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Metabolism Clinical and Experimental 54 (2005) 1368–1373

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Endothelial nitric oxide synthase content in adipose tissue from obese and lean African American and white American women

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Received 27 January 2005; accepted 12 April 2005

Abstract

It has been demonstrated that the enzyme endothelial nitric oxide synthase (eNOS) is present in adipose tissue, resulting in nitric oxide production and subsequent inhibition of lipolysis. A higher eNOS content has also been reported in the subcutaneous abdominal adipose tissue of obese than in that of lean white men. Furthermore, a lower lipolytic rate in obese than in lean women and a lower lipolytic rate in African American (AA) than in white American (WA) women have been demonstrated. The purpose of this study was to determine if eNOS protein content is higher in the subcutaneous and omental adipose tissues of obese than in those of lean women and if eNOS protein content is higher in the subcutaneous and omental adipose tissues of AA than in those of WA women. Whole tissue homogenates were prepared from frozen omental and subcutaneous adipose tissue samples obtained from lean and obese and AA and WA elective abdominal surgery patients and were analyzed for eNOS protein content using enzyme-linked immunosorbent assay. The adipose tissue eNOS protein content was approximately 40% higher in obese than in lean individuals (omental, 326.9 ± 40.5 pg/mL lean and 445.3 ± 38.0 pg/mL obese; subcutaneous, 246.8 ± 20.8 pg/mL lean and 343.1 ± 19.0 pg/mL obese; $P < .05$). There was no difference between the races for eNOS protein content in omental adipose tissue. In subcutaneous adipose tissue, there was a higher eNOS content in obese (417.1 ± 78.9 pg/mg total protein) than in lean (216.7 ± 29.9 pg/mg total protein) ($P < .05$) WA women, but there was no difference in subcutaneous adipose eNOS content between obese and lean AA women (250.7 ± 47.4 and 294.1 ± 42.2 pg/mg total protein, respectively). The higher eNOS content in the adipose tissue of obese than in that of lean WA women in the fasted state may contribute to the reduced lipolytic activity in WA women; however, eNOS protein content probably does not contribute to differences in lipolytic rates between AA and WA women.

1. Introduction

Cellular and molecular alterations in the metabolism of lipids by adipose tissue play an important role in the development and maintenance of obesity. Previous studies have demonstrated the importance of lipolysis in the maintenance of obesity and have reported basal in vivo lipolysis to be decreased in obesity [1-3]. However, there are multiple regulators of lipolysis in human beings such as catecholamines, insulin, and the more recently proposed nitric oxide (NO). L-NMMA, an inhibitor of nitric oxide synthase (NOS), has been used to successfully increase lipolytic rates in adipose tissue [4,5]. Endothelial nitric oxide synthase (eNOS) messenger RNA (mRNA) and protein content of subcutaneous adipose tissue have also been shown to be higher in obese than in lean men [6,7]. Furthermore, increased NO production has been positively correlated with body mass index (BMI) in both males and females [8]. Despite these previous findings and the reported lower lipolytic rates in obese than in lean women, the eNOS protein content has not been determined in women and has not previously been determined in the omental adipose tissue of lean individuals.

The alterations affecting obesity are not necessarily the same in African American (AA) and white American (WA) women. African American women tend to gain weight faster

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0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.04.028

and at an earlier age than WA women do; they also tend to lose less weight at a slower rate than WA women do [9,10]. Data from our laboratory and those from other laboratories suggest that there are impairments in fat mobilization in AA women that are not related to catecholamines [11,12]. Although there are previous reports of reduced lipolysis in AA compared with WA individuals, there have been no reports of eNOS content in the adipose tissue of AA women to implicate potential differences in NO production in the lower lipolytic rate in AA than in WA women. Therefore, the purpose of this study was to determine if there is a higher eNOS content in obese than in lean women within subcutaneous and omental adipose tissues. A further analysis was undertaken to determine if there is a higher eNOS content in these adipose depots in AA than in WA women.

