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Effect of a Single High-Fat Meal and Vitamins on the Circulatory Response to Local Heat in Koreans and Caucasians

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LOMA LINDA UNIVERSITY
School of Allied Health Professions
in conjunction with the
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Effect of a Single High-Fat Meal and Vitamins on the Circulatory Response to Local Heat in Koreans and Caucasians

by

JongEun Yim

A Dissertation submitted in partial satisfaction of
the requirements for the degree
Doctor of Science in Physical Therapy

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Each person whose signature appears below certifies that this dissertation in his/her opinion is adequate, in scope and quality, as a dissertation for the degree Doctor of Science.

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ABBREVIATIONS

EF	Endothelial Function
HTG	Hypertriglyceridemia
NO	Nitric Oxide
BFR	Blood Flow Response
HF	High Fat
LF	Low Fat
MDA	Malondialdehyde
APTA	American Physical Therapy Association
SP	Substance P
CGRP	Calcitonin Gene Related Peptide
TRPV4	Transient Receptor Potential Vanilloid 4
cGMP	Cyclic Guanosine Monophosphate
Ca	Calcium
DNA	Deoxyribo Nucleic Acid
DM	Diabetes Mellitus
CVD	Cardiovascular Disease
ANOVA	Analysis of Variance

ABSTRACT OF THE DISSERTATION

Effect of a Single High-Fat meal and Vitamins on the Circulatory Response to Local Heat in Koreans and Caucasians

by

JongEun Yim

Doctor of Science, Graduate Program in Physical Therapy
Loma Linda University, June 2012
Dr. Jerrold Petrofsky, Chairperson

Blood flow mediated by the vascular endothelium plays an important role in removing heat from the skin. Asians who have thrifty genes have impaired endothelial function (EF) due to the westernized high fat (HF) dietary lifestyle. Hypertriglyceridemia (HTG) can produce free radicals which reduces the bioavailability of the endothelium derived relaxing factor, nitric oxide (NO). EF, as measured by the blood flow response (BFR) to heat, occlusion and the free radical concentration after a single HF meal has not been studied in Korean-Asians compared to Caucasians. The purpose of this study was to evaluate baseline and postprandial EF as related to oxidative stress from a single HF and low fat (LF) meal and the effects of vitamins on scavenging free radicals and improving EF in Korean-Asians compared to Caucasians. This was done by assessing skin BFR to vascular occlusion and local heat and analyzing malondialdehyde (MDA) levels after ingestion of a HF and LF meal before and after vitamin intake.

Ten Caucasians and ten Koreans participated in the study (mean age 25.3 ± 3.6 years). BFR to vascular occlusion and local heat and oxidative stress was assessed after a single HF and LF meal at 2 hours compared to baseline. After administration of vitamins

(1000mg vitamin C, 800IU vitamin E, and 300mg Coenzyme Q-10) for 14 days, the same measurements were repeated.

The results of this investigation showed that the skin BFR to vascular occlusion and local heat at baseline was significantly lower in Koreans than Caucasians. The skin BFR to vascular occlusion and local heat following a HF meal significantly decreased and free radicals significantly increased at 2 hours compared to baseline in Koreans ($p<.001$), but not in Caucasians. Also, the skin BFR to vascular occlusion ($p=0.001$) and local heat ($p=0.003$) was significantly lower and free radicals ($p=0.004$) were significantly higher at 2 hours after the HF meal in Koreans than Caucasians. When vitamins were given, the BFR to vascular occlusion and local heat before and after HF meal was not significantly different in both Koreans and Caucasians.

These findings suggest that Koreans may have lower EF than Caucasians which may be explained, in part, by genetic variation. Also, even a single HF meal can reduce EF through an oxidative stress mechanism but can be blocked by antioxidant vitamins in Koreans.

In conclusion, Korean-Asians are probably more susceptible to skin damage during heat application due to lower endothelial function compared to Caucasians if Koreans eat high fat foods. However, reduced endothelial function may be prevented by taking antioxidant vitamins.

CHAPTER ONE

INTRODUCTION

Heat has been used as a therapeutic modality for thousands of year and it is one of the most common therapeutic modalities used in physical therapy after the initial acute phase of orthopedic injuries. [1,2] The use of heat is suggested as a potential intervention for the treatment of musculoskeletal conditions in the American Physical Therapy Association, guide to physical therapist practice (APTA 2001). Heat is used for its hemodynamic, neuromuscular, metabolic, and connective tissue effects. [3] It is also known for its pain reducing and relaxation properties. [4] Typically, local heating is used to increase circulation thereby promoting healing in the target tissue through several physiologic effects on tissue, including increasing delivery of the nutrients and oxygen necessary for the healing of tissues, stimulating the breakdown and removal of tissue debris and inflammatory metabolites, facilitating soft tissue repair through an accelerated metabolic rate, and accelerating inflammation by increasing delivery of leukocytes. [1,4,5] While heat has positive effects on tissues, there is also a chance of burning the skin. [6] Therefore, the ability of the skin to remove heat is very important for protecting the skin from damage. [7]

When heat is applied to the skin, the heat is removed by two different ways. First, the skin itself removes heat into the underlying tissue through conductive heat loss. [8,9] The second and better means of removing heat is through skin circulation. [10-12] For a rapid heat exposure (one second or less), the skin circulation does not have time to

respond, and therefore conductive heat loss is most critical in keeping the skin from being damaged. [13-15] However, as heating of intact skin progresses, the influence of the increased blood flow becomes more pronounced, finally causing a decrease in skin temperature in spite of continued irradiation. This phenomenon does not occur when blood flow is occluded as skin temperature continues to increase. Therefore, the values of conductive heat loss for living skin are not constant and will depend upon the skin blood flow. [7,16] For this reason, in sustained heat exposure to a continuous heat source, the skin blood flow does play an important role by continually removing heat. [7,17] Importantly, the rapid rise in the skin blood flow in response to local heating plays a pivotal role in protecting the skin from burns. For example, less heat will be transferred to the tissues when there is an increase in blood flow, thereby protecting the skin from damage. Local tissue damage will result if there is a diminished ability to rapidly increase skin blood flow in response to directly applied heat. [18]

However, the increase in skin blood flow in response to sustained local heat is biphasic. [7,19-21] In the first few minutes, tactile sensors in the skin cause a progressive vasodilatation mediated by substance P (SP) and calcitonin gene related peptide (CGRP) released from sensory nerves. [22-26] As this initial response subsides, there is a more prolonged increase in skin blood flow mediated by nitric oxide released from vascular endothelial cells. Endothelial nitric oxide synthetase is activated by transient receptor potential vanilloid 4 (TRPV4) voltage gated, temperature sensitive calcium channels. These channels increase calcium influx exponentially into the endothelial cells from the blood when the temperature reaches above 35°C. Calcium, in turn, activates the enzyme endothelial nitric oxide synthetase mediating the production of nitric oxide from amino

acid L-arginine. [27] Nitric oxide (NO), a potent vasodilator, diffuses into the surrounding smooth muscle activating cyclic guanosine monophosphate (cGMP). cGMP increases potassium permeability, reduces Ca^{++} permeability and blocks intracellular actomyosin ATPase causing vascular smooth muscle to relax. [15,28-33] Results from a series of studies clearly demonstrate that NO is importantly involved in the BFR to local heating of the skin. [34]

The sustained BFR to heat is mediated by vascular endothelial cells of the blood vessels. In different disease states, there is endothelial dysfunction and reduced BFR to heat. Endothelial dysfunction is caused by reduced NO bioavailability due to either reduced formation or accelerated degradation of NO. Degradation of NO by reacting with free radicals is a major mechanism of endothelial dysfunction. [35]

Free radicals are not always physiologically detrimental. They are commonly produced and neutralized in the body via the activity of radical generating enzymes and inflammatory cells, as well as through cellular metabolism involving the processing of macronutrients. [36] Beneficial effects of free radicals occur at low/moderate concentrations and involve physiological roles in promoting cellular signaling, aiding in phagocytic immune defense, as well as promoting apoptosis. [37] The harmful effect of free radicals causing potential biological damage is termed oxidative stress. [38] This occurs in biological systems when there is an overproduction of free radicals on one side and on the other side, a deficiency of enzymatic and non-enzymatic antioxidants. The excess free radicals can damage cellular lipids, proteins, or deoxyribo nucleic acid (DNA) inhibiting their normal function. [37] For example, high free radical concentration in the body increases cellular oxidation and can biodegrade NO and prostacyclin into inactive forms.

[39] Especially, these radicals bioconvert NO into peroxynitrite, thereby reducing the bioavailability of NO as a vasodilator and this can further damage the tissue by lipid peroxidation of membranes. [37] This is believed to be one of the mechanisms associated with reduced circulation in older people and diabetics. [39]

Evidence that dietary factors can affect the control of vascular tone is emerging in the literature, with dietary fat emerging as a potentially important modulator. [40] Many recent studies demonstrate that hypertriglyceridemia (HTG) is one of the risk factors in cardiovascular disease and can cause endothelial dysfunction, which is considered as an early process of atherosclerosis. [3,41,42] The mechanisms of postprandial HTG-induced endothelial dysfunction have been suggested that HTG significantly stimulates leukocytes to produce free radicals. Because free radicals reduce endothelium- derived NO, cause cell membrane injury, and induce low-density lipoprotein oxidation, which has cytotoxic effects on the vascular endothelial cells and can induce endothelial dysfunction. [35,43]

From the adoption of a more westernized lifestyle, higher dietary fat consumption is common in Asia. In addition, Asians are more susceptible to a HF food due to the influence of thrifty genes. The gene developed in populations where food supply was limited due to famine. It allows fat to be stored easily when food is available. This genotype, which is composed of many single nucleotide polymorphisms (SNPs), is a genetic difference regulating lipid metabolism and fat storage, and differs different depending on ethnicity. [22,24,44,45] For example, one of the thrifty SNPs related to lipid metabolism, fatty acid-binding protein 2 (FABP2), has been associated with obesity because it enhances fat absorption. The allelic frequency of FABP2 is 55% in Asians and

27.1% in Caucasians. Thus, if Asians consume the same amount of fat, a higher body fat deposit at a lower or the same BMI will be observed in Asians. [24,44] Also, evidence has been found that Asians having the thrifty genotype are less able to cope with changing dietary patterns of HF food resulting in obesity, diabetes mellitus (DM) and cardiovascular disease (CVD) because they have the ability to store fat better than others. [25] A recent study [46] showed that ingesting even a single HF meal impairs the endothelial function (EF) and hence, tissue blood flow in Asians, but not in Caucasians.

A standard method of assessing EF is post ischemic reactive hyperemia. [47,48] The reactive hyperemia to anoxia is the sudden rise in blood flow that can be measured by a Laser Doppler Imager or ultrasound after a 4 minute occlusion of arterial blood flow. [49] With clinical relevance, lower BFR to vascular occlusion have also been found in ageing, DM, smoking, and CVD. [49,50] Another method of assessing EF is the skin BFR to local heat mediated by the endothelial cells. [18,51] It is well established that when heat is applied to the skin, there is an increase in skin blood flow. [52,53] Both of these stressors can test EF but in different ways. Further, heat exposure is a practical issue in our daily lives and has tremendous clinical relevance.

There is some evidence that EF can be protected by vitamins. Numerous studies have demonstrated that antioxidant vitamins play an important role in increasing EF and decreasing oxidative stress. [39,54,55] Especially, antioxidant vitamins C and E improve vascular defense against oxidative stress by reducing free radicals and protecting NO from inactivation, thereby exerting beneficial effects on vascular function and structure. [39,55] Several studies showed that antioxidant vitamins improved EF in people with DM, coronary artery disease, and hypertension. [56,57] In a study of chronic smokers, a low

response to endothelium-dependent flow-mediated dilation was reversed with 25days administration of vitamin C (1000 mg) and E (500 IU) [58]. However, in other studies, antioxidant vitamins did not improve normal endothelial response to stressors in healthy people. [56,59]

Many studies showed that combined administration of vitamins C and E significantly increases endothelium-dependent vasodilation, while monotherapy with vitamin C alone is ineffective. [54,55,58] It is known that vitamins C and E have synergistic antioxidant actions, since vitamin E can have pro-oxidant properties and appropriate concentrations of vitamin C are necessary for the regeneration of vitamin E, thus increasing its antioxidant capacity. [55]

The purpose of this investigation was first, to identify whether there were any differences in EF between Koreans and Caucasians. In this case, EF was assessed by BFR to vascular occlusion and local heat. The second purpose was to look at evaluating postprandial EF by measuring the BFR to vascular occlusion and local heat before and after a HF, meal and the interventional effects of anti-oxidant vitamins on improving EF in young Korean-Asians compared to Caucasians.

CHAPTER TWO

DIFFERENCES IN ENDOTHELIAL FUNCTION BETWEEN KOREAN-ASIANS AND CAUCASIANS

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Summary

Background: The vascular endothelium plays an integral role in maintaining vascular homeostasis including the regulation of blood flow, vascular tone, and platelet aggregation. The aim of this study was to see if there were any differences in endothelial function between Koreans and Caucasians.

Materials/Methods: This was accomplished by 2 measures of endothelial function; the response to local heat and the response to vascular occlusion. Ten Caucasian and ten Korean male and female subjects participated (<35 years old). Endothelial function was assessed by the skin blood flow response to local heat using a thermode for 6 minutes at 3 temperatures (38°C, 40°C and 42°C) and by vascular occlusion for 4 minutes followed by release and measurement of skin blood flow for 2 minutes.

Results: When applying 6 minutes of local heat at 3 different temperatures 38°C, 40°C, and 42°C, the skin blood flows were significantly higher for all temperatures in Caucasians than Koreans with peak blood flow of 223 ± 48.1, 413.7 ± 132.1, and 517.4 ± 135.8 flux in Caucasians and 126.4 ± 41.3, 251 ± 77.9, and 398 ± 97.2 flux in Koreans respectively (p=0.001). Also, data in this study supported the idea that the skin blood flow response to occlusion was significantly higher in Caucasians (peak 411.9 ± 88.9 flux) than Koreans (peak 332.4 ± 75.8 flux) (p=0.016).

Conclusion: These findings suggest that Koreans may have lower endothelial function than Caucasians which may be explained, in part, by genetic variations between the two ethnic groups.

Key words: endothelial function, local heat, blood flow, ethnicity

Background

The vascular endothelium is a single layer of cells covering the internal surface of blood vessels in the body. It plays an important role in vascular growth, vasoregulation and vasoprotection [1]. Over the past three decades, endothelial function has emerged as a key topic because endothelial dysfunction is a leading cause of cardiovascular and age-related diseases and appears as an independent predictor of these diseases [2]. Therefore, the evaluation of endothelial function is very important and meaningful in the clinical setting. The most common clinical method for assessing endothelial function is post ischemic reactive hyperemia [3-6]. This is a noninvasive method for assessing and monitoring endothelial function in various populations and disease states, suitable for use in clinical practice [3, 5]. Another method of assessing endothelial function involves assessing the skin blood flow response to a heat source [7-9]. The two methods differ in that they test different metabolic pathways on endothelial cells. Studies looking at endothelial function, however, have been largely conducted in Caucasians and not much is known about endothelial function in Koreans or other Asian populations.

The vascular endothelium plays an integral role in maintaining vascular homeostasis including the regulation of blood flow, vascular tone, and platelet aggregation [10]. A balance between endothelium derived relaxing and contracting factors maintains vascular homeostasis [11]. Endothelium-derived relaxing factors are mainly nitric oxide (NO) and prostacyclin (PGI_2). Nitric oxide is produced by the enzymatic conversion of L-arginine to L-citrulline by nitric oxide synthase and possesses vasodilating, antithrombotic, and anti-proliferative properties. Prostacyclin (PGI_2), with similar biological effects as nitric oxide, is synthesized from arachidonic acid [3, 10].

Endothelial dysfunction, which is the disruption of a balance between vasodilation and vasoconstriction is common with ageing and diabetes. While there is little impairment in the ability to vasoconstrict, diminished nitric oxide bioavailability and reduced prostacyclin cause a shift in vascular homeostasis from vasodilation toward vasoconstriction [11]. Degradation of nitric oxide and prostacyclin by reacting with free radicals is a major cause of endothelial dysfunction [12].

