Age (years)	-.02 (.14) p=.88	-	.00 (.13) p=.98
HEBI (score)	-.06 (.03) p=.05	-.06 (.03) p=.05	-

[1] *Beta Coefficient and Standard Error derived from Linear Regression; Results did not appreciably vary when using log-transformed Leptin to account for skewness.*

[2] *HEBI (Healthy Eating and Behavioral Index) quantifies consumption of healthy and unhealthy foods as well as healthy behaviors, such as exercise. Scores range from -100 to 150, with higher scores corresponding to better adherence to the 2015 Dietary Guidelines for Americans (USDA).*

Table 5 demonstrates the Pearson Correlation Coefficients for baseline leptin values and Body Mass Index (BMI) before bariatric surgery (i.e., baseline), 6 months post-surgery, and 12 months post-surgery. BMI is defined as a person's weight (kg) divided by the square of their height (m) and is used to define normal weight, overweight, and obesity. Leptin appears to be a highly significant predictor of baseline BMI: for every unit increase in plasma leptin (ng/mL), an individual's BMI is about 0.08

kg/m^2 higher (p=.00). This correlation is unchanged in linear regression models adjusting for age and eating behavior survey responses. Leptin is also highly significant in predicting BMI at 6- and 12-months post-surgery. At 6 months after bariatric surgery, for every unit increase in plasma leptin (ng/mL), an individual's BMI is an average of 0.07 kg/m^2 higher (p=.00). This correlation is unchanged in linear regression models adjusting for age and eating behavior survey responses. This correlation weakens slightly at 12 months after bariatric surgery: for every unit increase in plasma leptin (ng/mL), an individual's BMI is 0.05 kg/m^2 higher (p=.02). In linear regression models adjusting for age and eating behavior responses, the correlation remains the same but becomes slightly less significant (i.e. 0.05 kg/m^2, p=.02 – .03). Plasma leptin levels seem to have a strong and significant correlation with BMI at baseline and 6 months post-surgery, and a slightly weaker correlation with BMI 12 months post-surgery. In the long term (12 months), other factors such as age, and eating behaviors appear to also significantly influence weight loss outcomes.

Compared to the significant predictive value of leptin, age seems to have a modest effect on baseline BMI and BMI outcomes. At baseline, age is modestly correlated with a decreased BMI: with additional year of age, baseline BMI decreases by 0.03 kg/m^2 (p=.81). However, at 6 months post-surgery, age has a slightly stronger correlation in the reverse direction: for each additional year of age, 6-month BMI increases by 0.05 kg/m^2 (p=.70). At 12 months post-surgery, the correlation reverses again to resemble that of the baseline BMI: for each additional year of age, 12-month

BMI decreases by 0.02 kg/m^2 (p=.88). These correlations are quite weak and are not statistically significant.

Exercise behaviors and dietary preferences have a consistently strong and significant correlation with baseline and post-surgery BMI. At baseline, the Healthy Eating and Behavioral Index (HEBI) is correlated with a lower BMI: for each point scored, BMI is an average of 0.06 kg/m^2 lower (p=.07). This correlation remains relatively steady over time. At 6 months post-surgery, each point on the HEBI questionnaire is correlated with a 0.07 kg/m^2 decrease in BMI (p=.02). Adjusting for age, this correlation remains at 0.07 kg/m^2 (p=.03). At 12 months post-surgery, the correlations weaken even further for the HEBI questionnaire (i.e., 0.06 kg/m^2, p=.05 unadjusted; 0.06 kg/m^2, p=.05 adjusted). This supports the idea that individuals with consistent exercise habits and a preference for healthy foods are leaner at baseline, giving them a significant advantage in achieving a healthy weight than those who score lower on the HEBI survey. However, since the correlations weaken significantly over time, it appears that these individuals are not able to maintain this advantage as well after surgery.

Discussion

Our data suggest baseline plasma leptin is inversely associated with % excess weight loss at 6 months post bariatric surgery while adjusting for age and indexes of diet quality and healthy behaviors. This association supports the hypothesis that elevated leptin (a putative reflection of leptin resistance), via dysfunctional appetite control, might impede weight loss even after bariatric surgery. Scaling the effect (β=-.14) into clinical terms, a baseline leptin value difference of 75 ng/mL might incur about 10 percentage points less excess weight lost over six months.

Baseline leptin levels had a somewhat weaker association with weight loss measures at 12 months post-surgery, suggesting that leptin resistance might be tapering off. While the correlation of baseline leptin with 12-month post-surgery BMI is similar to that of 6-month post-surgery BMI, the correlation of baseline leptin with EWL at 12 months is notably lower than at 6 months, and less significant. This indicates that baseline leptin plays a stronger predictive role for short-term outcomes than long-term outcomes.