2. Methods

2.1. Research participants

Demographic data were obtained from 57 lean AA, obese AA, lean WA, and obese WA premenopausal women who underwent gastric bypass surgery. Omental and/or subcutaneous adipose tissue samples were collected from each woman, although not all women contributed both tissue types. Omental adipose tissue samples were collected from lean AA (n = 5) and obese AA (n = 9) women and lean WA (n = 9) and obese WA (n = 12) women. Subcutaneous adipose tissue samples were collected from lean AA (n = 7) and obese AA (n = 8) women and lean WA (n = 11) and obese WA (n = 10) women. Participants were aged 18 years or older. Women with a BMI of 27.5 kg/m^2 or less were accepted into the nonobese groups; those with a BMI of 32.5 kg/m^2 or higher were placed in the obese groups. All subjects were from eastern North Carolina and were recruited through participating physicians performing abdominal surgery at the Pitt County Memorial Hospital. The AA and WA women were preceded by 2 generations whose ethnicity is homogenous to the group in which they were included; verification occurred by self-report. Obese individuals were undergoing gastric bypass whereas most pre-obese subjects were undergoing hysterectomy. Those having known metabolic disease or hypertension were excluded. This study has been approved by the East Carolina University Institutional Review Board. Each subject was thoroughly informed of the study procedures and about the handling of collected tissue before obtaining informed consent.

2.2. Study design

Before undergoing surgery, each participant's BMI was determined. Health histories were also obtained along with consent for surgery. Both subcutaneous and omental adipose tissue samples were excised during elective abdominal surgery and analyzed for eNOS protein content. Fasting blood samples were obtained on the day of surgery and analyzed for circulating estradiol, glucose, insulin, and glycerol concentrations.

2.3. Adipose tissue homogenate preparation

One gram of previously frozen (−80°C) adipose tissue pieces was placed in a glass-on-glass homogenizer along with 5 μL of a protease inhibitor cocktail (Sigma P-8340, Sigma-Aldrich Inc, St Louis, Mo) and 1 mL of a cell lysis buffer (2.5 mmol/L of $MgCl_2$, 1 mmol/L of $KHCO_3$, 2 mmol/L of Tris-HCl, pH 7.5). Samples were homogenized for a total of 30 strokes each. Two hundred microliter aliquots of each sample were stored at −80°C for analysis of eNOS and total protein content as described below.

2.4. Endothelial nitric oxide synthase protein quantitation

Adipose tissue eNOS protein content was determined on adipose tissue whole homogenates in triplicate using a Quantikine human eNOS enzyme-linked immunosorbent assay (ELISA; R&D Systems, Inc, Minneapolis, Minn) according to the manufacturer's instructions. Total protein content of the homogenate was determined using a bicinchoninic acid protein assay kit (Pierce-23225, Pierce Laboratories, Rockford, Ill). Absorbance was linearly related to the protein concentrations over a range of 20 to 2000 μg/mL. The coefficient of variation for the eNOS ELISA in our laboratory was determined to be 2.92% ± 0.5% (n = 22) for triplicate analyses. Hormone-sensitive lipase (HSL) protein content was previously analyzed for a different study [12] in some (n = 10 omental, n = 11 subcutaneous) of the samples used in the present investigation to determine if a correlation existed between eNOS protein content and HSL content.

2.5. Plasma estradiol, insulin, and glucose

17B-estradiol concentration was determined using an ELISA (R&D Systems). Plasma insulin and glucose were measured to determine the relationship between adipose tissue eNOS content and markers of glucose metabolism. Insulin was determined from frozen plasma samples using an automated Beckman-Coulter Access Immunoassay System (Fullerton, Calif). Glucose samples were determined from aliquots of the same plasma samples using a 2300 Stat Plus system (Yellow Springs Instruments, Inc, Yellow Springs, Ohio). An index of insulin sensitivity (homeostasis model assessment [HOMA]) was calculated from fasting blood samples by the following formula as described by Matthews et al [13]: fasting serum insulin (μU/mL) ×- fasting plasma glucose (mmol/L)/22.5.

