Athletic Amenorrhea and Endothelial Dysfunction

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ABSTRACT
Objectives: To determine if menstrual status changed in amenorrheic college runners over a 2-year period and what effect this had on brachial artery flow-mediated dilation.

Participants: Eighteen athletes first studied in our laboratory 2 years prior were available for follow-up. Nine of the 10 original women with athletic amenorrhea (mean ± SE, age 21.3 ± 1.2 yrs), and 9 of the 11 eumenorrheics/controls (age 20.1 ± 0.5 yrs) were studied 2 years after baseline measurements.

Methods: Questionnaires/personal interviews and blood draws were performed to determine menstrual status. A non-invasive ultrasound technique was used to determine brachial artery flow-mediated dilation (endothelium-dependent).

Results: Menstrual status changed in 7 of 9 original amenorrheic subjects (2 were taking hormone replacement, 2 were taking oral contraceptives, 3 had a natural menstrual period prior to testing, and 2 remained amenorrheic). Endothelium-dependent brachial artery dilation, measured as the percent change in maximal brachial artery diameter from baseline during reactive hyperemia, was improved in the original amenorrheic subjects (a 1.1% ± 1.0 increase in the original study versus 5.6% ± 1.1 increase in the current study, P=0.01) while in the eumenorrheic/control group there was no change (6.3% ± 1.7 versus 8.0% ± 1.3, P=0.42).

Conclusions: Menstrual status changed in 7 of the 9 original amenorrheic athletes, and this change was associated with an improvement in brachial artery flow-mediated dilation.

INTRODUCTION
Over the past 35 years, there has been an explosion in women’s sports participation. In 2006, there were nearly 3 million girls playing high school sports, a 10-fold increase since 1972. There are countless well-known health and social benefits for women who participate in sports and/or regular aerobic activity. However, we have also discovered unique injury patterns and medical conditions in female athletes. All women athletes are at risk for exercise-associated amenorrhea if they are in a calorie deficit (inadequate caloric consumption in relationship to the amount they are exercising).1 Amenorrhea puts young athletes at high risk for decreased peak bone mineral density (BMD),2 stress fractures,3 brachial artery endothelial dysfunction,4 and potentially osteopenia/osteoporosis in the postmenopausal years. When athletic amenorrhea occurs in conjunction with disordered eating and decreased peak BMD, the interrelated condition is called the “female athlete triad” (Triad).5 All physically active girls should be screened for all 3 components of the Triad, since significant morbidity and mortality are associated with this condition. Comprehensive care is required to treat the Triad and more gender specific programs are being developed in the United States to address the unique issues that female athletes face.

Although the exact etiology of athletic amenorrhea is not fully understood, it is known to have a steroid hormonal profile similar to menopause, characterized by low estrogen levels, which are etiologic in the development of osteoporosis in postmenopausal women. However, the greatest medical consequence of menopause is cardiovascular disease.6

Cardiovascular disease is the number 1 cause of
death in women. One in 4 women will die secondary to a cardiac event in 2007. Cardiovascular risk increases significantly after menopause when estrogen levels drop. One of the earliest clinical signs of cardiovascular disease is reduced endothelial-dependent vasodilation, which is seen as early as 3 months after menopause. We recently have shown that college women runners with athletic-associated amenorrhea have reduced endothelial-dependent dilation of the brachial artery compared with eumenorrheic/control runners. The consequences of abnormal brachial flow-mediated dilation are well-known and profound. In patients with cardiac risk factors, an abnormal flow-mediated dilation is 95% predictive of abnormal coronary endothelial function, which is associated with future cardiovascular events.

The purpose of this study was to determine if menstrual status changed in amenorrheic college runners over a 2-year period and what effect this had on brachial artery flow-mediated dilation.

