The Effects of Vitamin D on Microvascular Endothelial Function in Bariatric Surgery Patients

BY

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<td>1-25(OH)$_2$D</td>
<td>1,25 hydroxyvitamin D; biological active and hormonal form of vitamin D</td>
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<td>25(OH)D</td>
<td>25-hydroxyvitamin D; major circulating form of vitamin D</td>
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<td>1α-Ohase</td>
<td>25-hydroxyvitamin D-1-α-hydroxylase</td>
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<tr>
<td>AchID</td>
<td>Acetylcholine-Induced Dilation</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
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<tr>
<td>DCF-DA</td>
<td>dichlorodehydrofluorescein diacetate</td>
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<tr>
<td>eNOS</td>
<td>Endothelial Nitric Oxide Synthase</td>
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<tr>
<td>FID</td>
<td>Flow-Induced Dilation</td>
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<tr>
<td>H$_2$O$_2$</td>
<td>Hydrogen Peroxide</td>
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<tr>
<td>L-NAME</td>
<td>$N^\omega$-nitro-L-arginine methyl ester; NO synthase inhibitor</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
</tr>
<tr>
<td>PEG-CAT</td>
<td>polyethylene glycol catalase; H$_2$O$_2$ inhibitor</td>
</tr>
<tr>
<td>PI3K/Akt</td>
<td>phosphatidylinositol 3-kinase</td>
</tr>
<tr>
<td>PKA</td>
<td>Protein Kinase A</td>
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<tr>
<td>RA(s)</td>
<td>Resistance Artery(ies)</td>
</tr>
<tr>
<td>SUBQ</td>
<td>Subcutaneous Adipose Tissue</td>
</tr>
<tr>
<td>UIC CIC</td>
<td>University of Illinois at Chicago Clinical Interface Core</td>
</tr>
<tr>
<td>VDR</td>
<td>Vitamin D Receptor</td>
</tr>
<tr>
<td>VIS</td>
<td>Visceral Adipose Tissue</td>
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I. SUMMARY

A study of the effects of vitamin D on microvascular endothelial function in the obese state was carried out using an *ex situ* approach. Vitamin D deficiency and obesity are two major global public health concerns. Growing evidence suggests that these two modern health issues are interrelated (30, 34). There is a high prevalence of vitamin D deficiency in obese individuals compared to normal weight individuals (60). Moreover, both obesity and vitamin D deficiency are associated with cardiometabolic outcomes and events (2, 13, 30, 31, 33, 34, 44, 51, 55, 67).

Endothelial dysfunction serves as an important initiating step in the development of cardiovascular disease (CVD) and therefore, may be of prognostic value for predicting future cardiovascular events. Endothelial cells have the ability to not only produce the biologically active form of vitamin D in the presence of the precursor form of vitamin D, but they also have transmembrane vitamin D receptors (VDR). This suggests that vitamin D may play a role in endothelial function (64). Previous research from our lab and others suggests that visceral adipose tissue (VAT) accumulation is associated with endothelial dysfunction and excessive inflammation compared to subcutaneous adipose tissue (SAT) (28, 54).

Obesity is associated with excess VAT and vitamin D deficiency. The mechanism behind these relationships is yet to be established, but it is thought that vitamin D may be sequestered in the large amount of adipose tissue present in obese people, effectively reducing the amount of circulating vitamin D in the serum (12, 18, 24). Production of the potent endothelial vasodilator, nitric oxide (NO), by endothelial nitric oxide synthase (eNOS), plays a pivotal role in vascular health. Previous research suggests that there is an interaction between vitamin D, obesity and NO which may modulate vascular health.
However, little is known about the effect(s) of vitamin D on endothelial NO production in SAT and VAT resistance arteries (RAs) in a morbidly obese population. Furthermore, the role of weight loss following bariatric surgery in SAT and VAT RA NO production is also unexplored (14).

From the samples collected on the day of surgery, we hypothesized that 1) vitamin D would improve FID and AChID in arterioles from SAT and VAT and this effect would be abolished by $N^\omega$-nitro-L-arginine methyl ester (L-NAME), an eNOS inhibitor, but not by polyethyleneglycol catalase (PEG-CAT), an $H_2O_2$ scavenger and 2) in the presence of vitamin D, NO production measured by NO fluorescence would increase in both SAT and VAT and this effect would be abolished by L-NAME but not by PEG-CAT.

In comparing within subject day of surgery and post-surgery measures, we hypothesized that 1) due to the bariatric surgery, subjects would lose a significant amount of body weight, 2) SAT RAs obtained from the post-surgery visit would demonstrate improved FID and AChID and an enhanced sensitivity of L-NAME but a reduced sensitivity to PEG-CAT compared to SAT RAs from the day of surgery, 3) SAT RAs from post-surgery would demonstrate enhanced NO fluorescence compared to those from the day of surgery sample, 4) in the presence of L-NAME and L-NAME plus vitamin D, a greater relative reduction in NO fluorescence would be observed in post-surgery and 5) in the presence of vitamin D, no difference in NO fluorescence would be observed.

Fifteen morbidly obese subjects were recruited from the University of Illinois at Chicago Outpatient Care Center bariatric surgery clinic. SAT and VAT samples were obtained from the
abdomen at the time of surgery. Eight subjects completed the post-surgery visit which took place three months after surgery. At this time, a fasting blood draw, anthropometric measurements and a gluteal SAT sample for vascular measurements were obtained. Microvascular endothelial function was determined via flow-induced dilation (FID) and acetylcholine-induced dilation (AChID). Measurements of NO and hydrogen peroxide (H$_2$O$_2$) generation were made with fluorescence microscopy.

From the SAT and VAT samples collected on the day of surgery, the primary findings of this study were that 1) vitamin D improved FID and AChID in RAs from SAT and VAT and this effect was abolished by L-NAME but not by PEG-CAT and 2) in the presence of vitamin D, NO fluorescence increased in both SAT and VAT; this effect was abolished in the presence of L-NAME but not with PEG-CAT. Consistent with previous results from our lab, compared to RAs from SAT, those from VAT demonstrated reduced FID and AChID responses. Moreover, L-NAME reduced FID and AChID in SAT but had no significant effect on VAT (67).