The most common test of endothelial function is assessment of post ischemic reactive hyperemia [1, 6]. The reactive hyperemia to anoxia is the sudden rise in blood flow that can be measured by a Laser Doppler Imager or ultrasound after a 4 minute occlusion of arterial blood flow [13]. The reactive hyperemia is a result in part of myogenic and/or metabolic factors including adenosine, nitric oxide, and prostaglandins. Among them, vasodilator prostaglandins are an essential mediator of reactive hyperemia in the human skin [3, 4, 6]. In contrast, nitric oxide does not play a major role in causing vasodilation during peak reactive hyperemia in the human skin [3, 6]. However, although nitric oxide does not directly mediate reactive hyperemia in the skin, the possibility exists that NO could act in conjunction with one or more vasodilators to mediate the reactive hyperemia [3, 6]. Several investigators found that inhibition of prostaglandin synthesis significantly reduced the peak hyperemic response but had no effect on the total hyperemic response [3, 6]. However, when prostaglandin synthesis inhibition and nitric oxide synthesis inhibition were administered simultaneously, the total hyperemic response was significantly reduced but not eliminated [3, 6]. This suggests that prostacyclin and nitric oxide act synergistically to cause vasodilation during reactive hyperemia but other mediators are involved [3, 6]. With clinical relevance, lower blood

flow responses to vascular occlusion have also been found in ageing, diabetes, smoking, and cardiovascular diseases [13, 14].

Assessment of the skin blood flow response to local heat provides a convenient method of evaluating endothelial function [8, 9]. Local heat application to the skin results in a large increase in skin blood flow that is biphasic and characterized by a rapid initial peak followed by a more prolonged plateau [14-17]. The initial peak of blood flow response to local heat is primarily mediated by an axon reflex mechanism through the release of substance P and calcitonin gene related peptide. The secondary prolonged plateau of thermal hyperemic response is mediated primarily by nitric oxide synthase-mediated generation of nitric oxide [8, 14, 16-18]. However, due to impairment of endothelial function by free radicals associated with aging and diabetes to the nitric oxide pathway and also to the prostacyclin pathway, the blood flow response to local heat is reduced as an individual ages and in diabetics [14, 19].

Both of these stressors test endothelial function but in different ways. Further, heat exposure is a practical issue in our daily lives and has tremendous clinical relevance. However, although the blood flow response to vascular occlusion and local heat has been well documented in Caucasians, little has been done in Koreans or other Asians. According to a previous study, the blood flow response to occlusion is blunted in Asians compared to Caucasians possibly due to special genes, thrifty genes, which can affect endothelial function [20]. These genes are seen in Koreans. Therefore, the purpose of this study was to compare differences in endothelial function by assessing the blood flow response to local heat and vascular occlusion in Koreans compared to Caucasians.

Materials and Methods

Subjects

Healthy subjects were recruited and assigned to two groups based on self-reported ethnicity. Subjects did not have diagnosed cardiovascular disease, hypertension (>140/90mmHg) or diabetes, were non-smokers, were not taking any medications that would affect the cardiovascular system, and did not have any known peripheral circulatory diseases. The subjects included 10 Caucasians and 10 Koreans. The general characteristics of the subjects are shown in Table 1. There was no significant difference in age, height, weight, body mass index (BMI), skin thickness, subcutaneous fat thickness, and skin moisture between the two ethnic groups. All protocols and procedures were approved by the Institutional Review Board of Loma Linda University and all subjects signed a statement of informed consent.

Table 1. Mean (SD) of general characteristics of the 5 men and 5 women in each ethnic group.

	Caucasians	**Korean**	**p-value**
Age (years)	27.8 (2.4)	25.4 (4.2)	0.14
Height (cm)	176.1 (11.3)	168.8 (9.4)	0.14
Weight (kg)	73.6 (8.3)	70.5 (16.7)	0.60
BMI (kg/m^2)	23.8 (1.8)	24.5 (3.9)	0.60
Skin thickness (cm)	0.05 (0.01)	0.05 (0.01)	0.51
Fat thickness (cm)	0.11 (0.01)	0.10 (0.01)	0.15
Skin moisture (mg/cm^2)	40.8 (12.2)	41.4 (6.3)	0.89

Methods

Measurement of Skin Blood Flow

Skin blood flow was measured with a MOOR Laser Doppler Imager (Moor LTD, Oxford, England). The Imager used a red Laser beam to measure blood flow in the skin. The Laser was used in a single spot mode which was left on the skin and the reflected energy was used to measure the skin blood flow. Blood flow as measured by a Laser Doppler Imager was expressed in a unit called flux. The laser was warmed for 30 minutes prior to measurements. The stated reliability from the manufacturer is ± 5% from day to day. Possible potential sources of this reliability are aging and internal temperature of this machine. The Laser was calibrated before and in the middle and end of the study; there were no calibration changes noted.

Measurement of Skin Temperature

Skin temperature was measured with a thermistor manufactured by BioPac Systems (BioPac Inc., Goleta, CA). The thermistor output was amplified with an SKT 100 Thermistor amplifier (BioPac systems, Goleta, CA) and the output was then digitized at 1000 samples per second on a BioPac MP150 data collection system at 24 bits of resolution (BioPac Systems, Goleta, CA).

Control of Skin Temperature

Skin temperature was controlled with a thermode. The thermode consisted of a plastic box with a port on each end so that water could move through the box. The box was approximately 5 cm X 2.5 cm X 2.5 cm in size. On each end of the box, there was a

thermocouple such that as water circulated through the box from a controlled temperature water bath (Biopac systems Goleta California). The temperature difference across the box was measured. Water bath temperatures were kept at 38°C, 40°C, or 42°C. A final hole through the box, which was water insulated, allowed the Laser to scan through the center of the box onto the skin. This then provided blood flow data while the skin temperature was clamped at a set temperature. Further detail on the technique and the reliability and validity are published elsewhere [21, 22].

Measurement of Subcutaneous Fat and Skin Thickness

Subcutaneous fat and skin thickness were measured with a Mindray M7 (Mindray, Shanghai, China) using a linear L34256-element probe at a frequency of 10 MHz. A 0.5-cm standoff was used under the probe, and the probe was held vertical (90° to the skin) to avoid false echoes in the measurement of skin and subcutaneous fat thickness.

Procedures

Subjects were interviewed for inclusion and exclusion criteria. Those subjects that were eligible were placed into the study and read and signed the informed consent. Next, subjects rested for 15 minutes while skin and fat thickness, height, and weight were taken. Baseline skin blood flow was recorded for 1 minute. After this period of time, the thermode was applied upon the arm above the brachioradialis muscle to warm the skin to 38°C, 40°C, or 42°C on 3 separate days, respectively. The thermode was left on for 6 minutes. On another day, occlusion was applied by a blood pressure occlusion cuff

inflated to 200mmHg for 4 minutes followed by 2 minutes of additional blood flow recording. Skin temperature at this site was measured throughout the experimental period. Each experiment took approximately 10 minutes and was performed on 4 separate days.

Statistical Analysis

Baseline characteristics of Caucasians and Koreans were compared using an independent t-test (Table 1). Means and standard deviations of skin blood flow were calculated before and after 4 minutes of vascular occlusion and 6 minutes of thermode at different temperatures. A mixed factorial ANOVA was conducted to compare the effect of different temperatures and vascular occlusion on blood flow between Caucasians and Koreans over time. The level of significance was set at $p=0.05$.

Results

Blood Flow Response to Vascular Occlusion

The results of skin blood flow during the 4 minutes of vascular occlusion and then the first 2 minutes following occlusion are shown in Figure 1. There was a significant difference in the total blood flow response between minutes 4 and 6 between Caucasians and Koreans ($p=0.016$). The mean blood flow rapidly increased to a peak of 411.9 ± 88.9 flux in Caucasians compared to 332.4 ± 75.8 flux in Koreans at 20 seconds after releasing 4 minutes of vascular occlusion. The blood flow then decreased to a final value of 122.3 ± 42.4 flux in Caucasians compared to 84.8 ± 15.5 flux in Koreans at the end of the 2 minute period.

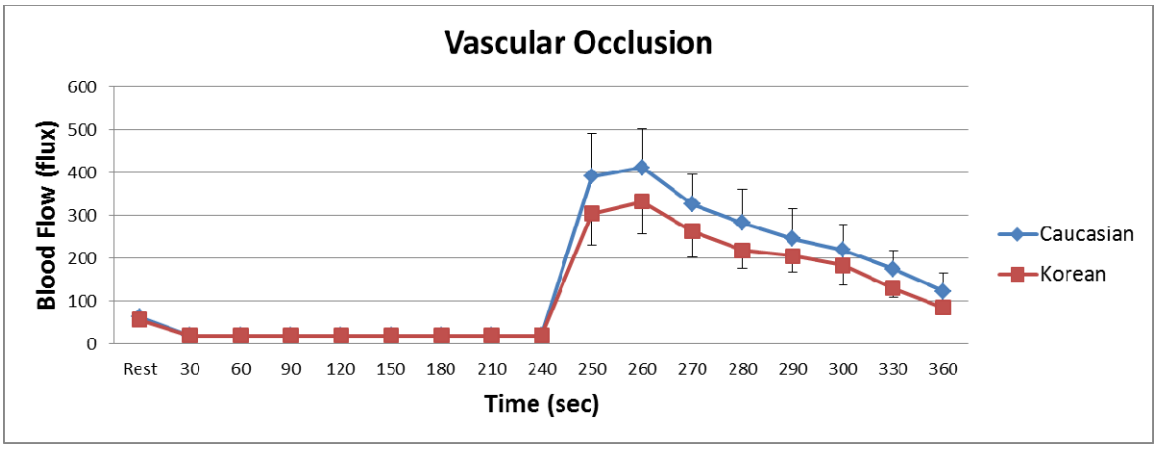

Figure 1. Mean ± SD of blood flow (flux) measured during the 4 minute period of occlusion and the 2 minute period following the release of the occlusion cuff in 10 Caucasians and 10 Koreans.

Blood Flow Response to Local Heat

When applying local heat on the forearm, skin temperature and skin blood flow were measured. When heat was applied on the forearm skin temperature significantly increased continually to a peak skin temperature at the 3 different thermode temperatures (38°C, 40°C, and 42°C) in Caucasians and Koreans ($p<0.001$) (Figure 2). However, skin temperature at rest and throughout the heat exposure (6 minutes) was not significantly different at all 3 different thermode temperatures between Caucasians and Koreans.

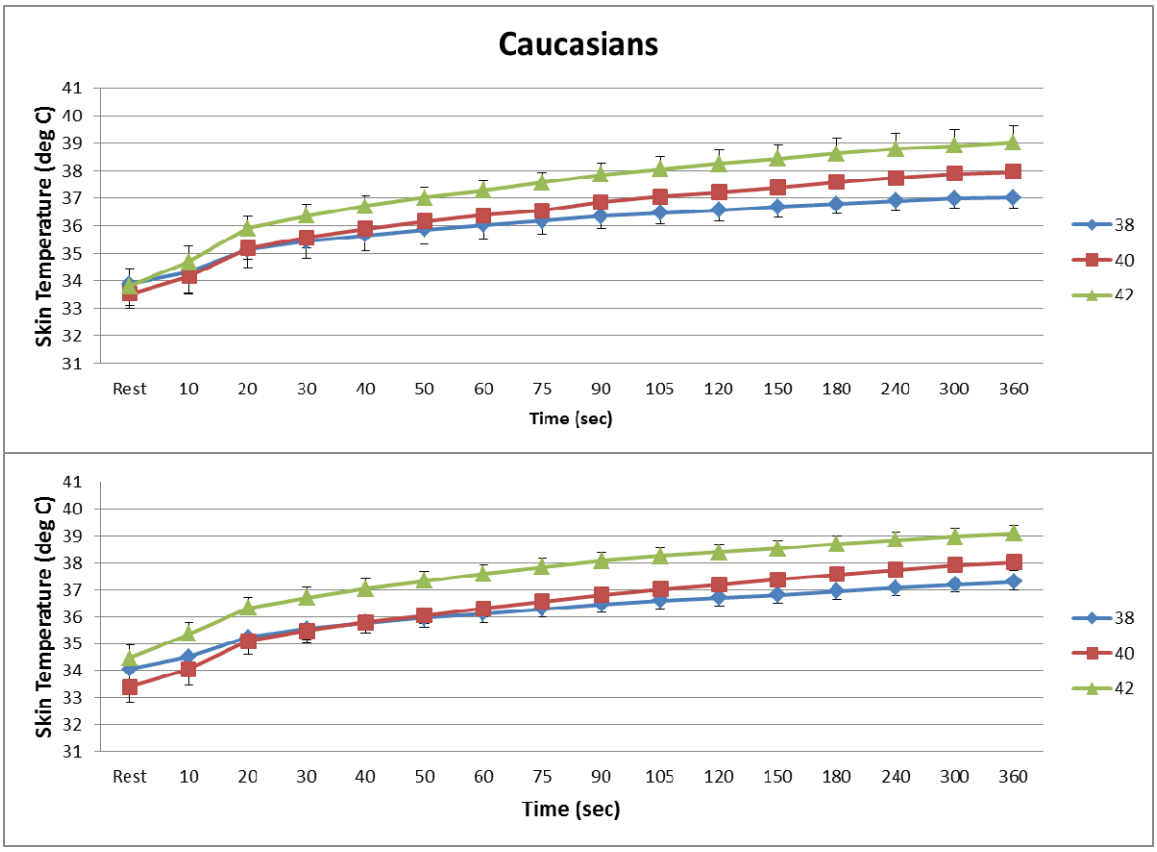

Figure 2. Mean ± SD of skin temperature (^{o}C) recorded throughout the exposure to a 38, 40, 42^{o}C thermode applied to the skin for period of 360 seconds in 10 Caucasians and 10 Koreans.

The results of skin blood flow during 6 minutes of heat exposure at 3 different thermode temperatures (38°C, 40°C, and 42°C) in both Caucasians and Koreans are shown in Figures 3, 4, and 5. When applying 6 minutes of local heat on the forearm at 3 different thermode temperatures (38°C, 40°C, and 42°C), skin blood flow continuously increased in both groups to the peak point at 240 seconds after the heat was applied and then decreased afterward (p=0.001). The total skin blood flow over the 6 minutes period at all 3 different thermode temperatures was significantly higher in Caucasians than Koreans (p=0.001). For the 38°C thermode, the skin blood flow was significantly higher in Caucasians than in Koreans after 60 seconds of heat exposure (p=0.004). The peak

blood flow after heat exposure was 223.0 ± 48.1 flux in Caucasians and 126.4 ± 41.3 flux in Koreans at 240 seconds respectively (Figure 3). For exposure to the 40°C thermode, the skin blood flow was significantly higher in Caucasians than in Koreans after 60 seconds of heat exposure (p=0.003). The peak blood flow after heat exposure was 413.7 ± 132.1 flux in Caucasians and 251.0 ± 77.9 flux in Koreans at 240 seconds respectively (Figure 4). For exposure to the 42°C thermode, the skin blood flow was significantly higher in Caucasians than in Koreans after 90 seconds of heat exposure (p=0.015). The peak blood flow after heat exposure was 517.4 ± 135.8 flux in Caucasians and 398.0 ± 97.2 flux in Koreans at 240 seconds respectively (Figure 5).

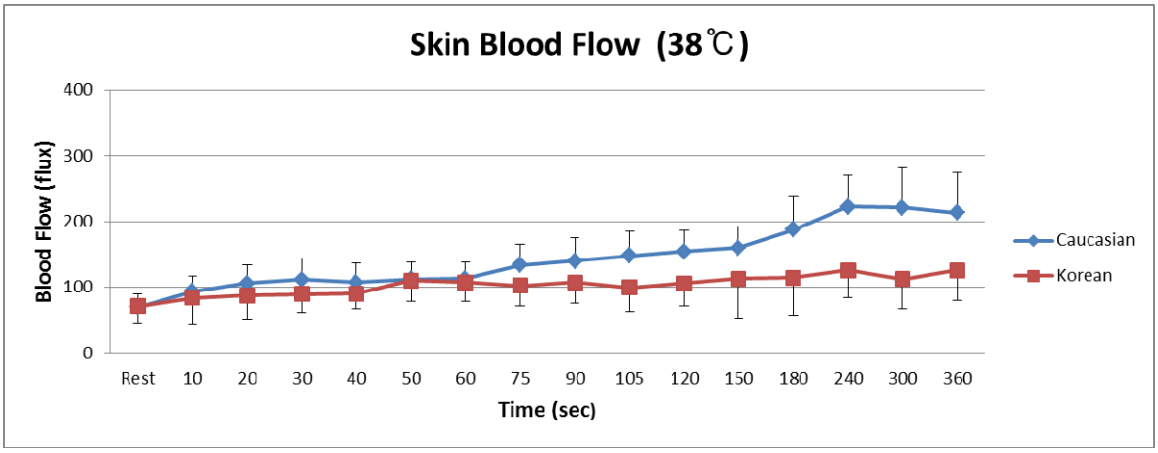

Figure 3. Mean ± SD of blood flow (flux) measured during the exposure to heat at 38°C in 10 Caucasians and 10 Koreans at rest and over a period of 360 seconds.