Across all outcome measures, healthy eating behaviors and diet quality seemed to have the most predictive effect on long-term weight loss outcomes. In this analysis, the Healthy Eating and Behavior Index (HEBI) questionnaire was used to assess health behaviors (Zoghbi et al., 2019; Hubert et al., 2019; Xu et al., 2020). Higher scores on this assessment indicates better adherence to recommended healthy behaviors and preferences for healthier foods. At 12 months post-surgery, each point increase on the HEBI questionnaire is correlated with a 0.26% increase in EWL (p=.06) as well as an

.07% loss of baseline BMI (p=.18) and 0.06 kg/m^2 decrease in the patient's BMI (p=.05). Although these correlations seem relatively modest, the HEBI has a wide range of scores, so a significant difference in scoring accumulates into a significant impact on weight loss outcomes. It is well-known that while a myriad of physiological, psychological, socioeconomic, and environmental factors affects an individual's health outcomes, maintaining healthy behaviors especially with regards to diet and exercise is the most effective way to combat obesity. This is not to say that other factors should not be considered; weight loss is very complex. However, for those who are able, it is still recommended that the first step to weight management and reversal of chronic disease is to develop and maintain healthy behaviors.

Leptin has a strong correlation with baseline BMI. Each unit increase in leptin (ng/mL) was correlated with a 0.08 kg/m^2 increase in the patient's BMI (p=.00). This correlation remained significant after adjusting for age and healthy eating behaviors as well as diet quality. Additionally, leptin was a significant predictor of short-term weight loss outcomes: at 6 months post-surgery, each unit increase in leptin (ng/mL) was correlated with a 0.07 kg/m^2 increase in the patient's BMI (p=.00). This correlation decreased slightly to 0.05 kg/m^2 increase in the patient's BMI (p=.02) at 12 months post-surgery. It is evident that leptin has a strong correlation with weight-loss outcomes and should be considered in the holistic approach to bariatric surgery. The fact that leptin has such a strong, consistent, and significant correlation with baseline BMI indicates that leptin may play a role not only in weight loss outcomes post-surgery, but

also the metabolic dysfunction that leads up to an individual reaching a state in which they required bariatric weight loss surgery.

Because leptin was so highly correlated with baseline BMI, a sensitivity analysis was run to examine the collinearity of these variables with Percent EWL at 6 months. The standardized and unstandardized beta values for leptin were -.271 and -.88 respectively (p=.25). The standardized and unstandardized beta values for baseline BMI were -.189 and -.47 respectively (p=.44). Therefore, we can conclude that leptin has a greater importance when predicting EWL than baseline BMI. However, this close association elicits further investigation in future experiments with larger samples.

Since the study sample consists entirely of women, these data can only confidently be extrapolated to females who undergo bariatric surgery. Morbid obesity is nearly twice as prevalent in females (10.0%) as in males (5.6%) in the United States (CDC, 2020). It follows that from 2002-2011, an overwhelming 81% of bariatric surgery patients were female (ASMBS, 2018). Although metabolism is thought to be a relatively consistent system across genders, women experience many physiological changes, societal barriers, and environmental stressors that the vast majority of males do not share, so it would be interesting to investigate any potential sex difference in future studies. For instance, during puberty, women accumulate adipose tissue in the breasts, hips, and thighs, while men increase muscle mass. While it is well-known that body fat is strongly liked to reproductive health, studies show that the increasing rate of childhood obesity is related to earlier onset of puberty in girls (Freedman et al., 2002). This is especially problematic considering that early puberty and obesity during puberty

are related to health issues later on in life including a higher risk of breast cancer and polycystic ovarian syndrome (PCOS), as well as a higher BMI later in life (Solorzano and McCartney, 2010).

Leptin itself has been proven to play an important role in regulating menstrual cycles in women. Matkovic et al. tracked leptin levels over time in pre-pubescent girls and noted that a unit increase in leptin (ng/mL) lowered the onset of puberty by one month, and each additional kg of body fat decreased the onset of puberty by nearly 2 weeks (Matkovic et al., 1997). This study also determined a serum leptin threshold of 12.2 ng/mL that was necessary for the onset of puberty, implying that adipose tissue directly regulates menstruation via leptin.