In comparing the measures from pre-and post-surgery, the findings were that 1) subjects lost a significant amount of their body weight three months post-surgery, 2) SAT RAs from post-surgery demonstrated improved FID and AChID and had a larger L-NAME component of their FID and AChID. The H$_2$O$_2$ scavenger, PEG-CAT, reduced FID and AChID to a lesser extent than SAT RAs from the day of surgery, 3) compared to pre-surgery SAT RAs, post-surgery SAT demonstrated enhanced NO fluorescence, 4) in the presence of L-NAME and separately, L-NAME plus vitamin D, there was a greater reduction in NO fluorescence following surgery compared to those from the day of surgery and 5) there was no effect of vitamin D on NO fluorescence in pre or post RAs.
II. INTRODUCTION

A. Background

Vitamin D deficiency and obesity are two major global public health concerns. Vitamin D insufficiency is characterized as blood serum levels of 30-50nmol/l (12-20ng/ml). Vitamin D deficiency is characterized as serum vitamin D levels of less than 30nmol/l (<12ng/ml). Adequacy of vitamin D is considered to be at least 50nmol/l (≥20ng/ml) (34). Individuals with a body mass index (BMI) of 25-29.9 kg/m² are considered to be overweight whereas those with a BMI of 30-39.9 kg/m² are considered to be obese. Individuals with a BMI of at least 40 kg/m² are characterized as morbidly obese.

The prevalence of both obesity and vitamin D deficiency are significant. In 2011, the prevalence of vitamin D deficiency in the United States was 24%. Obesity is also a very prevalent condition. In the United States in 2012, 69% of the population was either overweight or obese (45, 61). Between 2011 and 2012, more than one-third of adults in the United States were obese (61). Growing evidence suggests that these two modern health issues are related (12, 18, 24). A recent study assessed the prevalence of vitamin D deficiency in a sample of children aged 6-18 who were enrolled in the National Health and Nutritional Examination Survey. It was found that 21% and 49% of healthy weight and morbidly obese children, respectively, were deemed to be deficient in vitamin D (61). Both obesity and vitamin D deficiency negatively affect the cardiovascular system. Endothelial dysfunction may serve as an important initial step in the development of CVD in the context of vitamin D deficiency and obesity and therefore, may be a prognostic factor for future cardiovascular events (14, 65).
B. **Purpose of the study**

Endothelial dysfunction may serve an important initiating step in the development of CVD during morbid obesity and associated vitamin D deficiency may also serve as a comorbidity leading to the development of CVD. Therefore, vitamin D status may also have a prognostic value in predicting future cardiovascular events. (28). Vitamin D deficiency and obesity are two prevalent conditions with serious health problems (66). Therefore, it is important to gain a better understanding of the relationship between these two conditions. The purpose of this study is to study the effects of vitamin D on microvascular endothelial function from both SAT and VAT in obese adults before and after undergoing bariatric surgery. To the best of our knowledge, this is the first study of its kind.

From the samples collected on the day of surgery, *we hypothesized that* 1) in vitro vitamin D would improve FID and AChID in arterioles from SAT and VAT and this effect would be abolished by \(\text{NO}\)-nitro-L-arginine methyl ester (L-NAME), an eNOS inhibitor, but not by polyethyleneglycol catalase (PEG-CAT), an \(\text{H}_2\text{O}_2\) scavenger and 2) in the presence of vitamin D, NO production measured by NO fluorescence would increase in both SAT and VAT and this effect would be abolished by L-NAME but not by PEG-CAT.

In comparing (within subject) between the day of surgery and post-surgery measures, *we hypothesized that* 1) due to the bariatric surgery, subjects would lose a significant amount of body weight, 2) SAT RAs obtained from the post-surgery visit would demonstrate improved FID and AChID and an enhanced sensitivity of L-NAME but a reduced sensitivity to PEG-CAT compared to SAT RAs from the day of surgery, 3) SAT RAs from post-surgery would demonstrate enhanced NO fluorescence compared to those from the day of surgery sample, 4) in
the presence of L-NAME and L-NAME plus vitamin D, a greater relative reduction in NO fluorescence would be observed in post-surgery and 5) in the presence of vitamin D, no difference in NO fluorescence would be observed.
III. RELATED LITERATURE

1. Vitamin D

   a. Structure and forms of vitamin D

   Vitamin D is a group of fat-soluble secosteroids, which are molecules similar to steroids except that they have a broken B-ring. Multiple isoforms of vitamin D exist in vivo. Ergocalciferol, or vitamin D$_2$, is obtained by humans via dietary sources. Vitamin D$_2$ is produced by irradiation of ergosterol, a membrane sterol found in fungus. Dietary sources of vitamin D$_2$ include fish, egg yolks, dairy products and mushrooms. Cholecalciferol, or vitamin D$_3$, is endogenously produced in the epidermis of the skin via ultraviolet irradiation of 7-dehydrocholesterol, a precursor of cholesterol. Vitamin D obtained from food sources and via cutaneous production is not biologically active (1, 19).

   In order to become biologically active, vitamin D must undergo two hydroxylations. The first hydroxylation occurs in the liver where vitamin D is converted into 25-hydroxyvitamin D (25(OH)D) by mitochondrial and microsomal enzymes. 25(OH)D is the major circulating form of vitamin D. Less than 0.05% of 25(OH)D is free in the blood circulation and the remainder is bound to serum proteins. The second hydroxylation occurs mostly in the kidneys, whereby 25(OH)D is converted into the biologically active and hormonal form of vitamin D, 1,25 hydroxyvitamin D (1-25(OH)$_2$D or calcitriol), through 25-hydroxyvitamin D-1-α-hydroxylase (1α-OHase), a tightly controlled enzyme found in the cells of the proximal convolutedly tubule of the kidney. In addition to the kidneys, other cells also express 1α-OHase including cardiomyocytes, macrophages, cells from the colon, pancreas and immune system and endothelial cells. These extra-renal sources of 1α-OHase also locally produce
1-25(OH)₂D (1, 19).

The activity of renal 1α-OHase, unlike that produced by extra-renal sources, is under close modulation by a hormonal control loop that keeps 1-25(OH)₂D within a narrow range. Moreover, the concentration of 1-25(OH)₂D is also closely regulated by calcium, phosphate and parathyroid hormone. Whereas the half-life of 25(OH)D is 2 to 3 weeks, the half-life of 1-25(OH)₂D is only several hours. Therefore, serum levels of 1-25(OH)₂D do not usually decrease until vitamin D insufficiency becomes very severe. The combination of calcitriol’s short half-life and its narrow physiological range, make it inappropriate as a clinical measure to define vitamin D status.