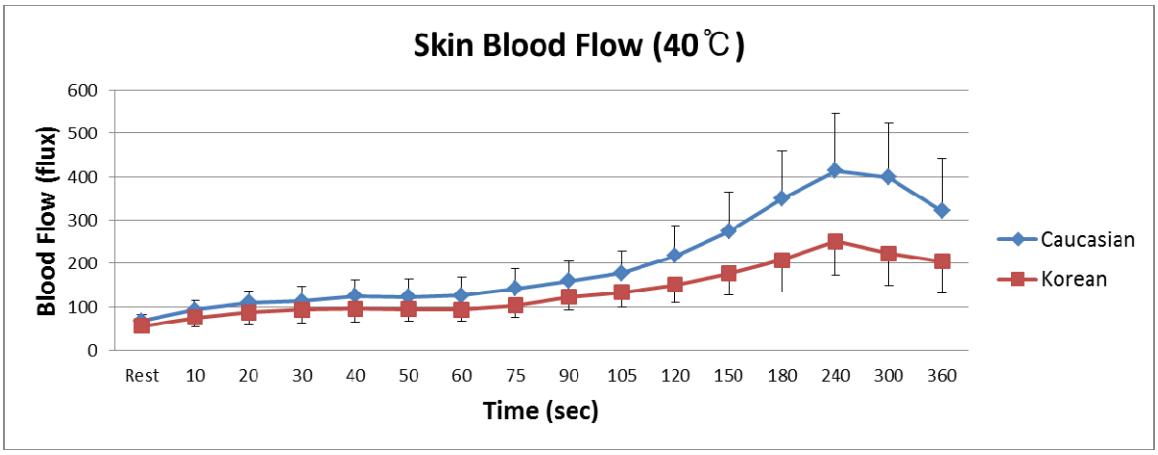

Figure 4. Mean ± SD of blood flow (flux) measured during the exposure to heat at 40°C in 10 Caucasians and 10 Koreans at rest and over a period of 360 seconds.

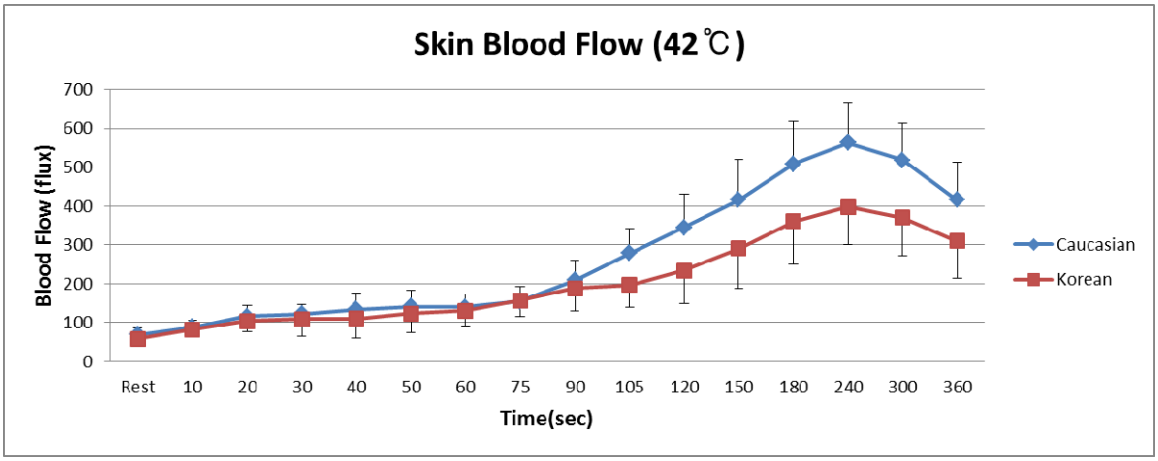

Figure 5. Mean ± SD of blood flow (flux) measured during the exposure to heat at 42°C in 10 Caucasians and 10 Koreans at rest and over a period of 360 seconds.

Discussion

Endothelial dysfunction implies an imbalance between endothelium-derived relaxing and contracting factors maintaining vascular homeostasis. Endothelial dysfunction plays an important role in the pathogenesis of most known cardiovascular diseases and diabetes mellitus and is an independent predictor of future cardiovascular

diseases [8, 23]. The human skin circulation is a useful and an appropriate model for generalized microvascular endothelial function [24]. The skin blood flow response to reactive hyperemia following occlusion and local heating are commonly used to evaluate microvascular endothelial function in normal subjects and patients with cardiovascular diseases, diabetes mellitus, and other stressors that affect the blood flow in the body [6, 24].

The present study examined vascular endothelial function in 10 young Caucasian subjects and 10 young Korean subjects. Endothelial function was assessed by the blood flow response to both occlusion and local heating. In the reactive hyperemia after 4 minutes of vascular occlusion, the skin blood flow response to occlusion was significantly higher in Caucasians than Koreans. In the response to 6 minutes of local heating at 3 different thermode temperatures (38°C, 40°C, and 42°C), the skin blood flow response to local heating was significantly higher in Caucasians than Koreans. These findings suggest that the Korean population has lower vascular endothelial function than Caucasians. Several previous studies have demonstrated reduced or impaired vascular endothelial function in Asians compared with Caucasians [25, 26]. However, this study is the first to compare endothelial function between Koreans and Caucasians by measuring the skin blood flow response to both occlusion and local heating. The ethnic differences in endothelial function, particularly in the bioavailability of endothelium-derived nitric oxide, have been suggested to play an important role. These differences are likely to be clinically important because endothelium-derived nitric oxide plays a major role in vascular homeostasis as a vasodilator and as an inhibitor of platelet activity, monocyte adhesion, and smooth muscle proliferation [11]. Furthermore, a recent study has

demonstrated that impaired endothelial function in the microvasculature is predictive of future cardiovascular disease events [27]. According to recent studies, the predicted prevalence of metabolic syndrome is 25% for non-Hispanic Whites compared to 45% of Asians (Korean, Asian Indian, Chinese, Filipino, Japanese, Vietnamese) and prevalence of diabetes in Asian Americans was 60% higher than in non-Hispanic whites [28, 29]. Such ethnic difference could be either due to a natural lower blood flow in Koreans or the influence of the thrifty genotype on endothelial function [25, 30, 31]. Due to a modern high fat diet, this genotype may have caused endothelial damage.

The thrifty genotype developed in populations where food supply was limited due to famine. People with thrifty genes could store fat better than others and were therefore more likely to survive prolonged food shortages [20]. This thrifty genotype, which is composed of many single nucleotide polymorphisms (SNPs), is a genetic difference regulating lipid metabolism and fat storage, and is different depending on ethnicity [25, 26, 30, 31]. For example, one of the thrifty SNPs, a peroxisome proliferator activated receptor-gamma2 Pro12Ala (PPAR-γ2 Pro12Ala), has been reported to have a preventive role in diabetes mellitus by decreasing insulin resistance in Caucasians, but not in Asians [20, 30]. The frequency of PPAR-γ2 Pro12Ala polymorphism has been shown to be considerably lower in Asians than in Caucasians [30, 31]. Other possible thrifty genes that may contribute to the difference in fat and energy metabolism between Koreans and Caucasians are the uncoupling protein-3 (UCP3) gene and the intestinal fatty acid binding protein-2 (FABP2) gene. The recessive homozygote of UPC3 gene is associated with a higher BMI, and is seen more frequently in Asians than Caucasians (48% and 22%, respectively). The UCP3 is also related to blood glucose and seems to significantly affect

sugar metabolism and the onset of diabetes mellitus [31]. The intestinal FABP2 related to lipid metabolism has been associated with obesity because it enhances fat absorption. The allelic frequency of FABP2 is 55% in Asians and 27.1% in Caucasians. Thus, if Asians consume the same amount of fat, a higher body fat deposit at a lower or the same BMI would be observed in Asians [25, 31] South Korea is one of the countries where the socioeconomic environment has changed rapidly to reflect more Westernization as well as its negative health consequences. For the adoption of a more westernized lifestyle, higher dietary fat consumption and less physical activity are common in Korea, and hence, these thrifty genes heighten the susceptibility to insulin insensitivity and cardiovascular diseases in relation to increased body fat and dyslipidemia in Koreans [20, 25, 26]. Increased body fat and dyslipidemia are associated with the induction of proinflammatory cytokines, adhesion molecules, and reactive oxygen species (ROS) within the vascular walls [32]. Increased superoxide anion radicals by leukocytes cause reduced nitric oxide bioavailability due to either reduced formation or accelerated degradation of nitric oxide, causing cell membrane injury, and induce low-density lipoprotein oxidation, which has cytotoxic effects on the vascular endothelial cells [33]. These factors, taken together, can induce endothelial dysfunction leading to an impaired skin blood flow response to occlusion and local heating. The interaction of thrifty genes and rapidly changed lifestyles may explain why Koreans have a lower skin blood flow response to occlusion and local heating due to endothelial dysfunction than Caucasians. International studies conducted among different Asian national populations in China, Korea, Philippines, Singapore, and Taiwan have shown increased risk of Type 2 diabetes and cardiovascular disease at lower BMI than European populations [28].

Previous studies show that when heat is applied to the skin, the ability of heat transfer through the skin into deeper tissues is impaired in individuals with a thinner dermal layer, thicker subcutaneous fat layer, and lower skin moisture, leading to the reduced skin blood flow response in local heating [14, 18, 34]. For example, a recent study from Petrofsky et al. showed a 100% increase in skin blood flow when the skin was moist versus when it was dry, as well as a reduced sensitivity for changes in blood flow with local heat applications [18]. Also, subcutaneous fat impairs the ability of circulation to transfer heat into deeper tissues which may contribute to higher skin temperatures during local heating [14, 18, 34]. For these reasons, in this study, we measured characteristics of the skin such as skin thickness, subcutaneous fat thickness, and skin moisture. However, there was no significant difference between Koreans and Caucasians. Thus, the difference in skin blood flow may not be due to characteristics of the skin in this study.

Local heat application to the skin results in a large increase in skin blood flow that is biphasic and characterized by an initial response followed by a more prolonged response [14-17]. In the first few minutes, the initial blood flow response to local heat is primarily mediated by tactile sensors in the skin through the release of substance P and calcitonin gene related peptide from sensory nerves. The secondary sustained blood flow response to local heat is mediated primarily by nitric oxide released from vascular endothelial cells [8, 14, 16-18]. Endothelial nitric oxide synthetase is activated by TRPV4 voltage gated, temperature sensitive calcium channels [8, 15]. In the present study, skin blood flow response to local heat was higher in Caucasians than in Koreans after 60 seconds of heat exposure at 38°C and 40°C and after 90 seconds of heat exposure at 42°C

(Figures 3, 4, and 5 respectively). In other words, in the first 60 or 90 seconds of the initial blood flow response mediated by tactile neurons, there was no significant difference between Koreans and Caucasians. After the 60 or 90 seconds of the initial response, however, there was significantly higher blood flow response in Caucasians than in Koreans during the sustained blood flow response mediated by nitric oxide. Thus, this result would seem to imply that Koreans have lower vascular endothelial function than Caucasians primarily due to the reduced bioavailability of nitric oxide, not the tactile receptors.

The result of this study may also infer greater susceptibility to skin burns in Koreans. Many studies indicated that it was both the conductive heat loss through the skin itself and skin blood flow that are important in dissipating heat from the skin [16, 18, 35]. However, conductive heat loss for living skin is not constant (a second or less) and the skin blood flow does play a progressively greater role in continually removing heat for protecting the skin from burns [14, 16, 35]. According to a recent study from this laboratory, older people and people with diabetes are more susceptible to skin damage and burns due to changes in the structures of the skin and a reduction in skin blood flow linked to reduced bioavailability of nitric oxide by free radicals in vascular endothelial cells [14]. In the present study, as mentioned before, there was no significant difference in skin thickness, subcutaneous fat thickness, and skin moisture between Koreans and Caucasians. As shown in Figures 3, 4, and 5, however, Korean subjects had significantly lower blood flow response to local heating than Caucasians and it is strongly believed that Koreans are more susceptible to skin burns than Caucasians.

In this study, Korean subjects had been living in the United States (US), rather than in Korea. Although the mean period of stay in US was 7.1 ± 2.3 months in Korean subjects, the results might be different in Koreans who did not reside in the US. In addition, due to different diets especially high-fat diet and environment, Koreans who have been in the US for longer periods may have more reduced endothelial function than those with shorter residing periods. Therefore, further studies need to be conducted with Koreans for those who have been in the US for long periods and those who have not been to US. These results are only in 10 subjects and therefore have limited power in themselves. However, similar results have been published for the vascular response to occlusion in people from India [36] and Thailand [20], increasing the strength of these conclusions. Seemingly contradicting these results are the results of a recent study showing the death rates from heart disease are much lower in Korea than in the United States [37]. However, the prevalence of cardiovascular disease and diabetes in African-Americans and Asian-Americans is much higher than in non-Hispanic whites in the US, skewing the Caucasian's data [28, 38, 39]. Also, the incidence of obesity and diabetes is increasing at a much higher rate in Koreans than in non-Hispanic whites in the US [28, 29] as it is across Asia. It has only been in the last 10 years that diabetes and obesity have increased dramatically in Asia to the point where the World Health Organization (WHO) has called this an epidemic. It will be interesting to see if, due to an increase in high fat food, the death rates remain this low 10-20 years from now in Korea. It will certainly take time to see the effects of this western diet on Asians.

Conclusions

In the present investigation, endothelial function was assessed by the blood flow response to local heat and vascular occlusion. In the reactive hyperemia after 4 minutes of vascular occlusion, the skin blood flow was significantly higher in Caucasians than Koreans. In the response to 6 minutes of local heating at 38°C, 40°C, and 42°C, the skin blood flow was significantly higher in Caucasians than Koreans. These findings suggest that the Korean population has lower vascular endothelial function than Caucasians.

Acknowledgements

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CHAPTER THREE

PROTECTIVE EFFECT OF ANTI-OXIDANTS ON ENDOTHELIAL FUNCTION IN YOUNG KOREAN-ASIANS COMPARED TO CAUCASIANS

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Summary

Background: Previous studies show that Asians have an impaired blood flow response (BFR) to occlusion after a single high fat (HF) meal. The mechanism is believed to be the presence and susceptibility to high free radicals in their blood. The free radical concentration after a HF meal has not been examined in Asians. Further the BFR to heat after a single HF meal in Koreans has not been measured.

Materials/Methods: This study evaluated postprandial endothelial function by measuring the BFR to vascular occlusion and local heat before and after a HF meal and the interventional effects of anti-oxidant vitamins on improving endothelial function in young Korean-Asians (K) compared to Caucasians (C) with these assessments. Ten C and ten K participated in the study (mean age 25.3 ± 3.6 years old). BFR to vascular occlusion and local heat and oxidative stress were assessed after a single low fat (LF) and HF meal at 2 hours compared to baseline. After administration of vitamins (1000mg of vitamin C, 800IU of vitamin E, and 300mg of Coenzyme Q-10) for 14 days, the same measurements were made.

Results: This study showed that the skin BFR to vascular occlusion and local heat following a HF meal significantly decreased and free radicals significantly increased at 2 hours compared to baseline in K ($p<.001$), but not in C. When vitamins were given, the BFR to vascular occlusion and local heat before and after HF meal were not significantly different between K and C.

Conclusion: These findings suggest that even a single HF meal can reduce endothelial response to stress through an oxidative stress mechanism but can be blocked by antioxidants, probably through scavenging free radicals in K. Since endothelial

function improved even before a HF meal in K, endothelial damage from an Americanized diet may be reduced in K by antioxidants.

Key words: antioxidant, Asian, endothelial function, heat, occlusion

Background

The vascular endothelium plays an integral role in maintaining vascular homeostasis including the regulation of blood flow, vascular tone, and platelet aggregation [1]. A balance between endothelial derived relaxing and contracting factors maintains vascular homeostasis [2]. Studies show that different ethnic populations have different genes that can alter endothelial function [3, 4]. For example, people from Korea and other Asians have a "thrifty" gene that was developed in populations where food supply was limited due to famine, thus allowing fat to be stored easily when only limited foods are available [5, 6]. Today, Koreans are eating more HF foods than a decade ago and the mortality rate due to diabetes mellitus (DM) has doubled during the last decade, increasing from 17.2 per 100,000 persons in 1995 to 24.5 per 100,000 persons in 2005 [7]. "Thrifty" genes seem to heighten the susceptibility to endothelial dysfunction when eating high fat (HF) foods or gaining body weight [3, 4, 6]. A standard test of endothelial function is the response to vascular occlusion for 4 minutes, sometimes called flow mediated vasodilatation. A recent study in this lab showed that Koreans had a lower blood flow response (BFR) to 4 minutes of vascular occlusion and 6 minutes of local heating than Caucasians. This finding suggests that the Korean population has lower endothelial response to stress than Caucasians [8]. In another study, it was found that

blood flow was reduced even further in Asians with a single HF meal, again implying reduced endothelial response to stressors due to fats in the diet [6].