In addition to physiological factors, psychological and lifestyle stressors unique to women have also been correlated with obesity. One study investigating the relationship between depression and obesity in middle-aged women showed that the prevalence of obesity was 57.8% higher in women with moderate to severe depression, as well as decreased exercise and a significantly higher daily caloric intake (Simon et al., 2008). Women are twice as likely as men to experience depression, generalized anxiety or panic disorders, and post-traumatic stress disorders (APA, 2017), highlighting the link between depression and obesity as female specific. There is a myriad of reasons why women would be more likely to experience depression: relentless demands pertaining to careers, motherhood, and housework; body negativity from targeted advertising; hormonal imbalances from menstrual cycles, post-partum depression, and menopause; and so much more. On a societal level, increasing access to affordable childcare as well

as adequate mental health care may ease some of these burdens and, in turn, lower obesity rates.

It is important to recognize that women are not a homogenous group. Women of color, as well as those from less fortunate socioeconomic backgrounds, face larger barriers to weight management. The prevalence of obesity is highest in Non-Hispanic Black women (56.9%), compared to Hispanic women (43.7%), non-Hispanic White women (39.8%), and non-Hispanic Asian women (17.2%). Additionally, non-Hispanic Blacks have the highest prevalence of morbid obesity (13.8%) compared to non-Hispanic whites (9.3%), Hispanics (7.9%), and non-Hispanic Asians (2.0%). These disparities can likely be attributed to a myriad of institutional shortcomings, including lack of adequate access to healthcare. When discussing a chronic disease such as morbid obesity, access to preventative care is specifically important because primary care providers are most influential in helping patients with these types of long-term health issues. In 2018, it was reported that nearly 1 in 5 Hispanic adults under the age of 65 were uninsured (19%) as well as 11% of non-Hispanic Blacks, 8% of non-Hispanic Whites, and 7% of non-Hispanic Asians (Kaiser Family Foundation, 2018). Furthermore, women of color and those who are socioeconomically disadvantaged consistently receive poor healthcare treatment. In the National Healthcare Disparities Report, 35% of Hispanics and low-income individuals could not get the care they needed, compared to 25% of White individuals and only 15% of high-income individuals. Additionally, non-Hispanic Blacks are three times more likely to be hospitalized for diabetes than non-Hispanic Whites; Hispanics are twice as likely (National Healthcare Disparities Report,

2013). Access to quality healthcare and treatment are just two of the many barriers that women of color and low-income face. Others include less access to fresh food, safe places to exercise, and free time.

Summary and Conclusion

In this paper, we were able to establish that baseline leptin is a valuable tool for predicting post-surgical weight loss success in morbidly obese patients. In this study of adults who had undergone bariatric weight loss surgery, we found that those who had the most trouble losing weight at the 6- and 12-month post-op marks had high pre-surgical leptin values, which may be indicative of leptin resistance. These findings are consistent with our initial hypothesis, as physiological resistance to the satiety hormone was predicted to cause overeating and therefore impede weight loss, even after bariatric surgery. While this is the first study of its kind investigating leptin levels in patients who had undergone surgery, it is consistent with other literature suggesting that plasma leptin, especially at consistently elevated levels, plays a role in weight management.

These findings have meaningful clinical implications as well. The results of our sample of individuals with morbid obesity may be applicable to the 7.6% of adults across the United States with morbid obesity (Hales et al., 2020); however, the results may only definitively be extrapolated to women. In patients who undergo bariatric weight loss surgery, measuring baseline leptin values may help the physician identify potential barriers to successful outcomes and modify the long-term care plan accordingly. Losing weight can be difficult even beyond the scope of bariatric surgery, and patients and physicians alike may become frustrated when typical diet and exercise guidelines do not solve the problem. However, measuring baseline leptin values may help physicians identify those who could be at increased risk for struggling with weight loss. However,

before these clinical methods can be put into widespread practice, thorough research must be completed to determine the clinical threshold value that defines leptin resistance. Many studies regarding leptin change over time note a common baseline range of about 17.0 to 19.4 ng/mL in healthy subjects, although more research is needed for validation (Huang et al., 2016).

This study contributes to the scientific dialogue regarding ways to improve the chances of weight loss success after bariatric surgery and has far-reaching implications in the public health sphere. It is well known that obesity is a fast-growing problem in the United States today, and the more we know about its etiology, the more informed our public recommendations can be. Weight loss has a significant psychological component, and failure to meet physician-recommended weight loss goals can often lead to internalized feelings of inadequacy, self-blame, and eventually hopelessness. Considering the phenomenon of leptin resistance may alleviate some of those feelings by factually attributing decreased success to a physiological mechanism rather than a character flaw, thereby increasing an individual's autonomy and motivation. This pattern was demonstrated in the recent literature regarding insulin resistance, the condition by which the body maintains consistently high levels of insulin and receptor cells do not adequately respond. Insulin resistance became popular among the American public in books by Dr. Jason Fung including *The Obesity Code* and *The Diabetes Code*. Dr. Fung, a prominent Canadian nephrologist, proposed intermittent fasting as a way to "reset insulin sensitivity" and has now become a common dietary pattern among those aiming to lose weight and/or manage type II diabetes (Fung, 2016). Perhaps, with

proper research, intervention, and public education, leptin resistance may follow a similar pattern.