METHODS

Twenty-one non-smoking female athletes who participated in a study in this laboratory 2 years prior (original study) were contacted via telephone and e-mail for this study (2-year follow-up). The athletes were categorized as having primary amenorrhea, secondary amenorrhea, or eumenorrhea. Primary amenorrhea is defined as the absence of menses by age 16; secondary amenorrhea is defined as the absence of 3 consecutive menstrual periods after menarche. Eumenorrhea is defined as menstrual cycles every 28–30 days for at least the last 12 months. Two original eumenorrheic/control women had moved out of state and could not participate in the 2-year follow-up study. One original amenorrheic subject was pregnant and was therefore excluded from the 2-year follow-up study. Nine original amenorrheic (2.3 ± 0.5 yrs) women athletes (age 21.3 ± 1.2 yrs) and 9 original eumenorrheics/controls (age 20.1 ± 0.5 yrs) volunteered for this investigation and signed an informed consent in accordance with our Institutional Review Board policy. At the end of the original study, all amenorrheic subjects underwent a 1-time educational intervention. They were instructed to increase their calories by 300 kcal/day and reduce their training duration by 10% per week. If they did not achieve natural menses in 9-12 months, they were instructed to follow-up with their physician to discuss hormone replacement or oral contraceptives. The subjects in this study all had a “team” or primary care physician, and treatment was on an individual basis without involvement from the investigators in this study.

At 2-year follow-up, each subject reported to the General Clinical Research Center on one occasion for approximately 3 hours. Each subject underwent a structured interview and filled out questionnaires regarding menstrual status, exercise history, and medical conditions over the last 2 years. A urine sample was collected to test for pregnancy prior to entry into the study. Fasting whole venous blood samples were obtained the morning of testing, including estradiol, progesterone, follicle stimulating hormone, thyroid stimulating hormone, and prolactin.

Height (cm) and weight (kg) were measured on the same scale (Scaletronix, Model #5005, Wheaton, IL) by the same technologist. Whole-body absorptiometry (GE Lunar Prodigy densitometer, version 2.15, Madison, WI) was used to determine total body fat percentage. Brachial artery studies were performed after an overnight fast (8 hours). Ambient temperature was recorded for each subject since a higher temperature can increase flow-mediated dilation. All subjects were tested at approximately the same time of day by the same cardiologist. With the subject supine, the dominant arm, used for ultrasound testing, was placed in a comfortable position with the arm abducted approximately 80 degrees from the body and the forearm pronated. A high frequency (12.0mHz) ultrasound (GE Pro Logiq 500) probe was positioned to visualize the brachial artery in the longitudinal axis approximately 1-3cm proximal to the antecubital fossa, in similar fashion to that described by other authors. The ultrasound probe was used to measure both diameter and central flow velocity (pulsed Doppler).

Endothelium-dependent vasodilation was measured as the peak percent change in brachial artery diameter from baseline and during reactive hyperemia, measured 30 seconds and 1 and 2 minutes after release of a 4.5 minute forearm occlusion (cuff inflation to 40 mmHg above systolic pressure). The hyperemic flow response was measured as the percent change from baseline brachial artery flow to maximal reactive hyperemic flow following forearm cuff release. Ten minutes after baseline was reestablished, 0.4mg of sublingual nitroglycerin was administered, the brachial artery was imaged, and the maximal diameter was averaged each minute for an additional 5 minutes. Nitroglycerin-induced brachial artery dilation is considered an endothelium-independent process. Our technique of measuring flow-mediated dilation is patterned after that of Celermajer, who found the technique to have a reproducibility coefficient of variation of 1.4% and a repeatability coefficient of variation of 2.3%.
A paired comparison of brachial artery measurements and serum chemistries were made between the original and 2-year follow-up amenorrheic and eumenorrheic runners. For the brachial artery studies, the average of each individual’s peak percent flow-mediated dilation was compared between the 2 groups. Values are expressed as the mean $\pm$ SE. A $P$ value of <0.05 was set as the level of statistical significance.

**RESULTS**

Menstrual status, as established in the initial study, changed significantly among the original amenorrheic group. This was determined by questionnaires, an interview, and hormonal blood tests at the 2-year follow-up. Two subjects were using hormone replacement (ethinyl estradiol 5ug/norethindrone acetate 1mg and estradiol 1mg/norethindrone 0.5mg), 2 were using oral contraceptives (ethinyl estradiol 35ug/norgestimate 0.18mg, 0.21mg, 0.25mg), 3 were menstruating naturally every 28-30 days for the last 12 months prior to testing, and 2 remained amenorrheic (no menstrual periods) over the 24-month period. Therefore, menstrual status changed in 7 of the 9 returning original amenorrheic subjects. For the 2 subjects who remained athletic amenorrheic, they were told by their primary care physician that “it was normal” not to have a menstrual period while running competitively. Menstrual status of the eumenorrheic/control group revealed that 4 of the 9 athletes remained eumenorrheic with normal menstrual cycles every 28 days. Five of the 9 subjects started oral contraceptives for personal reasons.