Making matters worse, higher dietary fat consumption is now more common in Asia. Many recent studies demonstrate that hypertriglyceridemia (HTG) is one of the risk factors in DM and cardiovascular disease (CVD) and can cause endothelial dysfunction [6, 9]. The mechanisms of postprandial HTG-induced endothelial dysfunction have been suggested that HTG significantly stimulates leukocytes to produce free radicals increased oxidative stress. Free radicals reduce production of endothelium derived relaxing factors such as nitric oxide and prostacyclin causing cell membrane injury, and induce low-density lipoprotein oxidation, which has cytotoxic effects on the vascular endothelial cells and can cause endothelial dysfunction [10, 11].

Free radicals are not always physiologically detrimental. They are commonly produced and neutralized in the body via the activity of radical generating enzymes and inflammatory cells, as well as through cellular metabolism involving the processing of macronutrients [12]. Beneficial effects of free radicals occur at low/moderate concentrations and involve physiological roles in promoting cellular signaling, aiding in phagocytic immune defense, as well as promoting apoptosis [13]. The detrimental effect of free radicals inducing potential biological damage is termed oxidative stress [14]. An overproduction of free radicals or a deficiency of enzymatic and non-enzymatic antioxidants induces the oxidative stress in biological systems. The excess free radicals can damage cellular lipids, proteins, or DNA inhibiting their normal function [13]. For example, high free radical concentration in the body increases cellular oxidation and can biodegrade nitric oxide and prostacyclin into inactive forms [15]. Both compounds are

released by vascular endothelial cells to relax blood vessels. These radicals bioconvert nitric oxide into peroxynitrite, thereby reducing the bioavailability of nitric oxide as a vasodilator and this can further damage the tissue by lipid peroxidation of membranes [13]. This is believed to be one of the mechanisms associated with reduced circulation in older people and diabetics [15].

One purpose of the present investigation was to quantify the effect of a HF meal on the BFR to heat and occlusion in Koreans compared to Caucasians. Unlike other studies measuring flow mediated vasodilatation at rest and after a HF meal, we wanted to measure free radicals in the blood to see how this correlated to flow mediated vasodilatation and the BFR of the skin to heat. In addition, we wanted to assess the effects of vitamins on these vascular responses.

Numerous studies have examined the benefits of vitamins in reducing free radicals in the body [15-17]. However, little is known about measuring the effects of a HF meal and vitamins on the level of free radicals and endothelial function. A standard method of assessing endothelial function is post ischemic reactive hyperemia [18, 19]. The reactive hyperemia to anoxia is the sudden rise in blood flow that can be measured by a Laser Doppler Imager or ultrasound after a 4 minute occlusion of arterial blood flow [20]. Another method of assessing endothelial function is the skin BFR to local heat mediated by the endothelial cells [21, 22]. It is well established that when heat is applied to the skin, there is an increase in skin blood flow [23, 24]. High concentrations of free radicals neutralize nitric oxide and prostacyclin, the two principal vasodilators, and reduce blood flow in response to vascular occlusion and local heat. Malondialdehyde (MDA) can be used to assess the degree of feeding-induced oxidative stress. MDA is a

highly reactive three carbon chain aldehyde produced as a byproduct of the decomposition of a lipid hydroperoxide and is commonly used as an indicator of lipid peroxidation [25].

The purpose of this study was to evaluate postprandial endothelial function as related to enhanced oxidative stress in a single HF and LF meal and more importantly the interventional effects of vitamins on scavenging free radicals and improving endothelial function in Korean-Asians. These data were compared to Caucasians. This was done by measuring skin BFR to vascular occlusion and local heat and analyzing MDA levels in both groups after ingestion of a LF and HF meal before and after vitamin intervention.

Materials and Methods

Subjects

Healthy young subjects were recruited by word of mouth and assigned to one of two groups based on self-reported ethnicity. Subjects did not have diagnosed cardiovascular disease, hypertension (>140/90mmHg) or diabetes, were non-smokers, were not taking any medications that would affect the cardiovascular system, and did not have any known peripheral circulatory diseases. The subjects included 10 Koreans and 10 Caucasians. They were in the age range of 20-35 years old and with a BMI of less than 40. The general characteristics of the subjects are shown in Table 2. All protocols and procedures were approved by the Institutional Review Board of Loma Linda University and all subjects signed a statement of informed consent.

Table 2. Mean (SD) of general characteristics of the 5 men and 5 women in each ethnic group.

	Koreans	Caucasians	p-value
Age (years)	25.4 (4.2)	27.8 (2.4)	0.14
Height (cm)	168.8 (9.4)	176.1 (11.3)	0.14
Weight (kg)	70.5 (16.7)	73.6 (8.3)	0.60
BMI (kg/m^2)	24.5 (3.9)	23.8 (1.8)	0.60
Skin thickness (cm)	0.05 (0.01)	0.05 (0.01)	0.51
Fat thickness (cm)	0.10 (0.01)	0.11 (0.01)	0.15
Skin moisture (mg/cm^2)	41.4 (6.3)	40.8 (12.2)	0.89

Methods

Measurement of Skin Blood Flow

Skin blood flow was measured with a MOOR Laser Doppler Imager (Moor LTD, Oxford, England). The Imager used a red Laser beam to measure blood flow in the skin. The Laser was used in a single spot mode. In this mode, the Laser did not scan but simply was left on the skin and the reflected energy used to measure the skin blood flow. Blood flow in a Laser Doppler Imager was expressed in units called flux. The Laser was warmed for 30 minutes prior to measurements. The reported reliability from the manufacturer is ± 5% from day to day. The Laser's calibration was checked just before, in the middle and at the end of the study; there was no calibration drift observed.

Measurement of Skin Temperature

Skin temperature was measured with a thermistor manufactured by BioPac Systems (BioPac Inc., Goleta, CA). The thermistor output was amplified with an SKT 100 Thermistor amplifier (BioPac Systems, Goleta, CA) and the output was then digitized at 1000 samples per second on a BioPac MP150 data collection system at 24 bits of resolution (BioPac Systems, Goleta, CA).

Control of Skin Temperature

Skin temperature was controlled with a thermode. The thermode consisted of a plastic box with a port on each end so that water could move through the box. The box was approximately 5 cm × 2.5 cm × 2.5 cm in size. On each end of the box, there was a thermocouple such that as water circulated through the box from a controlled temperature water bath (Biopac Systems, Goleta, California). The temperature difference across the box was measured. Water bath temperatures were kept at 38°C, 40°C, or 42°C. A final hole through the box, which was water insulated, allowed the Laser to scan through the center of the box onto the skin. This then provided blood flow while the skin temperature was clamped at a set temperature. Further details on the technique and the reliability and validity are published elsewhere [26, 27].

Test Meals

Isocaloric LF and HF meals (726 kcal) were given to the subjects at the study site in the morning after an overnight fast. The nutritional composition of HF meal was 50.1g total fat, 14g saturated fat, 443mg cholesterol, 22.3g protein, 43.8g carbohydrates.

The nutritional composition of isocaloric LF meal was 5.1g total fat, 1g saturated fat, 0mg cholesterol, 31.3g protein, 135.8g carbohydrates. To assure consistency across subjects, meals were ordered from the same commercial restaurant.

Measurement of Malondialdehyde

Malondialdehyde (MDA) was measured using the 2-ThioBarbituric Acid Reactive Substances (TBARS) assay kit (Oxford Biomedical Research, Inc., Oxford, MI, USA). This assay is based on the reaction of a chromogenic reagent, 2-thiobarbituric acid, with MDA at 95°C. One molecule of MDA reacts with 2 molecules of 2-thiobarbituric acid *via* a Knoevenagel-type condensation to yield a chromophore with absorbance maximum at 532 nm.

Procedures

All subjects were asked to abstain from dietary supplements for 1 week prior to starting the study and avoid engaging in physical exercise 48 hours prior to the experimental meal. Subjects fasted 12 hours prior to participating in the study. The order of HF and LF breakfast meals was randomly assigned and separated with a one week wash out period. Subjects began their experiment between 8-10 am and entered a thermally neutral room (22°C) and rested comfortably for 20 minutes. In the first experiment, the test breakfast meal was given to subjects and endothelial function was assessed by two methods at baseline and after 2 hours following the meal. The first was occlusion of the circulation on upper arm for 4 minutes followed by release and measurement of skin blood flow for 2 minutes. The second was assessment of the skin

blood flow response to local heat by applying a thermode of 42°C to the forearm. Each procedure was performed on different arms. Since the metabolic effect of a meal peaks at 2 hours on endothelial function, the measurements were repeated after 2 hours following the meal. A 10mL blood sample was taken at baseline and after 2 hours following the meal for measuring oxidative stress. After a wash out period of 7 days, the same procedure was performed but the subjects were given the other test meal and data was collected at baseline and after 2 hours following the meal. A 10mL blood sample was taken after 2 hours following the meal. Finally, to see if antioxidants can reduce the damage to endothelial cells from a HF meal, after a wash out period of 7 days from the last previous meal, a final series of experiments had the subjects ingest anti-oxidant supplements daily of 1000mg of vitamin C, 800IU of vitamin E, and 300mg of Coenzyme Q-10 for 14 days. After 14 days following anti-oxidant supplements intake, on the fifteenth day, measurements were repeated in baseline and followed by ingestion of a HF meal. A 10mL blood sample was taken at baseline and after 2 hours following a HF meal. The blood sample was not drawn more than 2 times per week and was assayed for oxidative stress.

Statistical Analysis

Data was summarized as Means and standard deviations. Baseline characteristics of Caucasians and Koreans were compared using an independent t-test (Table 1). A mixed factorial ANOVA was conducted to compare the BFRse to 4 minutes of vascular occlusion and 6minutes of local heat before and after two different meals with or without vitamins in Koreans and Caucasians. Also, a paired t-test was conducted to compare the

MDA concentration before versus after the meals with or without vitamins. The level of significance was set at p=0.05. Effect sizes were calculated to account for group variability. A post power analysis with an effect size of 1.6 according to the change in skin blood flow between Korean and Caucasian groups, an alpha level of 0.05, and a sample size of 20 indicated that the power was 0.91.

Results

Data is presented as baseline measurements and the results after a LF and HF meal and pre and post vitamins intervention as given below:

Baseline

The results of skin blood flow during 4 minutes of vascular occlusion and then the first 2 minutes following occlusion at baseline are shown in Figure 6A. There was a significant difference in the skin BFR after 4 minutes between Koreans and Caucasians (p=0.016). The mean blood flow rapidly increased to a peak of 332.4 ± 75.8 flux in Koreans compared to 411.9 ± 88.9 flux in Caucasians at 20 seconds after the release of the four minutes of vascular occlusion. The blood flow then decreased to a final value of 84.8 ± 15.5 flux in Koreans compared to 122.3 ± 42.4 flux in Caucasians at the end of the 2 minute period.

The results of BFR to local heat during 6 minutes at baseline are shown in Figure 6B. The skin blood flow was significantly lower in Koreans than in Caucasians after 60 seconds of heat exposure (p=0.003). The peak blood flow after heat exposure

was 251 ± 77.9 flux in Koreans and 413.7 ± 132.1 flux in Caucasians at 240 seconds, respectively.

To determine the level of lipid peroxidation and oxidative stress following meals, MDA concentration was measured using TBARS assay. When comparing the concentration of MDA at baseline, there were no significant differences between Koreans (4.6 ± 1.9 μM) and Caucasians (4.6 ± 1.4 μM) (Figure 7).

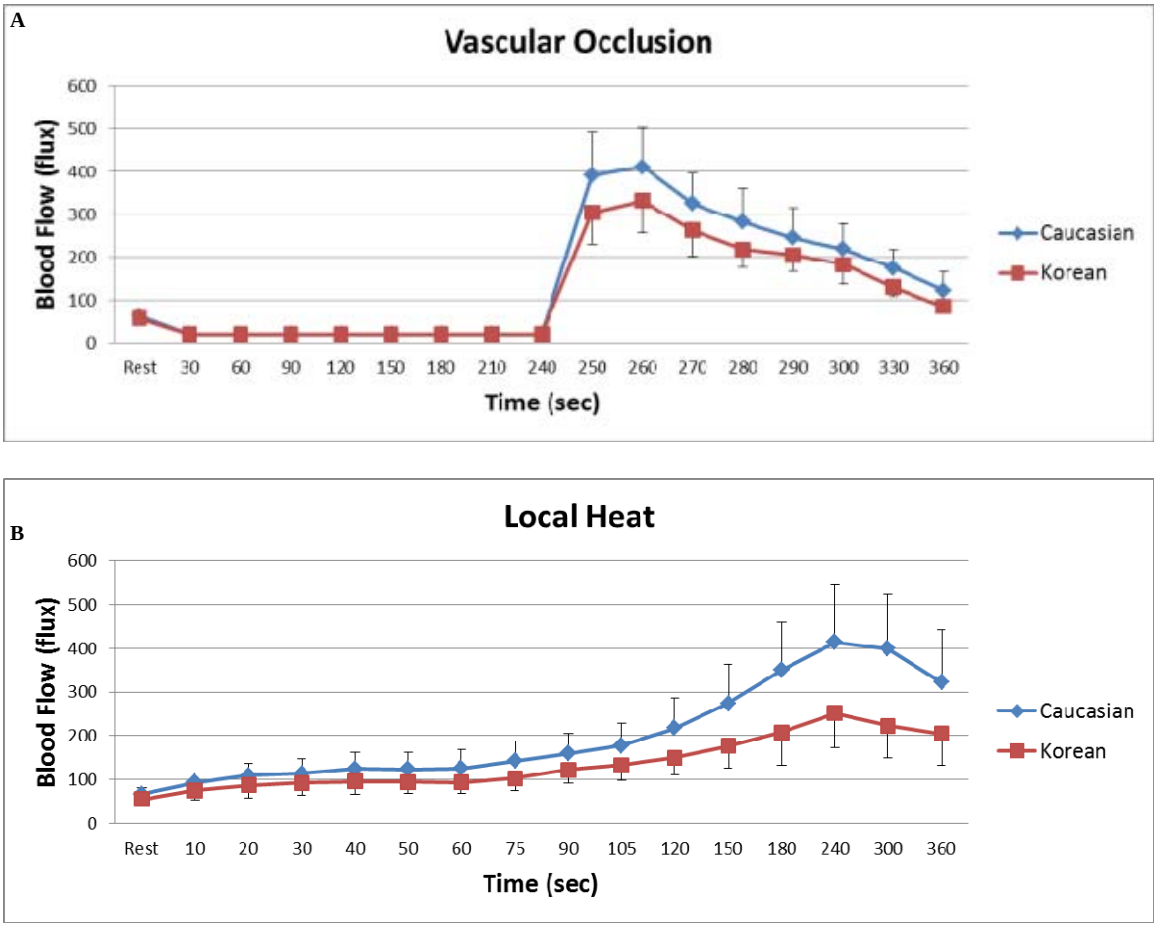


Figure 6. Mean ± SD of baseline blood flow (flux) measured during the 4 minutes period of vascular occlusion and the 2 minute period following the release of the occlusion cuff (A) and 6 minutes period of local heat (B) in 10 Koreans and 10 Caucasians.

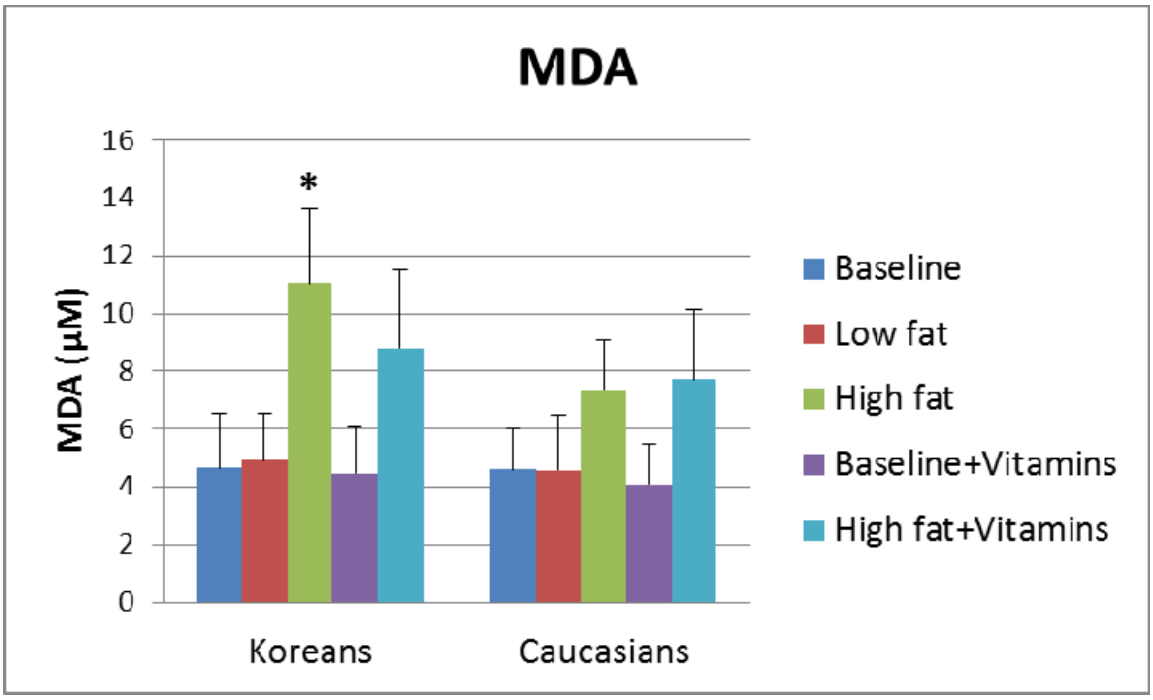


Figure 7. Mean ± SD of MDA concentration at baseline, after 2 hours of low fat meal, after 2 hours of high fat meal, at baseline with vitamins, and after 2 hours of high fat meal with vitamins in 10 Koreans and 10 Caucasians. * $p<0.01$ compared to Caucasians.