Although this novel study was able to provide valuable insight into the use of baseline leptin as a predictor of weight loss success in patients undergoing bariatric surgery, it was a relatively small pilot study. One limitation was the number of patients that were included in the analysis; future studies should include a larger sample size. Additionally, subsequent studies should include a relatively equal number of both males and females, a racially and ethnically representative sample, and should use data from hospitals across the country to account for geographical factors that may have contributed to weight loss success. These factors will help to create a sample that is more representative of individuals with morbid obesity. An extended timeline for longitudinal data may also help explain if and how leptin resistance impacts weight loss for years post-surgery. Overall, the study hypothesis was validated in its aim to establish baseline leptin values and potential leptin resistance as factors that should be considered in the holistic care of a patient with morbid obesity, as it proves to significantly impede weight loss. Future directions in this field of research should aim to better understand the physiological mechanisms of leptin resistance, then investigate strategies to combat leptin resistance.

References

1. Hales CM, Carroll MD, Fryar CD, Ogden CL. Prevalence of obesity and severe obesity among adults: United States, 2017–2018. NCHS Data Brief, no 360. Hyattsville, MD: National Center for Health Statistics. 2020
2. U.S. Department of Health and Human Services. The Surgeon General's call to action to prevent and decrease overweight and obesity. [Rockville, MD]: U.S. Department of Health and Human Services, Public Health Service, Office of the Surgeon General; [2001]. Available from: U.S. GPO, Washington. Ref https://www.cdc.gov/nccdphp/dnpa/pdf/CalltoAction.pdf
3. Adult Obesity Causes & Consequences. Division of Nutrition, Physical Activity and Obesity, National Center for Chronic Disease Prevention and Health Promotion*. United States. 2020.* Centers for Disease Control, Hyattsville, MD, 2020. Available online: https://www.cdc.gov/obesity/data/adult/causes.html (accessed on 4 April 2020).
4. Luotolahti, H., Viikari, J., Kantola, I. Attitudes that make weight loss management difficult. *Advances in Obesity, Weight Management & Control*. 2015; 2 (5). Ref http://medcraveonline.com/AOWMC/AOWMC-02-00031.pdf
5. Buchwald, H. Consensus conference statement bariatric surgery for morbid obesity: health implications for patients, health professionals, and third-party payers. Surg Obes Relat Dis 2005, 1(3), 371-81.
6. Panteliou, E.; Miras, A.D. What is the role of bariatric surgery in the management of obesity? Climacteric 2017, 20, 97-102, DOI: 10.1080/13697137.2017.1262638.
7. American Society for Metabolic and Bariatric Society. Estimate of bariatric surgery numbers, 2011-2017. Published June 2018. Available online: https://asmbs.org/resources/estimate-of-bariatric-surgery-numbers (accessed on 3 January 2020).
8. Zoghbi M, Stone A, Papasavas P, Swede H, Hubert P, Tishler D, Duffy VB**.** Evaluating taste preferences and dietary quality with a simple liking survey: Application in bariatric treatment settings. Bariatric Surgical Practice and Patient Care. 2019 14(1): 41-48.
9. Hubert, P.A., Papasavas, P., Stone, A., Swede, H., Huedo-Medina, T. B., Tisher, D., Duffy, V. B. Associations between weight loss, food likes, dietary behaviors and chemosensory function in bariatric surgery: A case-control analysis. Nutrients, 2019, 11.
10. Sharafi M, Rawal S, Fernandez ML, Huedo-Medina TB, Duffy VB. Taste phenotype associates with cardiovascular disease risk factors via diet quality in multivariate modeling. Physiol Behav 2018; 194:103-112.
11. Xu R, Blanchard BE, McCaffrey JM, Wooley S, *Corso LML, Duffy VB. Food liking-based diet quality indexes (DQI) generated by conceptual and machine learning explained variability in cardiometabolic risk factors in young adults. Nutrients accepted March 23, 2020.