25(OH)D’s longer half-life in serum allows it to serve as a biomarker of exposure to vitamin D (1). Circulating 1-25(OH)₂D is bound by a vitamin D binding protein, which allows it to circulate systemically until it reaches vitamin D target cells that contain the vitamin D receptor (VDR) (25).

1. **Vitamin D**

   b. **Vitamin D receptor**

   1-25(OH)₂D acts through an intracellular ligand-activated transcription factor receptor protein, the vitamin D receptor (VDR). The VDR was first discovered in the chick intestine in 1975 (11). It was later discovered to be widely present throughout the human body in almost all cells and tissues, including vascular smooth muscle cells, cardiomyocytes, intestinal epithelial cells, osteoblasts and endothelial cells (10, 25).

   The VDR is a macromolecule protein that is comprised of three distinct regions, an N-terminal, a C-terminus and an unstructured region. It’s N-terminus is comprised of a dual zincfinger DNA binding domain. It’s C-terminus is the ligand-binding activity domain. Its
structure is comprised of 12 alpha helices. Amino acids within this region form a ligand binding pocket. The unstructured region serves to connect the C and N terminal domains (25).

In the presence of 1-25(OH)₂D, the VDR binds to vitamin D response elements in the promoter region of target genes (39). There, it acts to nucleate the formation of protein complexes whose functional activities are essential for changes in transcription. Specifically, when 1-25(OH)₂D comes into contact with the ligand binding pocket of the C terminus, it leads to the formation of two independent protein interaction surfaces on the VDR. One of these proteins initiates interaction with a heterodimer partner required for specific DNA binding and the second protein plays a role in gene modulation as it is critical for the recruitment of large coregulatory complexes (59). In many target cells, these actions prompt the expression of networks of target genes whose functional roles combine to coordinate specific biological responses (10).

VDRs have the ability to synthesize 1-25(OH)₂D in the presence of 25(OH)D, which may account for many of the emerging non-traditional roles of vitamin D in the human body (69). Discovery of the VDR has widened the scope of biological effects that vitamin D plays in human health (10).

1. Vitamin D

c. Role of vitamin D in the human body

Vitamin D plays a variety of roles within the human body. The main role of vitamin D is to promote calcium absorption in the gastrointestinal tract. In addition to influencing calcium concentrations, it also plays a role in monitoring serum phosphate concentrations, thereby regulating the mineralization of bone, bone growth, bone density and bone remodeling.
Similarly, humans with a loss of function of the VDR exhibit a phenotype of rickets and alopecia. Vitamin D deficiency is related to several medical problems including osteoporosis, osteomalacia, rickets, fractures and falls. Beyond calcium and phosphate homeostasis, vitamin D plays other important physiological functions (7, 9).

Vitamin D plays a variety of roles within the human body including neuromuscular and immune functions, regulation of cell growth, reduction of inflammation, anti-carcinogenic actions, control of insulin secretion and effects on skeletal muscle and cardiovascular benefits (19, 27, 30, 60).

Vitamin D deficiency is a major public health concern. Epidemiological data from normal subjects, as well as in subjects with kidney disease and type II diabetes, have shown that vitamin D deficiency is associated with various chronic illnesses including metabolic complications and CVD and complications such as left ventricular hypertrophy, hypertension, increased arterial stiffness and endothelial dysfunction. The pathophysiological mechanisms underlying these associations remain mostly unexplained (2, 13, 30, 33, 44, 51, 55, 67).

The association between vitamin D deficiency and hypertension serves as the most convincing evidence for the involvement of vitamin D in the pathogenesis of CVD (50). Vitamin D deficiency is associated with a marked increased risk for preeclampsia, a disease in pregnant women characterized by hypertension (8). A recent study examined the effects of dietary salt loading and found that those that were deficient in vitamin D were subjected to greater increases in blood pressure compared to those with adequate levels of vitamin D (63). In a study performed in an elderly population, an association between high blood pressure and inadequate vitamin D levels was found (3). High-dose vitamin D therapy of 15,000 IU/day for one month in
an obese, hypertensive patient population was shown to decrease mean blood pressure (64). Moreover, a higher augmentation index, a measure of arterial stiffness which contributes to the development of hypertension, was observed in healthy subjects with lower 25(OH)D levels (47). Other research has found that loss of VDR signaling, via investigation of a VDR receptor mutant murine model, was associated with increased arterial stiffness and resulted in elevated systolic and diastolic blood pressures, independent of the renin-angiotensin-aldosterone system (20). Another study found VDR knockout mice have increased arterial stiffness through decreased bioavailability of NO (4). It is proposed that the mechanism driving the association between vitamin D deficiency and CVD and complications is related to the effects of vitamin D on the vascular endothelium (4, 43).

1. Vitamin D

d. Effects of vitamin D on the vascular endothelium

In a recent study, mice with a mutant, functionally inactive VDR were characterized with lower bioavailability of NO due to reduced expression of eNOS. Expression of mRNA and translation of protein for 1α-OHase, as well as the presence of intracellular VDRs in endothelial cells, has been demonstrated. Therefore, endothelial cells are able to produce vitamin D. It was also determined that 1-25(OH)₂D, acting through the VDR, is a direct transcriptional regulator of eNOS (39, 69).

The VDR is activated after binding to its ligand, 1-25(OH)₂D. It was recently demonstrated for the first time that 1-25(OH)₂D stimulates NO production in HUVEC cultures via activation of the VDR, thereby stimulating transcription of eNOS. The effect of vitamin D on NO production is concentration-dependent and the greatest effect was detected at 1nM
concentration after 1 minute of stimulation. Moreover, this same concentration was found to increase phosphorylation of eNOS and upstream effectors, phosphatidylinositol 3-kinase (PI3K/Akt) and protein kinase A (PKA) (34, 42). eNOS, PI3K/Akt and PKA are known to be involved in NO production. Endothelial cells express β-adrenoreceptors, which contribute to vasodilation via stimulation of the production of NO. This stimulation is mediated through phosphorylation of eNOS through both PKA and PI3K/Akt, thereby activating eNOS in a Ca\(^{2+}\)-dependent manner via enhancing its sensitivity to Ca\(^{2+}\)-calmodulin. This stimulation is sustained by an increase in L-arginine, a substrate of eNOS, uptake as a result from NO-mediated membrane hyperpolarization (52). Vitamin D has also been shown to modulate vascular tone by decreasing calcium influx into endothelial cells. This decreases the production of endothelial-derived contracting factors, which serve as potent vasoconstrictors (65).