Results after Two Meals

Low Fat Meal

The results of skin blood flow during 4 minutes of vascular occlusion and then the first 2 minutes following occlusion at baseline and at 2 hours after the ingestion of the LF meal in both Koreans and Caucasians are shown in Figure 8A and B. When comparing blood flow at baseline and at 2 hours, the skin blood flow was not significantly different in both Koreans and Caucasians.

The results of skin BFR to local heat during 6 minutes at baseline and at 2 hours after the ingestion of the LF meal in both Koreans and Caucasians are shown in Figure

8C and D. There were no significant differences in the skin blood flow in both Caucasians and Koreans.

When comparing the concentrations of MDA at baseline and at 2 hours, there was no significant difference in both Koreans (4.6 ± 1.9 μM vs. 4.9 ± 1.6 μM) and Caucasians (4.6 ± 1.4 μM vs. 4.6 ± 1.9 μM) (Figure 7).

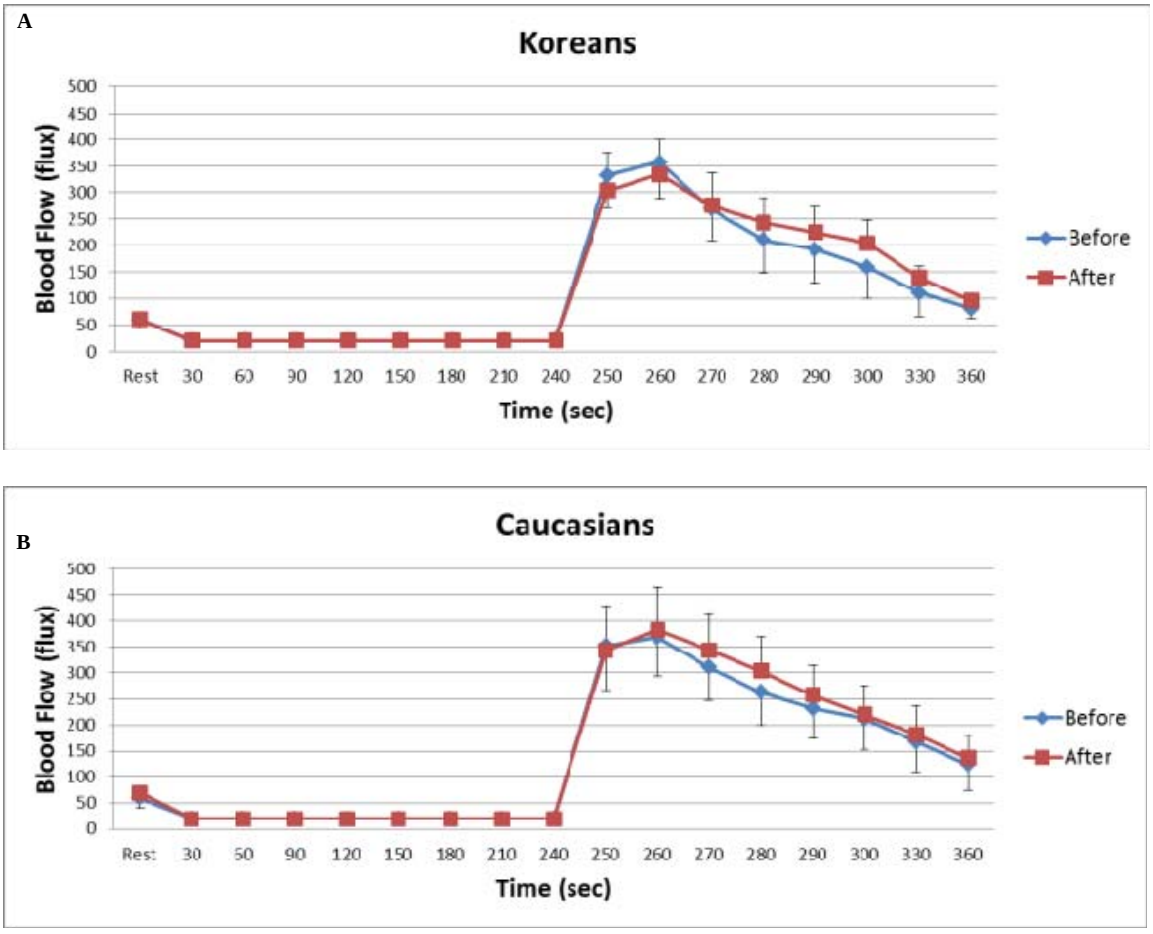


Figure 8. Mean ± SD of blood flow (flux) after a low fat meal measured during the 4 minutes period of vascular occlusion and the 2 minute period following the release of the occlusion cuff (A and B) and 6 minutes period of local heat (C and D, next page) at baseline and after 2 hours in 10 Koreans and 10 Caucasians.

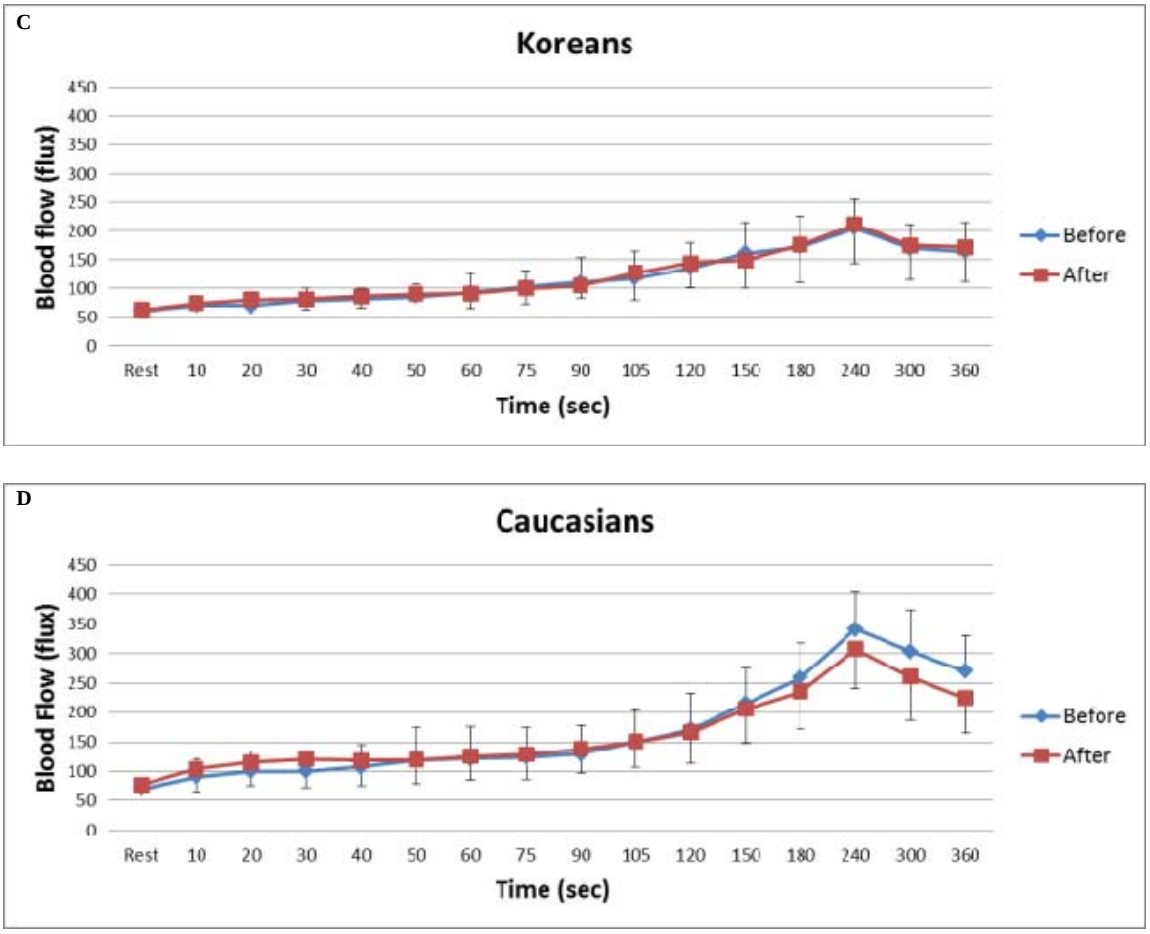


Figure 8. Mean ± SD of blood flow (flux) after a low fat meal measured during the 4 minutes period of vascular occlusion and the 2 minute period following the release of the occlusion cuff (A and B, previous page) and 6 minutes period of local heat (C and D) at baseline and after 2 hours in 10 Koreans and 10 Caucasians.

High Fat Meal

When comparing BFR after 4 minutes of vascular occlusion at baseline and at 2 hours after the ingestion of the HF meal, the total skin blood flow significantly decreased at 2 hours compared to baseline in Koreans ($p<0.001$). The peak blood flow after occlusion was 332.4 ± 75.8 flux at baseline and 266.2 ± 61.7 flux at 2 hours at 260

seconds respectively (Figure 9A). However, there were no significant differences in Caucasians after 2 hours compared to baseline (Figure 9B).

When applying 6 minutes of local heat at baseline and at 2 hours after the ingestion of the HF meal, the skin blood flow after 90 seconds of heat exposure significantly decreased at 2 hours compared to baseline in Koreans ($p<0.001$). The peak blood flow after heat exposure was 224.9 ± 48.5 flux at baseline and 173.5 ± 52.3 flux at 2 hours at 240 seconds respectively (Figure 9C). However, there were no significant differences in Caucasians after 2 hours compared to baseline (Figure 9D).

In the MDA measurements, there was a significantly higher mean MDA concentration at 2 hours than at baseline in both Koreans (4.6 ± 1.9 μM vs. 11.1 ± 2.6 μM) ($p<0.001$) and Caucasians (4.6 ± 1.4 μM vs. 7.4 ± 1.7 μM) ($p=0.002$) (Figure 7). Koreans had a 138.15% increase and Caucasians had a 60.22% increase from baseline.

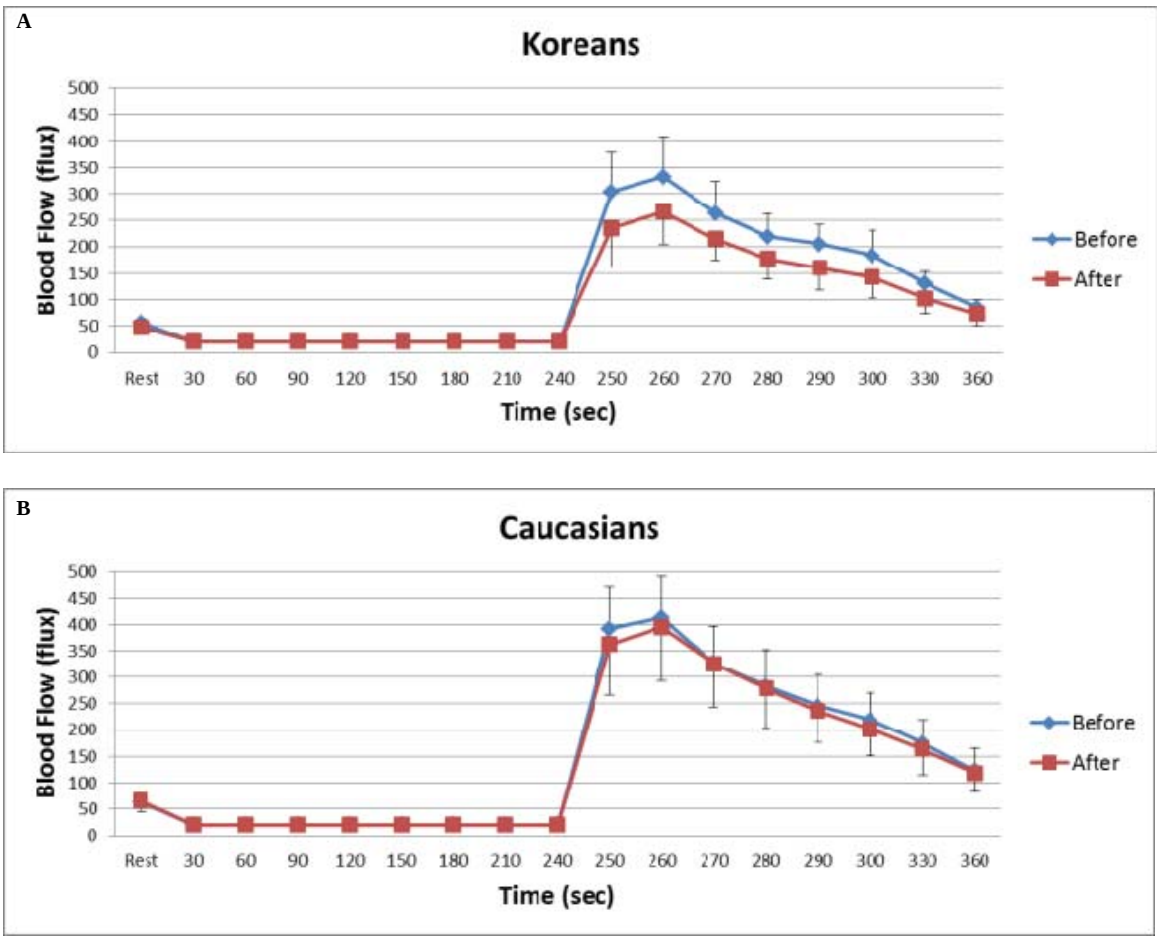
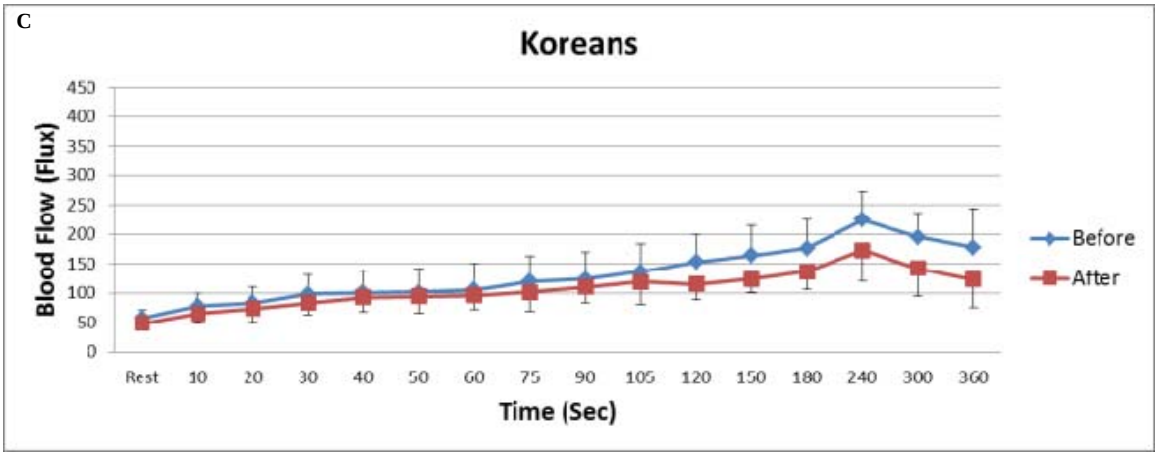


Figure 9. Mean ± SD of blood flow (flux) after a high fat meal measured during the 4 minutes period of vascular occlusion and the 2 minute period following the release of the occlusion cuff (A and B) and 6 minutes period of local heat (C and D, next page) at baseline and after 2 hours in 10 Koreans and 10 Caucasians.