2.6. Statistical analysis

Differences in eNOS protein content were compared using 3-way (race, obesity status, and adipose tissue depot) analysis of variance (ANOVA). Differences in each of the measured blood variables (plasma glycerol, plasma glucose, and plasma insulin) were compared using 2-way

Table 1
Demographic data

Variable	Lean WAs (n = 17)	Obese WAs (n = 15)	Lean AAs (n = 10)	Obese AAs (n = 15)
Age (y)	44.7 ± 2.6	44.8 ± 2.4	41.7 ± 0.8	42.4 ± 2.1
Height (in)	163.1 ± 1.5	165.0 ± 1.5	167.9 ± 5.6	164.8 ± 1.8
Weight (kg)	58.0 ± 5.7	144.8 ± 9.1	63.2 ± 9.5	121.7 ± 8.4
BMI (kg/m^2)	24.7 ± 0.6	52.8 ± 3.0	24.8 ± 0.8	45.3 ± 3.4

Data are expressed as mean ± SEM.

(race and obesity status) ANOVA. The Student-Newman-Keuls post hoc analysis was used when significance was obtained using ANOVA. Statistical significance was set at an α level of $P < .05$. Data for each group were expressed as mean ± SE.

3. Results

3.1. Subjects

Fifty-seven women were divided into 4 groups: lean WA (n = 17), obese WA (n = 15), lean AA (n = 10), and obese AA (n = 15). The BMIs were 24.7 ± 0.4 and 49.1 ± 0.5 kg/m^2 for all lean and all obese individuals, respectively ($P < .05$). The BMIs of the specific subgroups (lean and obese WA and lean and obese AA groups) are presented in Table 1. There were no significant differences in age or height.

3.2. Endothelial nitric oxide synthase protein content and obesity

The eNOS protein content in omental abdominal adipose tissue was higher in obese than in lean individuals irrespective of race ($P < .05$; Fig. 1). In subcutaneous adipose tissue, eNOS protein content was also higher in the groups of all obese than in the groups of all lean individuals ($P < .05$; Fig. 1). The eNOS protein content was higher in omental than in subcutaneous adipose tissue ($P < .05$; Fig. 1). There was no significant correlation between eNOS protein content and HSL protein content for either omental ($r = -.493$; n = 10) or subcutaneous adipose tissue ($r = .193$; n = 11).

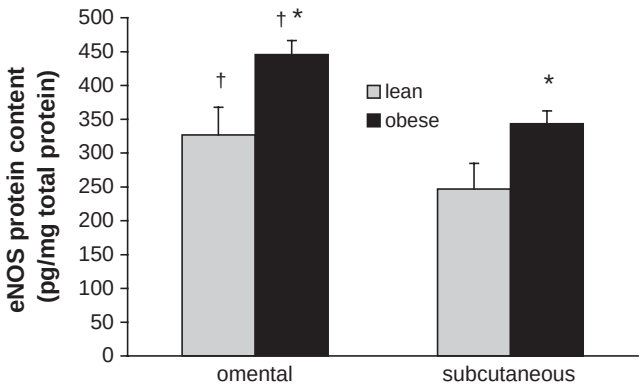

Fig. 1. Endothelial nitric oxide synthase protein content in omental and subcutaneous adipose tissues in lean and obese women. Omental adipose tissue samples were collected from lean AA and WA women (n = 14) and obese AA and WA women (n = 21). Subcutaneous adipose tissue samples were collected from lean AA and WA women (n = 18) and obese AA and WA women (n = 18). After homogenization, eNOS protein content was measured using ELISA. *Lean vs obese, $P < .05$. †Omental vs subcutaneous. Data are expressed as mean ± SE.