The concurrence of both the ability of endothelial cells to produce vitamin D and the presence of intracellular VDR in endothelial cells suggests that vitamin D is a regulator of endothelial function (65). Production of endothelial NO, by eNOS, plays a pivotal role in vascular health; NO is the most potent endothelial vasodilator that relaxes smooth muscle cells and reduces arteriolar stiffness. Some preliminary cell culture data have shown that vitamin D may modulate NO production and we are now looking at intact RAs. However, little is known about the effect(s) of vitamin D on endothelial NO production in SAT and VAT RAs in a morbidly obese population. Furthermore, the role of weight loss following bariatric surgery in SAT and VAT RA NO production in a morbidly obese population is also unexplored (14).
2. Obesity
   
a. **Relationship between adipose tissue and vascular function**

    Obesity is associated with various comorbidities including vitamin D deficiency and CVD (31, 35). Several mechanisms may contribute to the increased risk of CVD in obese individuals. Previous studies have found that adipose tissue is a regulator of vascular function. VAT has been found to be more pro-atherogenic and pro-inflammatory than SAT (56).

    Vasodilation is an important physiological regulator of tissue perfusion. The mediator of FID and AChID is vessel depot- and species- dependent, but typically involves the release if NO, prostaglandins, cyclooxygenase, bradykinin and endothelial derived hyperpolarizing factors. EDHF, which may include H$_2$O$_2$, is known to compensate for the reduced NO bioavailability in certain disease states such as obesity (32). RAs from VAT are associated with impaired endothelial function and large artery stiffness (21, 22). It is also known that obesity and weight gain is associated with low-grade inflammation that releases a number of inflammatory cytokines, such as IL-6 and CRP, effectively altering vascular function by disrupting the balance of vasoconstrictor and vasodilator agents (56). Previous work in our lab has found that FID is impaired in RAs from VAT in morbidly obese individuals due to an imbalance of NO and H$_2$O$_2$ (54). H$_2$O$_2$ may serve as a compensatory vasodilator during pathological conditions such as obesity (37, 38).

3. **Relationship between vitamin D and obesity**
   
a. **Mechanisms behind vitamin D deficiency in obesity**

    A number of mechanisms contributing to the high prevalence of vitamin D deficiency in obesity have been postulated. It has been suggested that obese individuals, overall, have
suboptimal levels of vitamin D because they tend to avoid exposure to sunlight. Solar ultraviolet radiation is required for cutaneous production of vitamin D (15). It has also been postulated that the lower bioavailability of vitamin D in obese individuals, compared to leaner individuals, is due to it being sequestered within adipose tissue, considering that vitamin D is lipid-soluble vitamin. Another idea is that obese individuals tend to wear more clothing than their leaner counterparts. This effectively covers up their skin, therefore also decreasing cutaneous production of vitamin D. Lastly, vitamin D deficiency may also be due to inadequate dietary intake and/or malabsorption of vitamin D (1, 51).
IV. METHODS

A. Study population

1. Subject selection, recruitment and characteristics

Morbidly obese patients who came to the University of Illinois Medical Center for a pre-bariatric surgery evaluation and concurrently met the study eligibility criteria, were eligible to be consented and participate in the study. All subjects were between 21-49 years of age and all female subjects were premenopausal. Eligibility criteria included a BMI of at least 40kg/m$^2$ and no presence of significant medical or inflammatory conditions to avoid any confounding effects on study outcomes. Excluded subjects included pregnant female subjects and individuals with current diabetes mellitus (I or II), cancer, heart disease, kidney disease, liver disease, gallbladder disease or acute or chronic inflammatory diseases other than obesity, such as rheumatoid arthritis.

Determination of subject’s eligibility criteria was completed prior to the subject’s pre-surgery evaluation clinic visit. At the clinic visit, eligible subjects had the details of the study described. Interested subjects provided written informed consent. The study protocol and procedures conformed to the standards set by the latest revision of the Declaration of Helsinki and were approved by the University of Illinois at Chicago Institutional Review Board. The surgery date was provided by the nurse and was noted. A date was also established with the subject to meet three months post-surgery at the University of Illinois Clinical Interface Core (UIC CIC; 2010-1010).

Fifteen morbidly obese men (n=2) and women (n=13), who underwent planned robotic and laparoscopic bariatric surgery at the University of Illinois Medical Center, were
included in the study. Written informed consent was obtained from each subject. Three subjects were taking medication for hypertension, two subjects were taking birth control medication and one was taking medication for bipolar disorder and depression. Four subjects were taking vitamin D supplementation as well. Three were taking 50,000 IU vitamin D/week and one subject was taking 10,000 IU vitamin D/week.

A. Study population

2. Physical and cardiometabolic parameters

Physical characteristics including age, sex, height, body weight, BMI and waist circumference and cardiometabolic risk factors including blood pressure and heart rate were assessed first. Fasting blood samples for biochemical parameters (blood glucose and insulin levels, lipid panel) were taken prior to biopsy acquisition.

B. Sample acquisition

1. Day of surgery

On the day of surgery, a blood sample was collected by the patient’s nurse prior to the administration of anesthesia. After the administration of anesthesia, but prior to the surgical procedure, matched samples of SAT and VAT samples were collected by the surgeon and immediately placed in 4°C HEPES buffer solution to maintain viability of the tissue. SAT was collected from the lower abdominal wall and VAT was obtained from the greater omentum. In the lab, the adipose tissue samples were dissected. RAs were carefully cleaned of fat and connective tissue and prepared for either continuous measurement of internal luminal diameter or fluorescence microscopy.
B. Sample acquisition

2. Post-surgery visit

Subjects were given a courtesy reminder call prior to their post-surgery visit (~3 months after surgery) at the UIC CRC. At this time, subjects were also reminded of the visit’s protocol and were reminded to fast from food and beverages for 12 hours prior to the study visit. At this visit, subjects’ height, weight, waist circumference, blood pressure and heart rate were measured. Subjects also underwent a fasting blood draw in order to measure total cholesterol, triglycerides, glucose and insulin. Lastly, a SAT biopsy was obtained from the gluteal region. Lidocaine, a local anesthetic, was administered to the biopsy site prior to obtaining the tissue sample. No stitches were required for the wound and Steri-Strip was placed over the wound. The biopsy was immediately placed in 4°C HEPES buffer solution. Once again, in the lab, the adipose tissue samples were dissected. RAs were carefully cleaned of fat and connective tissue and prepared for either continuous measurement of internal luminal diameter or fluorescence microscopy.