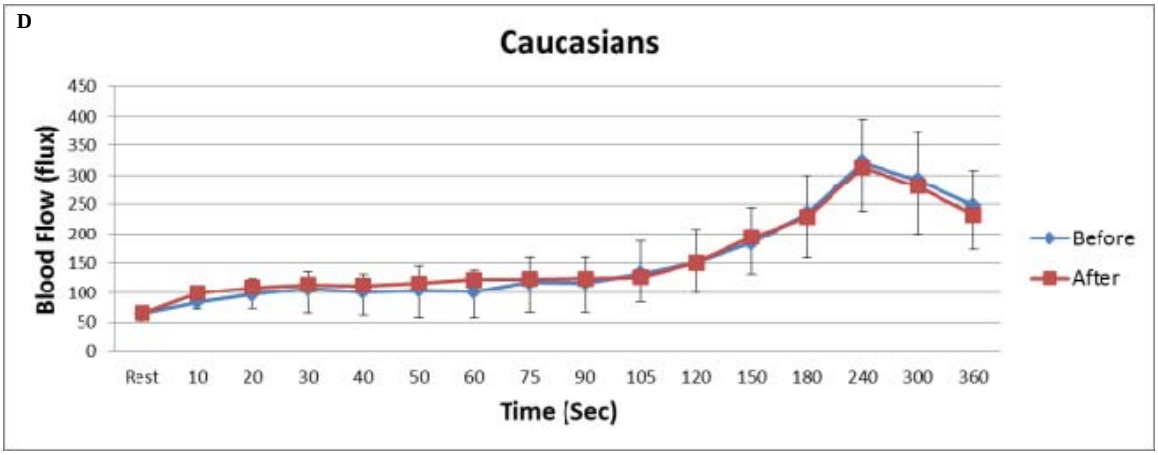


Figure 9. Mean ± SD of blood flow (flux) after a high fat meal measured during the 4 minutes period of vascular occlusion and the 2 minute period following the release of the occlusion cuff (A and B, previous page) and 6 minutes period of local heat (C and D) at baseline and after 2 hours in 10 Koreans and 10 Caucasians.

Results after the Intake of Vitamins for Two Weeks

In contrast, when comparing the BFR after 4 minutes of vascular occlusion at baseline and at 2 hours after the ingestion of the HF meal after the intake of vitamins for 14 days, there were no significant differences in both Koreans and Caucasians (Figure 10A and B).

Also, when comparing BFR to 6 minutes of local heat at baseline and at 2 hours after the ingestion of the HF meal after the intake of vitamins for 14 days, the skin blood flow was not significantly different in both Koreans and Caucasians (Figure 10C and D).

In MDA concentration, however, there were significant differences in both Koreans (4.5 ± 1.6 μM vs. 8.8 ± 2.7 μM) (p=0.001) and Caucasians (4.1 ± 1.4 μM vs. 7.7 ± 2.4 μM) (p=0.001) (Figure 7).

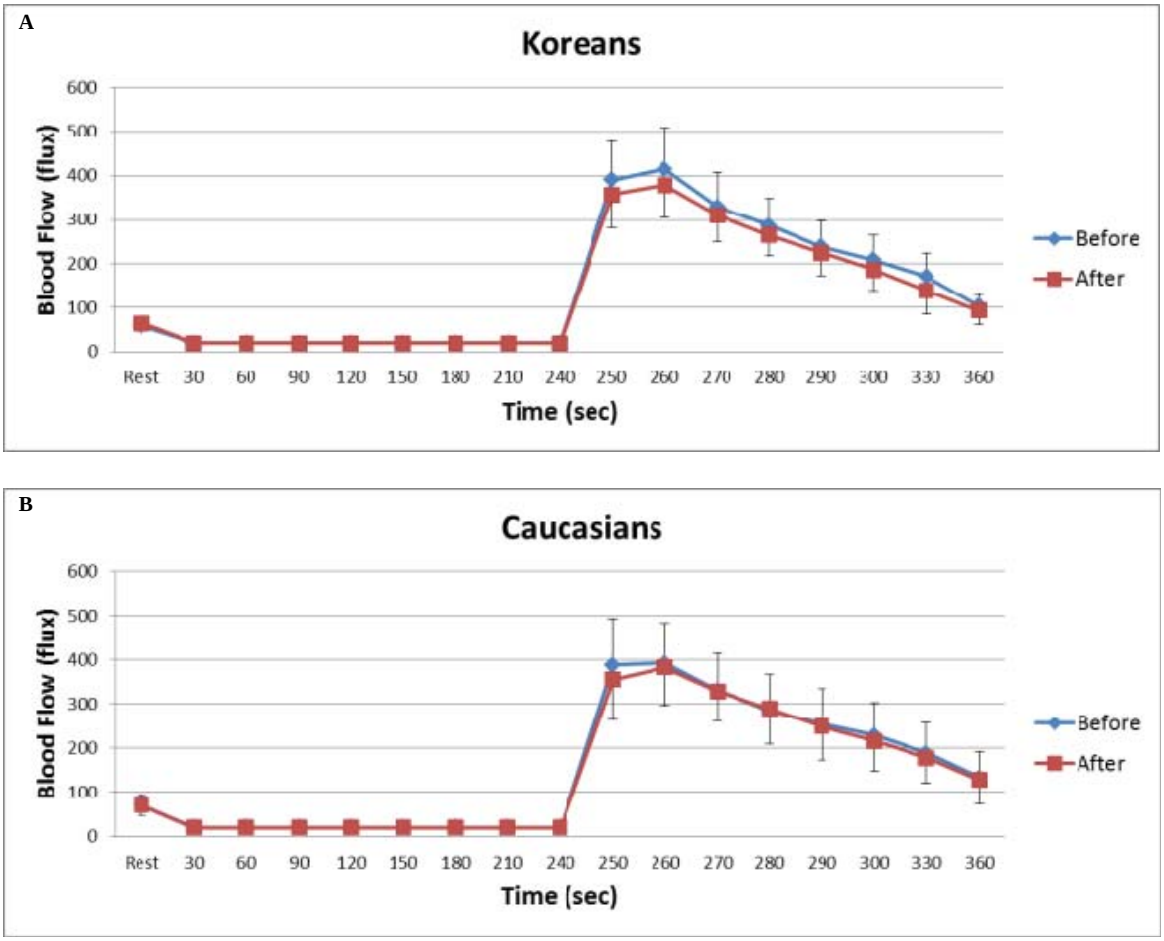


Figure 10. Mean ± SD of blood flow (flux) measured during the 4 minutes period of vascular occlusion and the 2 minute period following the release of the occlusion cuff (A and B) and 6 minutes period of local heat (C and D, next page) at baseline and at 2 hours after the ingestion of the high fat meal after the intake of vitamins for 14 days in 10 Koreans and 10 Caucasians.

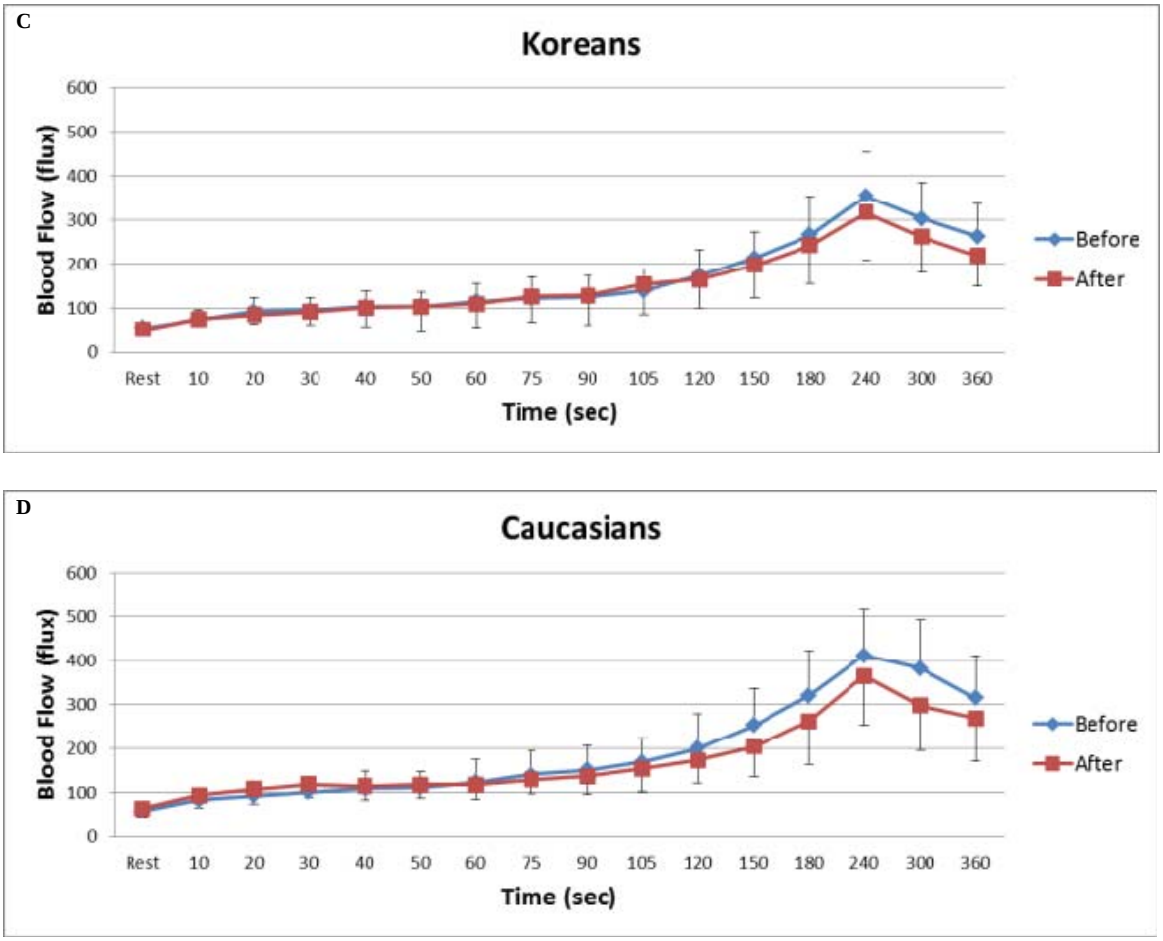


Figure 10. Mean ± SD of blood flow (flux) measured during the 4 minutes period of vascular occlusion and the 2 minute period following the release of the occlusion cuff (A and B, previous page) and 6 minutes period of local heat (C and D) at baseline and at 2 hours after the ingestion of the high fat meal after the intake of vitamins for 14 days in 10 Koreans and 10 Caucasians.

Comparison of Baseline with Vitamins and without Vitamins

When comparing the baseline BFR to 4 minutes of vascular occlusion between 14 days of vitamins and no vitamins in both ethnic groups, the baseline blood flow was significantly higher when vitamins were given compared to no vitamins in Koreans ($p=0.024$). The peak blood flow after occlusion was 414.7 ± 92.9 flux with vitamins

compared to 332.4 ± 75.8 flux without vitamins at 260 seconds, respectively (Figure 11A). However, no significant differences were observed in Caucasians (Figure 11B).

In regards to the baseline BFR to 6 minutes of local heat, the skin blood flow after 90 seconds of heat exposure significantly increased with vitamins compared to no vitamins in Koreans ($p=0.029$). The peak blood flow after heat exposure was 355.2 ± 128.6 flux with vitamins versus 224.9 ± 48.5 flux without vitamins at 240 seconds, respectively (Figure 11C). However, there were no significant differences in the skin blood flow in Caucasians (Figure 11D).

For the MDA concentration, there were no significant differences in both Koreans (4.6 ± 1.9 μM vs. 4.5 ± 1.6 μM) and Caucasians (4.6 ± 1.4 μM vs. 4.1 ± 1.4 μM). MDA concentration decreased by 3.88% in Koreans and decreased by 10.86% in Caucasians (Figure 7).

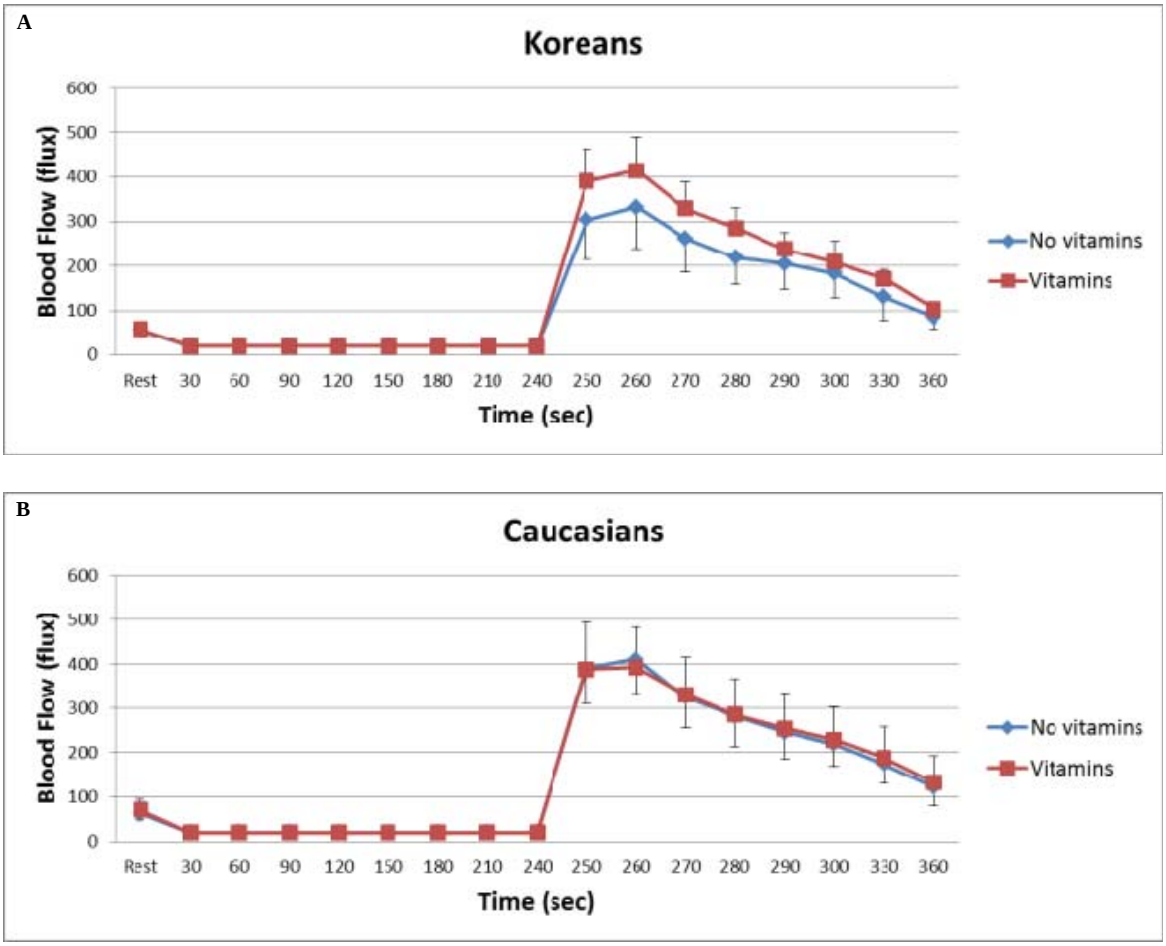
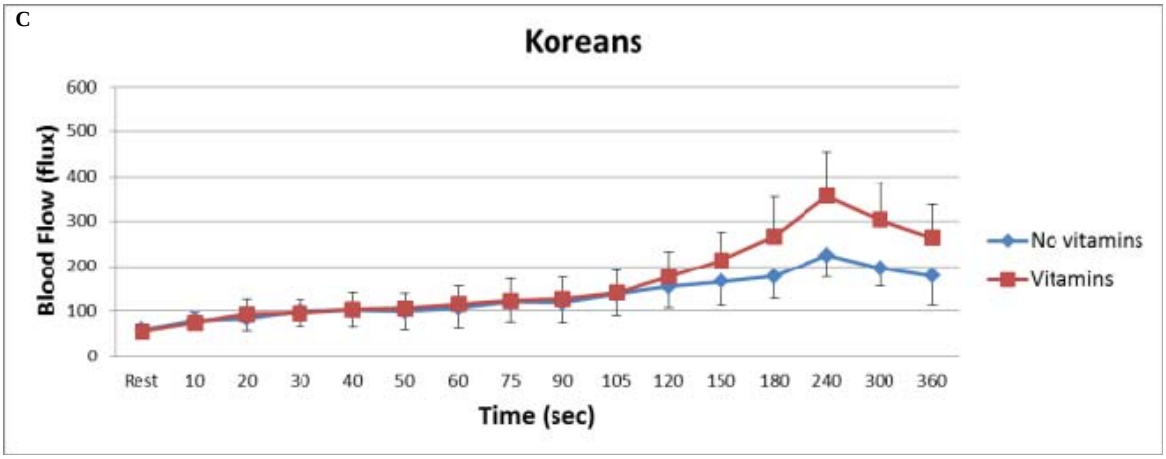


Figure 11. Mean ± SD of baseline blood flow (flux) measured during the 4 minutes period of vascular occlusion and the 2 minute period following the release of the occlusion cuff (A and B) and 6 minutes period of local heat (C and D, next page) before and after the intake of vitamins for 14 days in 10 Koreans and 10 Caucasians.