3.3. Endothelial nitric oxide synthase protein content: obesity and ethnicity

The eNOS content in omental adipose tissue was not different in lean WA and obese WA individuals (Fig. 2A). In AA individuals, the eNOS content of omental adipose tissue was also not different between lean AA and obese AA individuals (Fig. 2A). The eNOS content of subcutaneous abdominal adipose tissue was higher in obese WA than in lean WA individuals ($P < .05$; Fig. 2B), but the eNOS content of subcutaneous adipose tissue was not different in obese and lean AA individuals (Fig. 2B).

3.4. Plasma estradiol, insulin, and glucose

Mean estradiol concentrations were within the mid-to-high follicular range of 140.7 ± 11.6 pg/mL. Individual

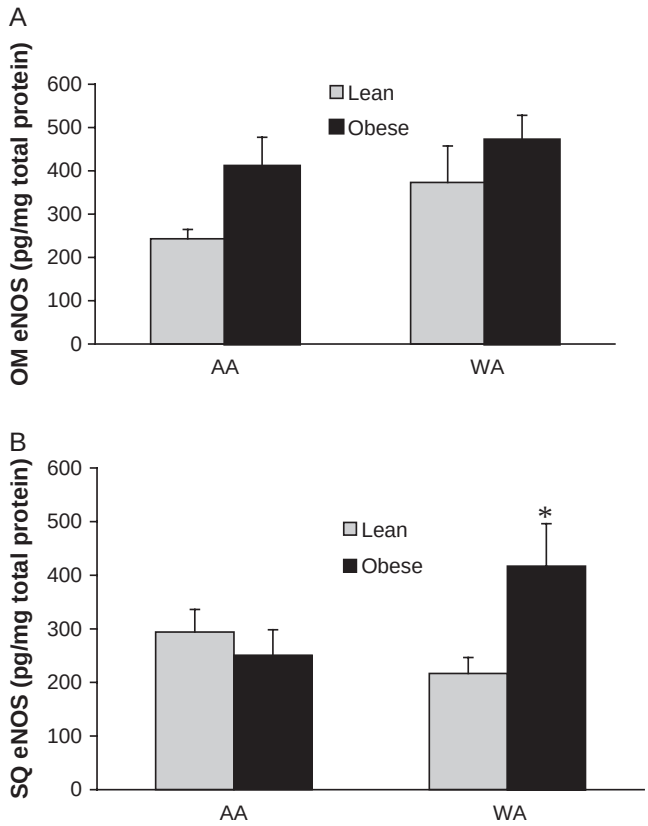

Fig. 2. Endothelial nitric oxide synthase protein content in omental (A) and subcutaneous (B) adipose tissues in AA and WA women. Omental adipose tissue samples were collected from lean AA (n = 5) and obese AA (n = 9) women and lean WA (n = 9) and obese WA (n = 12) women. Subcutaneous adipose tissue samples were collected from lean AA (n = 7) and obese AA (n = 8) women and lean WA (n = 11) and obese WA (n = 10) women. After homogenization, eNOS protein content was measured using ELISA immunoassay. *Lean WA vs obese WA subcutaneous adipose tissue, $P < .05$. Data are expressed as mean ± SE.

Table 2
Insulin sensitivity

Variable	Lean WAs (n = 17)	Obese WAs (n = 15)	Lean AAs (n = 10)	Obese AAs (n = 15)
Insulin	3.6 ± 0.8	9.2 ± 1.3[a]	3.6 ± 1.1	8.4 ± 2.0[a]
Glucose	81.9 ± 3.7	88.0 ± 5.2	78.8 ± 7.3	95.0 ± 6.0
HOMA	0.83 ± 0.20	2.60 ± 0.50[a]	0.74 ± 0.26	2.04 ± 0.48[a]

All data are presented as mean ± SE.
[a] Different from lean.

group means were 101.5 ± 13.6 pg/mL for lean WA women, 158.8 ± 30.3 pg/mL for obese WA women, 151.2 ± 46.8 pg/mL for lean AA women, and 142.4 ± 14.3 pg/mL for obese AA women. There were no differences between AA and WA or lean and obese individuals.