12. Young, M.T.; Phelan, M.J.; Nguyen, N.T. A decade analysis of trends and outcomes of male vs female patients who underwent bariatric surgery. J Am Coll Surg 2016, 222, 226-231, DOI: 10.1016/j.jamcollsurg.2015.11.033.
13. Gruzdeva, O., Borodkina, D., Uchasova, E., Dyleva, Y., Barbarash, O. Leptin resistance: underlying mechanisms and diagnosis. Diabetes Metab Syndr Obes 2019, 12, 191-198. DOI: 10.2147/DMSO.S182406
14. Wabitsch M, Funcke JB, Lennerz B, et al. Biologically inactive leptin and early-onset extreme obesity. N Engl J Med. 2015;372(1):48–54.
15. Friedman J. 20 years of leptin: leptin at 20: an overview. J Endocrinol. 2014;223(1):T1–T8.
16. Chumakova GA, Ott AV, Veselovskaya NG, Gritsenko OV, Shenkova NN. Pathogenetic mechanisms of leptin resistance. Russ J Cardiol. 2015;4(4):107–110.
17. Mantzoros CS. The role of leptin in human obesity and disease: a review of current evidence. Ann Intern Med. 1999;130(8):671–680.
18. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature. 1998;395(6704):763–770.
19. Landman RE, Puder JJ, Xiao E, Freda PU, Ferin M, Wardlaw SL. Endotoxin stimulates leptin in the human and nonhuman primate. J Clin Endocrinol Metab. 2003;88(3):1285–1291.
20. Mattace Raso G, Simeoli R, Russo R, et al. Effects of sodium butyrate and its synthetic amide derivative on liver inflammation and glucose tolerance in an animal model of steatosis induced by high fat diet. PLoS One. 2013;8(7):e68626.
21. Reseland, J.E., Anderssen, S.A., Solvoll, K., Hjermann, I., Urdal, P., Holme, I., Drevon, C.A. Effect of long-term changes in diet and exercise on plasma leptin concentrations. Am J Clin Nutr 2001, 73(2): 240-245. DOI: 10.1093/ajcn/73.2.240
22. Spiegel, K., Leproult, R., L'hermite-Balériaux, M., Copinschi, G., Penev, P.D., Van Cauter. E. Leptin levels are dependent on sleep duration: relationships with sympathovagal balance, carbohydrate regulation, cortisol, and thyrotropin. J Clin Endocrinol Metab 2004, 89(11): 5762-71. DOI: 10.1210/jc.2004-1003
23. Freedman DS, Khan LK, Serdula MK, Dietz WH, Srinivasan SR, Berenson GS. Relation of age at menarche to race, time period, and anthropometric dimensions: the Bogalusa Heart Study. Pediatrics. 2002;110:e43
24. Solorzano, C. M., McCartney, C. R. Obesity and the Pubertal Transition in Girls and Boys. *Reproduction*, 140(3), 399-410. September 2010. Ref https://pubmed.ncbi.nlm.nih.gov/20802107/
25. Matkovic, V., Ilich, J. Z., Skugor, M., Badenhop, N. E., Goel, P., Clairmont, A., Klisovic, D., Nahhas, R. W., Ladoll, J. D. Leptin is Inversely Related to Age at Menarche in Human Females. *The Journal of Clinical Endocrinology & Metabolism*, 82 (10), 2329-3245. October 1997. Ref https://academic.oup.com/jcem/article/82/10/3239/2823183
26. Simon, G. E., Ludman, E. J., Linde, J. A., Operskalsi, B. H., Ichikawa, L., Rohde, P., Finch, E. A., Jeffrey, R. W. Association between obesity and depression in middle-aged women, *Gen Hosp Psychiatry*, 2008; 30 (1): 32-39. Ref https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2675189/

27. Mental Health Disparities. Division of Diversity and Health Equity; Council on Minority Mental Health and Health Disparities. *American Psychiatric Association, 2017.* Available online: https://www.psychiatry.org/File%20Library/Psychiatrists/Cultural-Competency/Mental-Health-Disparities/Mental-Health-Facts-for-Women.pdf (accessed on 3 January 2020).
28. Kaiser Family Foundation estimates based on the Census Bureau's American Community Survey, 2008-2018.
29. National Healthcare Disparities Report, 2013. Content last reviewed May 2014. Agency for Healthcare Research and Quality, Rockville, MD. https://archive.ahrq.gov/research/findings/nhqrdr/nhdr13/index.html
30. Huang, T., Tobias, D. K., Hruby, A., Rifai, N., Tworoger, S. S., Hu, F. B. An increase in dietary quality is associated with favorable plasma biomarkers of the brain-adipose axis in apparently healthy US women. *J. Nutr* 2016; 146: 1101-8.
31. Fung, J. (2016). *The Obesity Code: Unlocking the secrets to weight loss*. Greystone Books.