The physical characteristics and exercise histories of the original amenorrheic athletes compared to the 2-year follow-up and the original eumenorrheic athletes compared to the 2-year follow-up are shown in Table 1. Differences between the amenorrheic athletes at 2-year follow-up include an increase in weight ($P=0.04$), height ($P=0.04$), body fat ($P=0.04$), and body mass index ($P=0.04$).
crease in flow-mediated dilation. Follow-up Eumenorrheic Athletes

Follicle stimulating hormone, thyroid stimulating hor-

cics/controls in the original or 2-year follow-up study

diameter, flow-mediated dilation of the brachial artery

to compare the original (white bars). There was no change in

Figure 1. Percent change in brachial artery diameter in

response to reactive hyperemia for the amenorrheic and

eumenorrheic groups. Brachial artery dilation improved signifi-
cantly in the follow-up amenorrheic subjects (grey bars) com-

pared with the original (white bars). There was no change in

brachial artery dilation in the follow-up eumenorrheic subjects

grey bars) compared with the original (white bars). *P=0.01

comparing original and follow-up amenorrheic athletes

index (P=0.05), and a decrease in training days (P=0.01)

and miles run per week (P=0.05). There was a trend to-

ward improvement in best 5-kilometer times (P=0.08)

(Table 1). There were no differences among the eumen-

orrheic/control athletes in height, weight, percentage

body fat, body mass index, and best 5-kilometer times

at the 2-year follow-up (Table 1). There was a signifi-
cant decrease in training days (P=0.004) and miles run

per week (P=0.02) in the eumenorrheic runners at 2-

year follow-up (Table 1).

The brachial artery vasodilator response to reactive

hyperemia improved significantly (P=0.01) in the ame-
norrheic runners at 2-year follow-up (5.6 ± 1.1%) com-

pared with the original amenorrheic group (1.1 ± 1.0%)

(Figure 1 and Table 2). The 2 subjects who remained

amenorrheic had the lowest flow-mediated dilation at

2-year follow-up (1.95% and 2.2%). If these subjects

are eliminated, the average increase in flow-mediated

dilation from 1.4% to 4.88% ± 2.2 after treatment.

These findings in athletes with amenorrhea are

encouraging since we know the accompanying reduced

BMD is largely irreversible and does not improve with

time or treatment. Our study found a 5-fold increase in

flow-mediated dilation in the returning original amenor-

rhic runners. Other studies in postmenopausal women

have found a significant reduction in cardiac risk factors

with only a 2-fold increase in flow-mediated dilation. Con-

versely, a higher risk of nonfatal cardiovascular

events in postmenopausal women can be predicted by

a lack of improvement in abnormal flow-mediated dil-

ation in as soon as 6 months. More longitudinal research

needs to be done to determine if endothelial dysfunction

is a novel risk factor for premature cardiovascular risk in

women athletes. Finally, this study and Rickenlund’s

study also suggest there may be a fourth component to

the Triad and a more appropriate title may be the Female

Athlete “Tetrad.”

Our study raises the question as to whether inter-

vention is necessary when impaired endothelial function is

observed in a young female athlete. Based on the im-

plications of altered endothelial function as a common

pathological pathway predisposing to the development

of the subjects were pregnant or had evidence of thy-

droid disease or an elevated prolactin level.