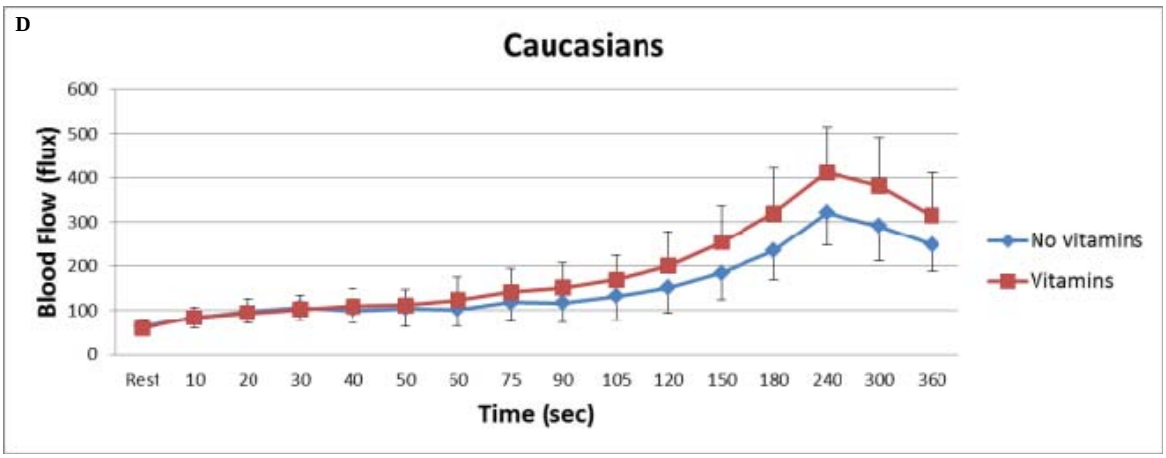


Figure 11. Mean ± SD of baseline blood flow (flux) measured during the 4 minutes period of vascular occlusion and the 2 minute period following the release of the occlusion cuff (A and B, previous page) and 6 minutes period of local heat (C and D) before and after the intake of vitamins for 14 days in 10 Koreans and 10 Caucasians.

Comparison of High Fat Meal and High Fat Meal with Vitamins

When comparing the BFR to 4 minutes of vascular occlusion at 2 hours after a HF meal with a previous intake of vitamins for 14 days to no vitamins in both ethnic groups, the skin blood flow was significantly higher when vitamins were given compared to no vitamins in Koreans ($p=0.001$). The peak blood flow after occlusion was 378.5 ± 73.7 flux with vitamins and 266.2 ± 61.7 flux without vitamins at 260 seconds,

respectively (Figure 12A). However, there were no significant differences in the skin blood flow in Caucasians (Figure 12B).

When comparing the BFR to 6 minutes of local heat at 2 hours after HF meal with a previous intake of vitamins for 14 days to no vitamins in both ethnic groups, the skin blood flow significantly increased with vitamins compared to no vitamins in Koreans ($p=0.024$). The peak blood flow after heat exposure was 317.2 ± 137.3 flux with vitamins and 173.5 ± 52.3 flux without vitamins at 240 seconds, respectively (Figure 12C). However, there were no significant differences in the skin blood flow in Caucasians (Figure 12D).

For the MDA concentration, there were no significant differences in both Koreans (11.1 ± 2.6 μM vs. 8.8 ± 2.7 μM) and Caucasians (7.4 ± 1.7 μM vs. 7.7 ± 2.4 μM). However, MDA concentration decreased by 20.36% in Koreans and increased by 4.75% in Caucasians (Figure 7).

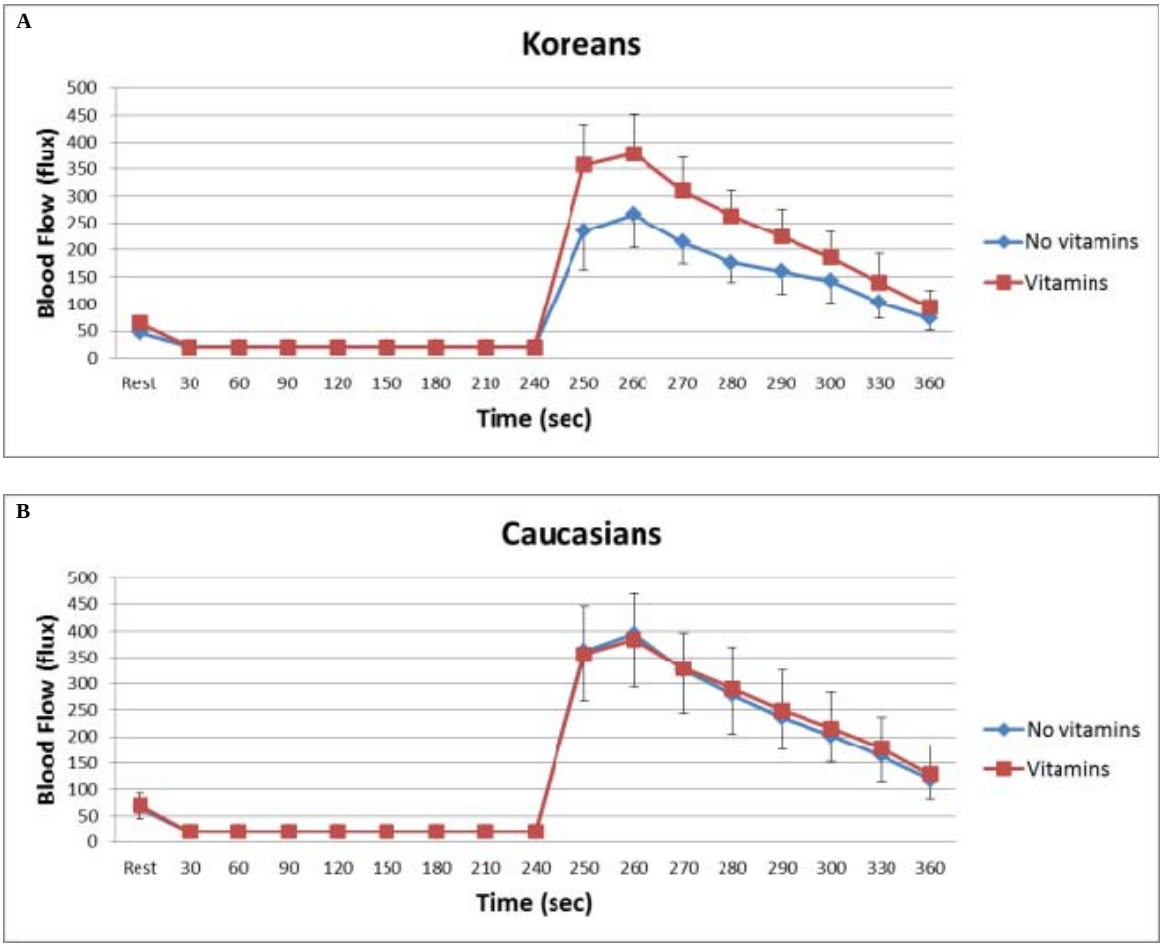


Figure 12. Mean ± SD of blood flow (flux) after a high fat meal measured during the 4 minutes period of vascular occlusion and the 2 minute period following the release of the occlusion cuff (A and B) and 6 minutes period of local heat (C and D, next page) before and after the intake of vitamins for 14 days in 10 Koreans and 10 Caucasians.

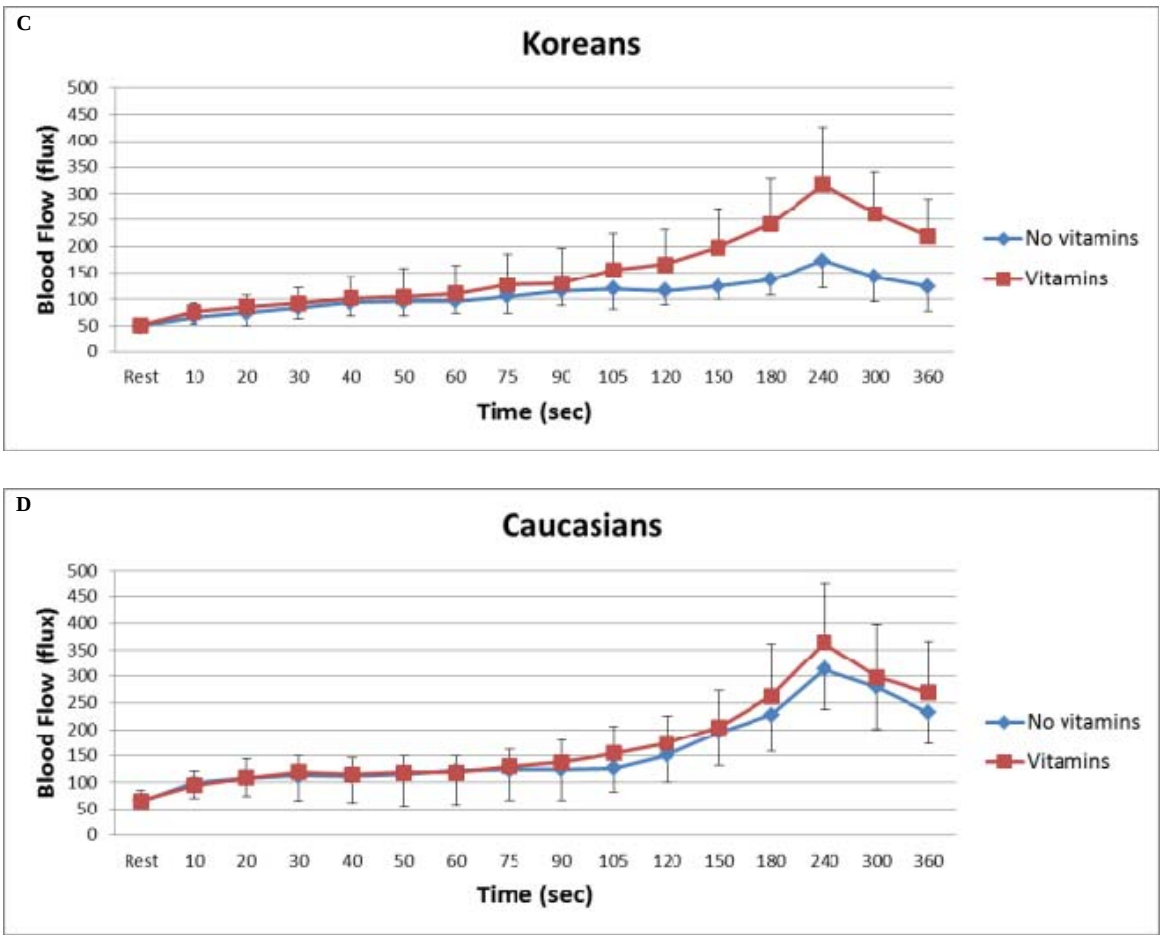


Figure 12. Mean ± SD of blood flow (flux) after a high fat meal measured during the 4 minutes period of vascular occlusion and the 2 minute period following the release of the occlusion cuff (A and B, previous page) and 6 minutes period of local heat (C and D) before and after the intake of vitamins for 14 days in 10 Koreans and 10 Caucasians.

Comparison of Koreans and Caucasians with High Fat Meal

When comparing the BFR to 4 minutes of vascular occlusion at 2 hours after a HF meal, the skin blood flow was significantly lower in Koreans compared to Caucasians ($p=0.001$). The peak blood flow after occlusion was 266.2 ± 61.6 flux in Koreans and 394.3 ± 100.7 flux in Caucasians, respectively (Figure 13A). However, there were no

significant differences in the skin blood flow at 2 hours after a HF meal with a previous intake of vitamins for 14 days between Koreans and Caucasians (Figure 13B).

When comparing the BFR to 6 minutes of local heat at 2 hours after high fat meal, the skin blood flow significantly lower in Koreans compared to Caucasians ($p=0.003$). The peak blood flow after heat exposure was 173.5 ± 52.3 flux in Koreans and 312.8 ± 74.2 flux in Caucasians at 240 seconds, respectively (Figure 13C). However, there were no significant differences in the skin blood flow at 2 hours after a HF meal with a previous intake of vitamins for 14 days between Koreans and Caucasians (Figure 13D).

For the MDA concentration, there were significant differences between Koreans and Caucasians (7.4 ± 1.7 μM vs. 11.1 ± 2.6 μM) at 2 hours after a HF meal ($p=0.004$). However, there were no significant differences between Koreans and Caucasians at 2 hours after a HF meal with a previous intake of vitamins for 14 days (Figure 7).

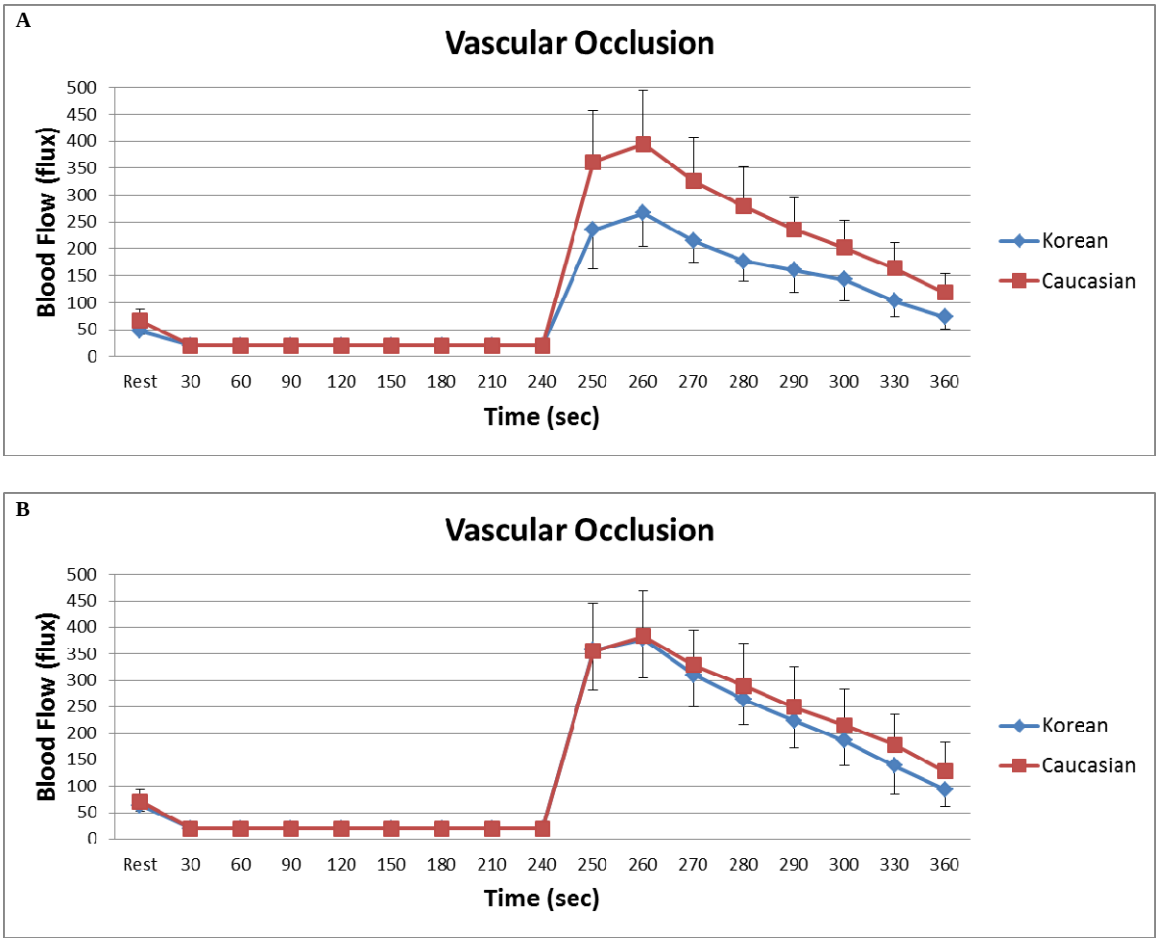


Figure 13. Mean ± SD of blood flow (flux) after a high fat meal measured during the 4 minutes period of vascular occlusion and the 2 minute period following the release of the occlusion cuff and 6 minutes period of local heat before (A and C) and after (B and D, next page) the intake of vitamins for 14 days between 10 Koreans and 10 Caucasians.