Fasting plasma insulin concentrations in the lean group (all lean individuals) were 3.6 ± 0.6 μU/mL whereas insulin concentrations in the obese group (all obese individuals) were 8.8 ± 0.5 μU/mL ($P < .05$). There were no differences in fasting plasma glucose values between or among the groups (Table 2). There was an effect of BMI on the HOMA, although there was no effect for race. The mean HOMA score was 0.79 ± 0.1 in the lean group and 2.31 ± 0.11 in the obese group ($P < .05$).

4. Discussion

The data from this study demonstrate that eNOS protein content in subcutaneous and omental adipose tissues is higher in obese than in lean women. This finding extends previous reports that eNOS mRNA and protein content in subcutaneous adipose tissue is higher in obese than in lean men [6]; eNOS protein content in omental adipose tissue has not previously been compared in obese and lean individuals. The most significant novel finding of the present investigation was that subcutaneous adipose tissue eNOS content was higher in obese than in lean WA women but was not different between obese and lean AA women. However, there was no difference in omental adipose tissue eNOS content between AA and WA women.

4.1. Endothelial nitric oxide synthase, lipolysis, and obesity

Previous studies have reported basal in vivo lipolysis to be decreased in obesity [1-3]. However, the precise cellular and molecular bases of these impairments have not been completely elucidated. It has recently been suggested that lipolysis may be influenced by NO. In vivo, L-NMMA, an inhibitor of NO, has been used to successfully increase lipolytic rates in adipose tissue [4]; furthermore, this study demonstrated an antilipolytic effect of NO that is independent of blood flow. Increased NO production has been positively correlated with BMI in both males and females [8]. Elizalde et al [6] also reported that increased fat mass was associated with increased eNOS expression in subcutaneous adipose tissue: eNOS mRNA was 56% higher in obese compared with lean men. Previous research by the same authors has shown this increased eNOS to be directly related to the adipocytes themselves and not to a byproduct of increased vascularity/endothelium present in adipose tissue [6]. The present data demonstrating an approximately 40% higher eNOS protein content in obese compared with lean women in both subcutaneous and omental adipose tissue depots are in line with previous findings.

4.1.1. Endothelial nitric oxide synthase content is higher in omental than in subcutaneous fat

There is sufficient evidence that abdominal obesity and the lipolytic activity of visceral adipose tissue play a major role in the development of the metabolic syndrome [14]. Ryden et al [15] compared eNOS and inducible NOS mRNA and protein content in the omental and subcutaneous abdominal tissues of obese white males to investigate the role of NOS in lipolysis in omental and subcutaneous adipose tissues. Endothelial nitric oxide synthase, but not inducible NOS, was found in both tissue types of the subjects, with the eNOS content higher in omental than in subcutaneous adipose tissue. They also reported that the basal lipolysis was higher in subcutaneous than in omental adipose tissue. In keeping with this previous report in obese men, eNOS protein content was also higher in the omental than in the subcutaneous adipose tissue of the women in the present study.

4.2. Endothelial nitric oxide synthase, lipolysis, and ethnicity

Efforts have previously been made to discover the source of lower lipolytic rates among AA women [11,16,17]. However, there are many factors that affect lipolysis. Data from our laboratory do not support the contention that differences in response to catecholamine stimulation are the origin of the lower lipolytic rate in AA individuals. Furthermore, adipocytes from AA women have the capacity to be stimulated to higher lipolytic rates in vitro despite the apparently lower in vivo lipolytic rates in AA women [11,12]. It therefore appears that an unknown endogenous source of inhibition that suppresses lipolysis to a greater extent in AA than in WA women is present in vivo. The lack of difference in eNOS protein content in subcutaneous abdominal adipose tissue between obese AA and lean AA women in the present study suggests that NOS is not related to differences in obesity status within AA women. The issue in omental adipose tissue is less clear because there was an indication that the eNOS content of omental adipose tissue was increased with increased adiposity in AA women ($P = .09$ obese vs lean AA women; Fig. 2A). If NOS is an important factor in the obesity of AAs, then it appears that omental adipose tissue is more important in this regard than subcutaneous adipose tissue in AA women.