C. Experimental protocol

1. Experimental chemicals

The NO Detection Kit was obtained from Enzo Life Sciences. The dichlorodehydrofluorescein diacetate (DCF-DA) dye was obtained from Life Technologies. The remaining pharmacological agents used (L-NAME, PEG-CAT, 1-25(OH)2D) were obtained from Sigma-Aldrich. None of the chemical agents produced significant changes in baseline RA internal luminal diameter and resulted in less than a 1% change in total volume (data not shown).
C. Experimental protocol

2. Microvascular function

In an organ chamber, RAs were cannulated and prepared as previously described (16). The internal luminal diameter of each RA was initially measured after 30 minutes of stabilization at 60 cm H₂O and following administration of endothelin-1 (ET-1; 100-200pM) to constrict RAs to 30-50% of their internal luminal diameter. This was followed by flow-induced changes of RA internal luminal diameter. Flow was produced by simultaneously changing the heights of the reservoirs in equal and opposite directions to generate a pressure gradient of Δ10, Δ20, Δ40, Δ60 and Δ100 cm H₂O. In separate experiments, arteriolar dilation to acetylcholine (ACh, 10⁻⁹ to 10⁻⁴ M) was also determined. FID and AChID of RAs were measured in both the absence and presence of the following chemical agents: a) L-NAME (10⁻⁴M), b) PEG-CAT (500 U/ml), c) 1-25(OH)₂D (1nM), d) L-NAME plus 1-25(OH)₂D and e) PEG-CAT plus 1-25(OH)₂D. Agents were added to the external bathing solution of the organ chamber 30 minutes prior to FID and ACh. Maximal diameter of every vessel was determined in the presence of papaverine (10⁻⁴M) at the end of each experiment.

C. Experimental protocol

3. Fluorescence detection of cellular NO and H₂O₂ production

Vascular NO was measured using an NO Detection Kit, which is a non-fluorescent, cell-permeable dye. It reacts with NO in the presence of oxygen with high specificity, sensitivity and accuracy and yields a water-insoluble red fluorescent product. The NO fluorescent product was excited by a 650 nm wavelength light with an emission spectrum of 670 nm with a krypton/argon fluorescent microscope (Nikon eclipse 80i). Application of DCF-DA (2 µM) was
used to measure $\text{H}_2\text{O}_2$. DCF-DA fluorescence was excited by 492 nm wavelength of light with an emission spectrum of 527 nm. RAs were cannulated and maintained at 37 °C at an equilibration pressure of 60 cmH$_2$O for 30 minutes and then exposed to flow (pressure gradient of Δ60 cmH$_2$O) in the presence or absence of L-NAME ($10^{-4}\text{M}$), PEG-CAT (500 U/ml) and 1-25(OH)$_2$D (1nM). Vessels were exposed to the NO detection dye or DCF-DA dye for the final 30 minutes, rinsed in HEPES buffer, and examined under the fluorescent microscope.

**D. Statistics**

1. **Statistical Analysis**

All data are reported as mean ± standard error with $p < 0.05$ considered to be statistically significant unless otherwise noted. Acquired images were analyzed for fluorescence intensity while correcting for background autofluorescence using NIH image software (Image J).

FID, AChID, NO fluorescence measurements and other cardiometabolic and physical characteristics were assessed using Student’s t-test for within-group comparisons. Dilation in dose response data is expressed as a percentage, with 100% representing the change from constricted diameter to the maximal diameter at 60 cm H$_2$O intraluminal pressure (usually in the presence of papaverine). Constriction responses to ET-1 were represented as a percentage, with the vessel diameter before the ET-1 dose response subtracted from the minimum diameter for each dose, divided by the pre-ET-1 dose response diameter -100% represented a closed lumen, with no visible dilation. Analyses were run with Microsoft Office Excel software (Version 2013, Microsoft Corporation, Redmond, WA). Figures were prepared using SigmaPlot (Version 12.0, San Jose, CA).
V. RESULTS

A. Physical and cardiometabolic parameters

Table I summarizes the subjects' physical characteristics. Fifteen subjects (2 males, 13 females; age: 37 ± 8 yrs.) were enrolled in the study. Eight subjects (1 male, 7 females; age: 35 ± 6 yrs.) returned for the post-surgery visit. On average, subjects lost 13.3 kg of their body weight (post: 119.2 ± 2 kg vs. pre: 132.5 ± 10.4 kg, p < 0.05). Subjects’ BMI decreased as well (post: 40.7 ± 5.6 kg/m² vs. pre: 47.1 ± 6.3 kg/m², p < 0.01). Subjects’ waist circumference also reduced (post: 115.4 ± 13.8 cm vs. 131.1 ± 12.6 cm, p < 0.01).

Table II summarizes the cardiometabolic parameters. Post-surgery, both systolic and diastolic blood pressures decreased (systolic post: 119.6 ± 9.8 mm Hg vs. pre: 130.3 ± 13.5 mm Hg, p < 0.05; diastolic post: 66.7 ± 3.7 mm Hg vs. pre: 78.1 ± 9.1 mm Hg, p < 0.01). On average, subjects’ total cholesterol also decreased three months after surgery (pre: 185.9 ± 20.8 vs. post: 168.7 ± 19.3 mg/dL; p < 0.05). Moreover, reductions in glucose were observed three months after surgery compared to the day of surgery (pre: 87.4 ± 15.4 vs. 83.8 ± 13.3 mg/d; p < 0.05).

B. FID and AChID

1. RAs from VAT and SAT obtained on the day of surgery

Vitamin D improved FID and AChID compared to baseline in RAs from both VAT and SAT obtained on the day of surgery (Figure 1, n = 15 p ≤ 0.01 (VAT), p ≤ 0.05 (SAT)). These enhancements in FID in the presence of vitamin D in RAs from VAT and SAT were abolished with the eNOS inhibitor, L-NAME (Figure 2, p ≤ 0.05), but not with the H₂O₂ scavenger, PEG-CAT (Figure 4). The enhancements in AChID in the presence of vitamin D in RAs from VAT
and SAT were also abolished with L-NAME (Figure 5, p ≤ 0.05); data of the effect of combined PEG-CAT and vitamin D on AChID was not obtained.