DISCUSSION

This is the first study to retrospectively follow-up with

amenorrheic athletes with reduced flow-mediated di-
lation. Interestingly, 2 athletes remained amenorrheic

without a menstrual period in 24 months, 3 regained

menses naturally by increasing calorie consumption and

decreasing training, and 4 started hormone replacement

or birth control. The 2 athletes who remained amenor-

rhic had the lowest flow-mediated dilation of the group

(1.95% and 2.2%). However, the group as a whole, de-

spite different treatments and 2 remaining amenorrheic,

had an increase in flow-mediated dilation. If the 2 who

remained amenorrheic are eliminated, the increase is

even greater. These results are similar to those found by

Rickenlund who treated 11 amenorrheic athletes with

known endothelial dysfunction with low dose, mono-

phasic, combined oral contraceptives (30µg ethinyl es-

tradiol and 150µg levonorgestrel on days 1-21) for a

period of 9 months and found an increase in flow medi-

ated dilation from 1.4% ± 1.0 to 4.88% ± 2.2 after treat-

ment. These findings in athletes with amenorrhea are

encouraging since we know the accompanying reduced

BMD is largely irreversible and does not improve with

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Athlete “Tetrad.”

Our study raises the question as to whether inter-

vention is necessary when impaired endothelial function is

observed in a young female athlete. Based on the im-

plications of altered endothelial function as a common

pathological pathway predisposing to the development
of coronary artery disease and cardiovascular events, it is prudent to consider altered flow-mediated dilation as a risk factor for cardiovascular mortality similar to tobacco use, hypercholesterolemia, or diabetes. Together with the fact that atherosclerosis begins in childhood, a case can be made to strongly consider treatments that improve endothelial function in amenorrheic women athletes. Hormone replacement, oral contraceptives, or lifestyle changes may be sufficient to reverse loss of menses and improve endothelial function. However, other treatment strategies, with less risk and easier implementation than hormones, could be considered. For example, folic acid in doses over 2.5mg/day has been shown to improve endothelial dysfunction in men with hypercholesterolemia, but this treatment would not address the risk of loss of bone mineral also associated with the amenorrhea. We have recently shown in a pilot study that folic acid increases brachial artery flow-mediated dilation in eumenorrheic runners. In this context, improving flow-mediated dilation may serve to prevent future events or at least delay onset of events in later years. However, this potential preventive benefit needs to be assessed with prospective large-scale clinical trials.

Mean arterial pressure, temperature and nitroglycerin-induced dilation increased in the eumenorrheic/control group at 2-year follow-up. Interestingly, baseline brachial diameter did not change over the same time. The increased vasodilatory response to nitroglycerin could represent vascular remodeling with changes in vessel wall distensibility and/or elasticity as a result of the changes in mean arterial pressure. Such remodeling could alter maximal vasodilator capacity without changing the ability of the vessel to respond to a less potent endothelium-dependent dilator stimulus. Alternatively, the slight increase in arterial pressure may have dampened the endothelium-dependent dilator response, but this could have been compensated by the increase in vascular responsiveness to nitroglycerin so that net dilation to flow was unchanged. Further studies will be needed to determine the mechanism of this change.

The general belief in the athletic community by coaches and athletes is that lower body weight and body fat result in improved running times. In fact, it is this perception that typically triggers women to develop restrictive eating patterns and eventually athletic-associated amenorrhea. Another important finding in this study is that the original amenorrheic runners decreased their training days per week (P=0.01), gained weight (P=0.08), and body fat (P=0.04) with a strong trend toward an actual decrease in best 5 kilometer times (P=0.08), therefore substantiating the concept that athletes can gain weight, resume normal menstrual periods, and at the same time improve their athletic performance. This contradicts the myth that thinner equals improved performance.

A limitation of this study is the retrospective design with no control of the treatment of athletic-associated amenorrhea in our subjects. However, this design does allow for insight into how athletic women approach treatment for this difficult problem without the benefit of a structured comprehensive program and continued close follow-up.

CONCLUSION

In summary, menstrual status changed in 7 of the 9 original amenorrheic athletes and brachial artery flow-mediated dilation improved significantly. It is unclear if improvement of flow-mediated dilation represents long-term cardiovascular prevention until large scale longitudinal studies are performed. These findings are potentially significant for long-term cardiovascular prevention, and more immediately, athletic performance. This study also further exemplifies the unique issues that affect female athletes and the need for heightened awareness among physicians to screen and treat for athletic-associated amenorrhea.

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