Contrary to our secondary hypothesis, the eNOS content was not higher in AA compared with WA women. Despite previous findings of lower lipolytic rates in obese AA than in obese WA women [1-3], the present data do not support the hypothesis that NOS plays a greater antilipolytic role in AA than in WA women.

4.3. Hormone-sensitive lipase

Previous work compared HSL mass and activity in obese AA and obese WA women. It was reported that obese AA women had significantly less HSL mass in both the subcutaneous and omental fractions [12]. Furthermore, a 73% lower HSL protein in the subcutaneous adipose of obese than in that of lean white men has been reported [6]. A negative correlation was also established between HSL protein content and eNOS mRNA but not between HSL protein content and eNOS protein content [6].

Some of the adipose tissues analyzed in the present study were previously analyzed for HSL protein content [12]. We used these HSL data to perform correlations between HSL content and eNOS content. Consistent with the findings of others [6], we found in our limited sample no correlation between eNOS protein content and HSL protein content. It can be therefore concluded from these studies that there is no relationship between HSL protein content and eNOS protein content in either subcutaneous or omental adipose tissue. Whether there is a relationship between eNOS activity and HSL activity remains to be investigated. Endothelial nitric oxide synthase activity was not measured on these samples because the conditions of collecting this adipose tissue in the clinical setting did not allow for immediate freezing of the sample to ensure valid eNOS activity measurements.

4.4. Plasma estradiol, glucose, and insulin

17B-estradiol was measured in this study because estrogen has a well-documented effect on eNOS mRNA and protein content. Estradiol has been positively correlated with increased presence of eNOS [18]. However, there were no differences in plasma estradiol with respect to race or BMI in the present study. Furthermore, no subjects appeared to be amenorrheic based on these data. It is therefore unlikely that the prevailing plasma estradiol concentration around the time of surgery had any effect on adipose tissue eNOS protein content in the subjects of this study.

There were no significant interactions between or among groups for plasma glucose, although plasma insulin concentrations were significantly higher in the obese as compared with the lean groups. The insulin sensitivity calculated using the HOMA was also not different between the AA and WA women. The HOMA was significantly different between lean and obese subjects primarily due to the significantly higher plasma insulin concentration in obese than in lean individuals. The antilipolytic effect of insulin has been suggested to be related to the difference in obesity rates between WA and AA subjects. Albu et al [11] reported that AA women were more responsive to the antilipolytic effect of insulin than were their WA counterparts. Our findings indicate that insulin is not the source of decreased lipolysis in AA women. The lack of difference in insulin sensitivity between AA and WA women suggests a need for further investigation into the etiology of decreased lipolytic activity among AA women.

4.5. Summary

The eNOS protein content in subcutaneous and omental adipose tissues was compared between lean and obese AA and WA women. The eNOS protein content was higher in the subcutaneous and omental adipose tissues of obese women than in those of lean women. There was no difference in omental adipose tissue eNOS content between AA and WA women. In subcutaneous adipose tissue, eNOS content was higher in obese than in lean WA women but was not different between obese and lean AA women. Although NO production in adipose tissue may be an appropriate avenue to pursue in terms of obesity treatment, the present data do not support a role of eNOS protein content in the decreased in vivo lipolytic rate in AA as compared with WA women. The adipose tissue samples of the present investigations were obtained from women during elective abdominal surgery after an overnight fast; the influence of a fed state on eNOS remains to be studied.

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