Consistent with previous data from our lab, dilator responses of VAT RAs were less sensitive to flow and acetylcholine compared to SAT RAs (Figures 1, 2). Moreover, L-NAME did not significantly affect FID and AChID in RAs from VAT (Figure 2A & 5A, respectively) but reduced FID and AChID in RAs from SAT were observed (Figures 2B & 5B; p ≤ 0.01) (53). Four subjects were taking prescribed vitamin D supplementation prior to surgery (10,000 IU, n = 1; 50,000 IU, n = 3). No differences in FID and AChID in RAs obtained from participants taking vitamin D supplementation prior to surgery versus those that weren’t were found (Figure 3).

B. FID and AChID

2. Comparison of RAs obtained from SAT pre- and post-surgery

RAs from the post-surgery SAT sample demonstrated improved FID at baseline and an enhanced effect of the eNOS inhibitor, L-NAME, compared to RAs from SAT collected on the day of surgery (Figure 7; (A; p ≤ 0.05) & (B; p ≤ 0.05), respectively). Moreover, RAs from the post-surgery SAT sample demonstrated improved AChID at baseline and an enhanced effect of L-NAME compared to RAs from the SAT sample collected on the day of surgery (Figure 8; (A; p ≤ 0.05) & (B; p ≤ 0.05), respectively). A greater percent absolute change in FID in the presence of L-NAME was observed at both 60 and 100 cm H$_2$O (Figure 10A, p ≤ 0.05).

In the presence of vitamin D, no difference in FID or AChID were observed in SAT RAs pre- vs. post-surgery (Figures 7C & 8C, respectively). An enhanced effect of combined L-NAME plus vitamin D in FID and AChID was observed in RAs post-surgery compared to pre (Figures 7D & 8D, p ≤ 0.05, respectively).
A reduced effect with PEG-CAT, a scavenger of H$_2$O$_2$, was observed in SAT RAs obtained three months post-surgery compared to those obtained from the day of surgery (Figure 9). A reduced percent absolute change in FID in the presence of PEG-CAT was observed at 100 cm H$_2$O (Figure 10B, p ≤ 0.05).

C. Fluorescence detection of NO production

1. RAs from VAT and SAT obtained on the day of surgery

In the presence of vitamin D, NO fluorescence increased in RAs obtained from VAT at the time of surgery (Figure 11, p ≤ 0.05). This effect was abolished by eNOS inhibitor, L-NAME (p ≤ 0.05) but not with the H$_2$O$_2$ scavenger, PEG-CAT (Figure 11).

In the presence of vitamin D, NO fluorescence increased in RAs obtained from SAT at the time of surgery (Figure 12, p ≤ 0.05). This effect was abolished with L-NAME (p ≤ 0.05) but not with PEG-CAT (Figure 12).

Consistent with previous data from our lab, SAT RAs obtained from the day of surgery exhibited higher baseline NO fluorescence than VAT RAs (Figures 11 & 12, p ≤ 0.05). Moreover, NO production was reduced in SAT RAs in the presence of L-NAME (p ≤ 0.05) but not in VAT RAs (53).

Through flow- and acetylcholine-induced dilation and NO production, we observed a decreased NO vasodilatory component in visceral resistance arteries from the day of surgery compared to those from subcutaneous tissue. H$_2$O$_2$ seems to have an effect, but we haven’t measured it directly before and after vitamin D (Figure 14A).
C. Fluorescence detection of NO production

2. Comparison of RAs obtained from SAT pre- and post-surgery

NO fluorescence was greater in SAT RAs obtained post-surgery compared to pre-surgery (Figures 12 & 13, \( p \leq 0.05 \)). In the presence of L-NAME, a greater reduction in NO production was observed in SAT RAs from post-surgery samples compared to those from the day of surgery (Figures 12 & 13, \( p \leq 0.05 \)). No difference between SAT RAs obtained pre-surgery and post-surgery was observed in the presence of vitamin D (Figures 12 & 13). In the presence of combined L-NAME plus vitamin D, a greater reduction in NO production was observed in SAT RAs from post vs. pre-surgery (Figures 12 & 13, \( p \leq 0.05 \)). Both NO and H2O2 play a role in vasodilation in subcutaneous tissue at the time of surgery. As we move toward surgical weight loss, the NO vasodilatory component is enhanced and the H2O2 compensatory component in reduced to flow- and acetylcholine-induced dilation. When subjects were treated with vitamin D at surgery, there is an enhancement in NO production but as subjects lose weight, no improvements with vitamin D were observed (Figure 14B).
VI. DISCUSSION

A. Conclusion

To the best of our knowledge, this is the first study of its kind to explore the effects of weight loss via bariatric surgery and of vitamin D on microvascular function in SAT and VAT. From the SAT and VAT samples collected at the day of surgery, the primary findings of the present study are that vitamin D improved FID and AChID in RAs from SAT and VAT and this effect was abolished by L-NAME but not by PEG-CAT. In the presence of vitamin D, NO fluorescence increased in both SAT and VAT. This effect was abolished in the presence of L-NAME but not with PEG-CAT.

In comparing the measures from pre-and post-surgery, the primary findings of the study are that 1) subjects lost a significant amount of their body weight after 3 months post-surgery, 2) SAT RAs from post-surgery demonstrated improved FID and AChID at baseline and an enhanced effect of L-NAME but a reduced effect with PEG-CAT compared to SAT arterioles from the day of surgery sample, 3) compared to RAs from the pre-surgery SAT sample, those from the post-surgery SAT demonstrated enhanced NO fluorescence, 4) in the presence of L-NAME, a greater reduction in NO fluorescence was observed in the post-surgery sample, 5) in the presence of vitamin D, no difference in NO fluorescence between SAT samples were observed, 6) incubation of combined L-NAME and vitamin D led to a greater reduction in NO fluorescence in post-surgery than in the pre-surgery sample.

The findings that vitamin D improves FID, AChID and NO fluorescence in SAT and VAT supports the notion that vitamin D is a regulator of endothelial function (65). Our findings
are in direct agreement with previous data suggesting that 1-25(OH)₂D is a direct transcriptional regulator of eNOS, effectively increasing the production of NO, the most potent vasodilator within the vasculature (39, 69). It is possible that the large response to vitamin D is due to low blood serum levels of vitamin D may be low. This may be perhaps due to the sequesterization of vitamin D within in the adipose tissue, therefore it is not free to circulate in the serum (66).

The findings that baseline FID, AChID and NO fluorescence improved 3 months post-surgery in RAs from SAT but no difference in NO fluorescence in the presence of vitamin D was observed suggests that endogenous NO bioavailability is enhanced following weight loss in obesity and therefore, this may explain why there is less of an effect of exogenous vitamin D which stimulates NO production. A decreased absolute change in FID in the presence of PEG-CAT was observed following post-surgery weight loss compared to RAs from the day of surgery samples. These findings are in agreement with previous evidence indicating that, in the presence of disease, such as morbid obesity, other endothelium-derived substances, such as H₂O₂ may compensate for the lack of NO (37, 38, 41, 49).

Previous evidence suggests that SAT is the main storage site for cutaneously produced vitamin D. It is possible that during the 3 months of following the bariatric surgery, subjects lost more weight in areas with VAT, such as the abdominal region and lost less SAT (66). In this case, weight loss 3 months post-surgery may not have affected the amount of SAT, therefore a significant amount of vitamin D sequestered in those tissues may have not been released in to the serum.

Exogenous vitamin D may help morbidly obese people with tissue perfusion via enhanced dilatory capacity in RAs. This may decrease CVD risk. Weight loss is associated with
decreased inflammation and reactive oxidative species and improved NO bioavailability, thus improving CVD risk. This study could be important to consider in light of the associations between inadequate levels of vitamin D and CVD in the morbidly obese population. Vitamin D and surgical weight loss may improve endothelial function and reduce CVD risk factors in the morbidly obese population by enhancing the NO component of vasodilation. Future studies are warranted to explore these associations.

B. Limitations

There are several limitations of this study. First, this study was limited to a young morbidly obese male and premenopausal female population, thereby limiting the generalizability of the findings to the population at large. Second, the study design made it unfeasible to control for menstrual cycle in the female subjects during sample acquisition. Menstrual cycle has been shown to affect the macrovasculature but its influence on the microcirculation is unknown. The surgery date could not be scheduled to control for this as a confounder a priori. Moreover, only 8 of the 15 recruited subjects returned after their surgery for their second visit and blood samples from the day of surgery were only collected for a limited number of subjects, therefore matched samples of blood and tissue pre- and post- surgery do not exist for each subject. No post-surgery VAT sample was obtained as this biopsy was unattainable in the absence of general anesthesia. On the day of surgery, the adipose biopsies were obtained after the administration of general anesthesia whereas at the post-surgery visit, the biopsies were obtained after administration of a local anesthesia. Another limitation of this study is that pre- and post- surgery adipose samples were obtained from different adipose depots (abdominal vs. gluteal SAT). However, previous
studies have shown that mechanisms of dilations in SAT or VAT depots are not dependent on the region of biopsy (49).
### TABLES

<table>
<thead>
<tr>
<th></th>
<th>Surgery (n = 15)</th>
<th>Post-Surgery (n = 8)</th>
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<tbody>
<tr>
<td><strong>Age (yr)</strong></td>
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<td>35 ± 6</td>
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<tr>
<td><strong>Sex</strong></td>
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<td><strong>Height (cm)</strong></td>
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<td>119.2 ± 3.5 *</td>
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<tr>
<td><strong>BMI (kg/m^2)</strong></td>
<td>47.1 ± 6.3</td>
<td>40.7 ± 5.6 *</td>
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<tr>
<td><strong>Waist Circumference (cm)</strong></td>
<td>131.1 ± 12.6</td>
<td>115.4 ± 13.8 *</td>
</tr>
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Table I. Physical characteristics of subjects both at the time of surgery (n=15) and three months post-surgery (n=8). Body weight (kg), BMI (kg/m^2) and waist circumference (cm) decreased post-surgery compared to the day of surgery. (*, p < 0.05).

<table>
<thead>
<tr>
<th></th>
<th>Surgery (n = 15)</th>
<th>Post-Surgery (n = 8)</th>
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</thead>
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<tr>
<td><strong>Systolic BP (mm Hg)</strong></td>
<td>130.3 ± 13.5</td>
<td>119.6 ± 9.8 *</td>
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<tr>
<td><strong>Diastolic BP (mm Hg)</strong></td>
<td>78.1 ± 9.1</td>
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<td><strong>Insulin (µIU/mL)</strong></td>
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<td>9.7 ± 1.3</td>
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Table II. Cardiometabolic risk factors of subjects both at the time of surgery (n=15) and three months post-surgery (n=8). Systolic and diastolic blood pressure (mm Hg), total cholesterol (mg/dL) and glucose (mg/dL) decreased post-surgery compared to the day of surgery (*, p ≤ 0.05).
Figure 1: Effects of vitamin D on flow-induced dilation (FID) and acetylcholine-induced dilation (AChID) in resistance arteries from both visceral adipose tissue (VAT; A & B respectively, n = 15) and subcutaneous adipose tissue (SAT, C & D respectively, n = 15) collected from the day of surgery. Vitamin D improves FID and AChID in VAT (A & B, respectively, †, p < 0.01). Vitamin D improves FID and AChID in SAT (C & D, respectively, †, p ≤ 0.05).
Figure 2. Effects of eNOS inhibition via L-NAME and vitamin D on flow-induced dilation (FID) of resistance arteries from visceral adipose tissue (VAT, n = 15, A) and subcutaneous adipose tissue (SAT, n =15, B) collected at the time of bariatric surgery. Vitamin D improves FID in both VAT and SAT at the time of surgery (A,B;†; p < 0.05) and this effect is abolished with eNOS inhibitor, L-NAME (A,B, Δ; p ≤ 0.05). In figure 2B, as expected, L-NAME reduced dilator responses to flow (*; p ≤ 0.01).
Figure 3. Effects of eNOS inhibition via L-NAME and vitamin D on flow-induced dilation (FID) of resistance arteries from visceral adipose tissue (VAT, n=4, A) and subcutaneous adipose tissue (SAT, n=4, B) collected at the time of bariatric surgery from patients taking prescribed vitamin D supplementation prior to surgery (10,000-50,000 IU). No difference in responses in subjects taking vitamin D at the time of surgery and those that were not was observed (Figure 2). Consistent with Figure 2, vitamin D improves FID in both VAT and SAT at the time of surgery (A,B; †; p < 0.01). This effect of vitamin D is abolished in both adipose depots with eNOS inhibitor, L-NAME (A,B; Δ; p < 0.01). As expected, L-NAME reduced dilator responses to flow in SAT (B, *; p < 0.01) but had no significant effect in VAT.
Figure 4. Effects of scavenging H$_2$O$_2$ via PEG-CAT and vitamin D on flow-induced dilation (FID) of resistance arteries from visceral adipose tissue (VAT, n=15, A) and subcutaneous adipose tissue (SAT, n=15, B) collected at the time of bariatric surgery. In resistance arteries from both VAT and SAT at the time of surgery, the effect of vitamin D (compared to baseline, A,B; †, p < 0.01) is not abolished by PEG-CAT. PEG-CAT decreased FID in both adipose depots (A,B,*; p ≤ 0.05).
Figure 5. Effects of eNOS inhibition via L-NAME and vitamin D on acetylcholine-induced dilation (AChID) of resistance arteries from visceral adipose tissue (VAT, n=15, A) and subcutaneous adipose tissue (SAT, n=15, B) collected at the time of bariatric surgery. In both VAT and SAT, vitamin D improves AChID (A,B; †; p < 0.05) and this effect is abolished with eNOS inhibitor, L-NAME (A,B; Δ; p ≤ 0.05). In SAT, L-NAME reduced dilator responses to flow (B; *; p ≤ 0.01) but had no significant effect in VAT.
Figure 6. Effects of eNOS inhibition via L-NAME and vitamin D on flow-induced dilation (FID, n=8, A) and acetylcholine-induced dilation (AChID, n=8, B) of resistance arteries from subcutaneous adipose tissue (SAT) collected three months after bariatric surgery. In both A and B, no difference between baseline and vitamin D dilator responses was observed. The effect of vitamin D is abolished with eNOS inhibitor, L-NAME (A,B; ∆; p < 0.05). L-NAME reduced dilator responses to flow (A,B; *; p ≤ 0.01).
Figure 7. Effects of baseline (A), eNOS inhibition via L-NAME (B), vitamin D (C) and combined L-NAME plus vitamin D (D) on flow-induced dilation (FID) of resistance arteries from subcutaneous adipose tissue (SAT) from both the time of surgery (n=8, A-D) and three months post-surgery (n=8, A-D). FID improves three months post-surgery compared to the time of surgery (A, ◊, p < 0.05). An enhanced effect of L-NAME is observed post surgery compared to pre (B, □, p < 0.05). No effect of vitamin D was observed (C). An enhanced effect of the combined L-NAME plus vitamin D on FID was observed post-surgery versus on the day of surgery (D, $, p \leq 0.05).
Figure 8. Effects of baseline (A), eNOS inhibition via L-NAME (B), vitamin D (C) and combined L-NAME plus vitamin D (D) on acetylcholine-induced dilation (AChID) of resistance arteries from subcutaneous adipose tissue (SAT) from both the time of surgery (n=8, A-D) and three months post-surgery (n=8, A-D). AChID improves three months post-surgery compared to the time of surgery (A, ◊, p ≤ 0.05). An enhanced effect of L-NAME is observed post surgery compared to pre (B, □, p ≤ 0.05). No effect of vitamin D was observed (C). An enhanced effect of the combined L-NAME plus vitamin D on FID was observed post-surgery versus on the day of surgery (D, $, p ≤ 0.05).
Figure 9. Effects of scavenging H$_2$O$_2$ and vitamin D on flow-induced dilation (FID, n=8) of resistance arteries from subcutaneous adipose tissue (SAT) collected three months post bariatric surgery. The effect of vitamin D (compared to baseline, †, p < 0.01) is not abolished by PEG-CAT. PEG-CAT decreased FID (*; p < 0.05).
Figure 10. Absolute change of eNOS inhibition via L-NAME (A) and of H$_2$O$_2$ scavenging via PEG-CAT (B) on flow-induced dilation (FID) of resistance arteries from subcutaneous adipose tissue (SAT) from both the time of surgery (n=8, A & B) and three months post-surgery (n=8, A & B). An enhanced effect of L-NAME on FID was observed post-surgery compared to the day of surgery (A, *, p < 0.05). A reduced effect with PEG-CAT post-surgery compared to SAT arterioles from the day of surgery was observed at 100 cm H$_2$O (B, *, p < 0.05).
Figure 11. Effects of eNOS inhibition, H$_2$O$_2$ scavenging and vitamin D on NO fluorescence of resistance arteries from visceral adipose tissue (VAT) collected at the time of bariatric surgery (n=15). In the presence of vitamin D, NO fluorescence increased compared to baseline (†, p < 0.05). This effect was abolished with L-NAME (Δ, p ≤ 0.05) but not with PEG-CAT. As expected, L-NAME had no effect.
Figure 12. Effects of eNOS inhibition, H₂O₂ scavenging and vitamin D on NO fluorescence of resistance arteries from subcutaneous adipose tissue (SAT) collected at the time of bariatric surgery (n=15). In the presence of vitamin D, NO fluorescence increased compared to baseline (†, p < 0.05). This effect was abolished with L-NAME (Δ, p < 0.05) but not with PEG-CAT. As expected, L-NAME reduced NO fluorescence (*, p ≤ 0.05).
Figure 13. Effects of eNOS inhibition, \( \text{H}_2\text{O}_2 \) scavenging and vitamin D on NO fluorescence of resistance arteries from subcutaneous adipose tissue (SAT) collected three months post-surgery (n=8). No difference in NO fluorescence was observed in the presence of vitamin D, however, vitamin D plus L-NAME reduced NO fluorescence compared to vitamin D alone (\( \Delta \), \( p \leq 0.05 \)). As expected, L-NAME reduced NO fluorescence (*, \( p \leq 0.05 \)).
Figure 14. Summary slide of the vasodilatory agents in visceral adipose tissue obtained on the day of surgery (A) and before and after weight loss in subcutaneous adipose tissue (B). Through flow- and acetylcholine-induced dilation and NO production, a decreased NO vasodilatory component was observed compared to subcutaneous tissue. H$_2$O$_2$ seems to have an effect, but it wasn’t measured directly before and after vitamin D. We also observed that vitamin D enhanced NO production (A). Both NO and H$_2$O$_2$ play a role in vasodilation in subcutaneous tissue at the time of surgery. As we move toward surgical weight loss, the NO vasodilatory component is enhanced and the H$_2$O$_2$ compensatory component in reduced to flow- and acetylcholine-induced dilation. When subjects were treated with vitamin D at surgery, there is an enhancement in NO production but as subjects lose weight, no improvements with vitamin D were observed (B).
CITED LITERATURE


27. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, 2011.


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