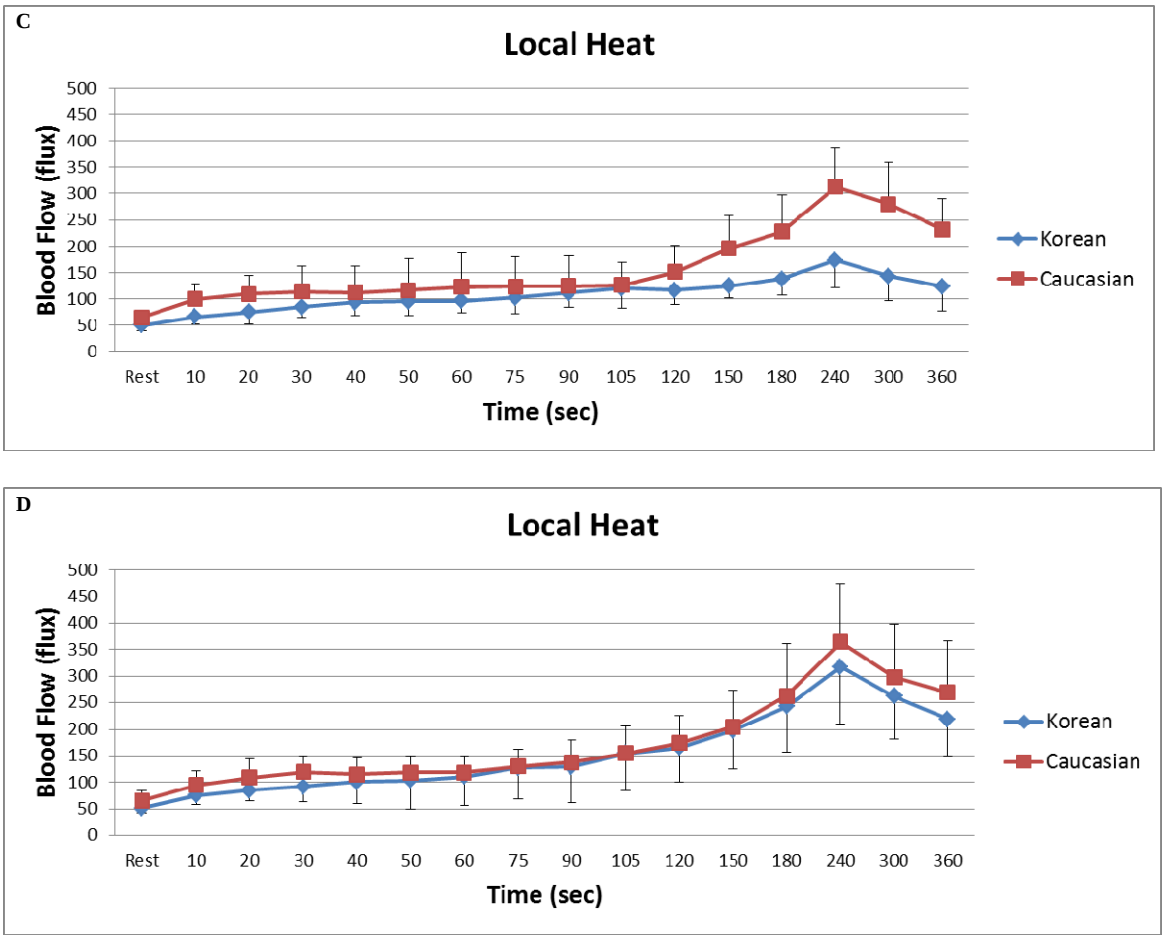


Figure 13. Mean ± SD of blood flow (flux) after a high fat meal measured during the 4 minutes period of vascular occlusion and the 2 minute period following the release of the occlusion cuff and 6 minutes period of local heat before (A and C, previous page) and after (B and D) the intake of vitamins for 14 days between 10 Koreans and 10 Caucasians.

Discussion

Hypertriglyceridemia (HTG) due to a HF diet is an independent risk factor of CVD and DM [28]. Furthermore, postprandial HTG induces endothelial dysfunction by enhanced oxidative stress [10]. Asians have a "thrifty" gene that heightens their susceptibility to a reduced endothelial response to stressors when eating HF foods or gaining body weight [3, 4, 6]. According to a recent study, even a single HF meal impaired endothelial response to stress in Asians but not in Caucasians [6]. A possible

mechanism of postprandial HTG-induced endothelial dysfunction is that HTG significantly stimulates leukocytes to produce free radicals. High concentrations of free radicals neutralize nitric oxide and prostacyclin, which in turn results in significantly decreased endothelial response to stressors that normally cause vasodilation [11, 15].

In the present investigation, we have found that Koreans have a lower baseline BFR to vascular occlusion and local heat compared to Caucasians. We further showed that just one single HF meal decreased the BFR to vascular occlusion and local heat in Koreans, but not in Caucasians and also increased oxidative stress, as measured by MDA in both Koreans and Caucasians. In addition, pre-treatment with antioxidant vitamins (vitamin C, vitamin E, and Coenzyme Q-10) for 14 days improved baseline BFR to vascular occlusion and local heat significantly in Koreans but not in Caucasians. Also, the vitamins markedly eliminated the decrease in the BFR to vascular occlusion and local heat following the HF meal in Koreans, but did not increase vasodilation in Caucasians. There are several intriguing findings in these data. First, the fact that the vascular response to 2 stressors, heat and occlusion were increased before a HF meal simply by taking vitamins for 2 weeks (antioxidants) in Korean but not Caucasians implies higher levels of harmful free radicals in the Korean group than the Caucasian group. This was confirmed in the MDA measurements. Since the Korean group had lower BMIs than the Caucasian group, it is not linked to obesity but must be linked to heredity of diet. The fact that endothelial function improved with antioxidants would seem to imply high free radicals due to their diet, which, as cited in the introduction, has been westernized in recent years. The higher concentrations of free radicals in the blood of the Koreans would not be surprising since, these Koreans had been in the United States for an average of 7

months and, unlike Caucasians, do not have the genetic makeup to allow for a westernized diet. As a result of the influence of the “thrifty" genes these subjects had higher free radicals and thus a diminished BFR to heat and occlusion. With vitamin administration, free radicals fell and the BFR to the stressors examined here matched that of Caucasians. Thus there seems to be no permanent damage to endothelial cells that could be reversed by vitamins or perhaps diet. And yet these were young subjects. Older subjects, after years of HF meals may have permanent damage that cannot be reversed by vitamins.

A second finding is that in Koreans, but not in Caucasians, a single HF meal can impair endothelial response to stressors through an oxidative stress mechanism and can be reversed by antioxidant vitamins probably through scavenging free radicals within the vasculature. This finding of decreased endothelial response to stressors after ingestion of a single HF meal in Koreans, but not in Caucasians, may also due to the influence of “thrifty” genes.) The “thrifty” genotype developed in populations where food supply was limited due to famine. People with “thrifty” genes could store fat better than others and were therefore more likely to survive prolonged food shortages [6]. In affluent societies, however, the “thrifty” genotype has become disadvantageous, leading to an increased risk of cardiovascular disease and diabetes. This “thrifty” genotype, which is composed of many single nucleotide polymorphisms (SNPs), is a genetic difference regulating lipid metabolism and fat storage, and differs different depending on ethnicity [5, 29-31]. For example, one of the “thrifty” SNPs related to lipid metabolism, fatty acid-binding protein 2 (FABP2), has been associated with obesity because it enhances fat absorption. The allelic frequency of FABP2 is 55% in Asians and 27.1% in Caucasians. Thus, if Asians

consume the same amount of fat, a higher body fat deposit at a lower or the same BMI will be observed in Asians [29, 30]. South Korea is one of the countries where the socioeconomic environment has changed rapidly to reflect more westernization as well as its negative health consequences. For the adoption of a more westernized lifestyle, higher dietary fat consumption and less physical activity are common in Korea, and hence, these "thrifty" genes heighten the susceptibility to insulin insensitivity and CVD in relation to increased body fat and dyslipidemia in Koreans [6, 30, 31]. Increased body fat and dyslipidemia stimulate leukocytes to induce free radicals. The high concentration of free radicals neutralizes NO and prostacyclin, the 2 principal vasodilators, and reduces BFR to vascular occlusion and local heat [6, 15]. These findings may explain why Koreans had a lower BFR to vascular occlusion and local heat and higher MDA concentration after ingestion of a single HF meal compared to Caucasians in this study. It should be pointed out that international studies among different Asian national populations in China, Korea, Philippines, Singapore, and Taiwan show increased risk of Type 2 DM and CVD at lower BMI than European populations [32].

Numerous studies have demonstrated that antioxidant vitamins play an important role in increasing endothelial function and decreasing oxidative stress [15, 33, 34]. To our knowledge, no study has examined the effects of antioxidant vitamins on endothelial function by ethnicity. In the present study, antioxidant vitamins of 1000mg of vitamin C, 800IU of vitamin E, and 300mg of Coenzyme Q-10 were given to Korean and Caucasian subjects for 14 days. Most investigators use 400-800 IU of vitamin E, 500-1000mg of vitamin C, and 60-300mg of Coenzyme Q-10. This pre-treatment of vitamins restored decreased endothelial response to stressors following a HF meal in Koreans but didn't

improve endothelial response to stressors in Caucasians. Koreans are more susceptible to reduced endothelial response to stressors when eating HF foods compared to Caucasians. Moreover, vitamins restore impaired endothelial response to stressors by scavenging free radicals, but may not improve normal endothelial response to stressors [6, 35]. Several studies showed that antioxidant vitamins improved endothelial function in people with DM, coronary artery disease, and smokers but not in healthy control groups [36, 37]. In some studies antioxidant vitamins did not change oxidative stress status in healthy athletes [35]. However, in a study of young health men and women, the response to heat, unlike the response to occlusion, was increased by administration of large doses of vitamins in young, fit students at similar dosages to the present investigation and also for 2 weeks [15].

Previous studies have suggested that antioxidant vitamins C and E improve vascular defense against oxidative stress by reducing free radicals and protecting NO from inactivation, thereby exerting beneficial effects on vascular function and structure [15, 34]. Especially, combined administration of vitamins C and E significantly increases endothelium-dependent vasodilation, while monotherapy with vitamin C alone is ineffective [33, 34, 38]. It is known that vitamins C and E have synergistic antioxidant actions, since vitamin E can have pro-oxidant properties and appropriate concentrations of vitamin C are necessary for the regeneration of vitamin E, thus increasing its antioxidant capacity [34]. These previous findings support the results from this study.

Baseline BFR was significantly lower in Koreans than Caucasians by 17.54%. After stress with heat, the blood flow response was 44.54% less in Koreans and with occlusion it was 32.49% less than that seen for Caucasians. Certainly, some of the

difference between the blood flow response to stress may be due to a lower baseline blood flow in Koreans. However, the difference with stress was proportionally much higher after heat and occlusion. Thus it would suggest that not only is the baseline BFR causing this difference but certainly some other factors are also involved to increase the difference with stress between Koreans and Caucasians. Perhaps the most telling evidence was the baseline blood flow after vitamin ingestion in the two groups of subjects. After taking the vitamins, there was no significant difference in baseline blood flow between the two groups. This shows that the difference in the blood flow response to stress is not only due to the difference in baseline blood flow but also due to higher oxidative level in Koreans, corrected by vitamin administration.

In this study, ten young Korean subjects had been living in the United States, rather than in Korea. Although the mean period of stay in US was only 7.1 ± 2.3 months in the Korean subjects, results may be different in Koreans who did not reside in the US. In addition, due to different diets especially HF diet and environment, Koreans who have been in the US for longer periods may have more reduced endothelial response to stressors than those with shorter residing periods. Therefore, further studies need to be conducted with Koreans for those who have been in the US for long periods and those who have not been to US. Moreover, in the present investigation, the reduced endothelial response to stressors was reversed by vitamins at baseline and even after a single HF meal in this young population. However, with longer periods of HF intake among young subjects or in the elderly, the recovery effects of vitamins on reduced endothelial response to stressors might be different. Prolonged high free radicals will probably cause permanent endothelial dysfunction.

Conclusions

This study evaluated postprandial endothelial function by measuring the BFR to vascular occlusion and local heat before and after a HF meal and the interventional effects of anti-oxidant vitamins on improving endothelial function in young Korean-Asians compared to Caucasians. The skin BFR to vascular occlusion and local heat following a HF meal significantly decreased and free radicals significantly increased at 2 hours compared to baseline in Koreans, but not in Caucasians. When vitamins were given, BFR to vascular occlusion and local heat before and after HF meal were not significantly lower in Koreans than Caucasians. These findings suggest that even a single HF meal can reduce endothelial response to stress through an oxidative stress mechanism but can be blocked by antioxidants, probably through scavenging free radicals in Koreans. Koreans and other Asians historically have a diet which is healthier than Caucasians because of the large number of vegetables and low fat intake. However, the present investigation shows that a westernized diet is causing high oxidative levels in Koreans impairing endothelial function. Since Asians have “thrifty” genes, if they continue consuming a westernized diet, they need to take vitamin supplements or go back to their historic low fat diets. These levels of vitamins can not be achieved by diet alone.

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CHAPTER FOUR

DISCUSSION

Heat has been used as a therapeutic modality for centuries to manage soft tissue and joint injuries with specific goals of relieving pain, promoting healing tissues, and affecting the plasticity of connective tissue, including muscle, tendon, ligament, and joint capsule. [4] While heat has positive effects on tissues, there is always a chance of burning the skin. [6] Therefore, the ability of the skin to remove heat is very important for protecting the skin from damage and skin blood flow plays a major role in removing heat. [7] Vascular endothelial cells mediate BFR to heat by releasing factors that cause the surrounding smooth muscle either to contract or dilate. [29,30,60] NO is one of the most predominant dilators. [15] Reduced NO bioavailability by reacting with free radicals is a major mechanism of endothelial dysfunction. [35] HTG in HF foods significantly stimulates leukocytes to produce free radicals. Generally, Asians are overlooked for the risk of cardiac health problems as they tend to be slimmer and smaller compared to Caucasians. However, incidence of CVD is rapidly becoming the major health problem in Asian countries and increasing incidence in Asians who have thrifty genes is known to be related to the adoption of a more Westernized lifestyle in which high dietary fat consumption is common. [25,61] Little is known about measuring the effects of a high fat meal and vitamins on the level of free radicals and EF in Koreans compared to Caucasians. The purpose of this study was to evaluate baseline and postprandial EF as related to enhanced oxidative stress in a single HF and low fat (LF) meal and more

importantly the interventional effects of vitamins on scavenging free radicals and improving endothelial function in Korean-Asians compared to Caucasians. This was done by measuring skin BFR to vascular occlusion and local heat and analyzing Malondialdehyde (MDA) levels in both groups after ingestion of a HF and LF meal before and after vitamin intervention.

In the present investigation, we found that Koreans had a lower baseline BFR to vascular occlusion and local heat compared to Caucasians. We further showed that just one single HF meal decreased the BFR to vascular occlusion and local heat in Koreans, but not in Caucasians and also increased oxidative stress, as measured by MDA in both Koreans and Caucasians. This finding in Koreans, but not in Caucasians, may also due to the influence of "thrifty" genes. For the adoption of a more Westernized lifestyle, higher dietary fat consumption is common in Korea, and hence, these "thrifty" genes heighten the susceptibility to endothelial dysfunction by increasing free radicals in Koreans. [24,45,46] Koreans had higher free radicals and thus a diminished blood flow response to heat and occlusion compared to Caucasians. According to a recent study, even a single HF meal impaired endothelial response to stress in Asians but not in Caucasians. [46]

Moreover, the results of this study showed that pre-treatment with antioxidant vitamins (vitamin C, vitamin E, and Coenzyme Q-10) for 14 days improved baseline BFR to vascular occlusion and local heat significantly in Koreans but not in Caucasians. Also, the vitamins markedly eliminated the decrease in the BFR to vascular occlusion and local heat following the HF meal in Koreans, but did not increase vasodilation in Caucasians. Antioxidant vitamins play an important role in increasing EF and decreasing oxidative stress through scavenging free radicals. [39,54,55] Therefore, with vitamin

administration, free radicals were reduced and the BFR to the stressors examined matched that of Caucasians. Moreover, vitamins restore impaired endothelial response to stressors by scavenging free radicals, but may not improve normal endothelial response to stressors. [46,59] Previous studies have shown that antioxidant vitamins improved EF in people with DM, coronary artery disease, and smokers but not in healthy control groups. [56,57] However, some studies using antioxidant vitamins did not change oxidative stress status in healthy athletes. [59]

In conclusion, even a single HF meal can reduce endothelial response to stress through an oxidative stress mechanism but can be blocked by antioxidants, probably through scavenging free radicals in Koreans by improving EF and preventing a skin burn during heat application. Since Asians have "thrifty" genes, if they continue consuming a Westernized diet, they need to take vitamin supplements or return to their historic low fat diets.

We identified several limitations in the present study. In this study, ten young Korean subjects had been living in the United States, rather than in Korea. Although the mean period of stay in US was only 7.1 ± 2.3 months in the Korean subjects, results may be different in Koreans who did not reside in the US. In addition, due to different diets especially HF diet and environment, Koreans who have been in the US for longer periods of time may have more reduced endothelial response to stressors than those with shorter residing periods. Therefore, further studies need to be conducted with Koreans for individuals who have been in the US for long periods of time and those who have not been to the USA. Moreover, in the present investigation, the reduced endothelial response to stressors was reversed by vitamins at baseline and even after a single HF meal in this

young population. However, with longer periods of HF intake among young subjects or in the elderly, the recovery effects of vitamins on reduced endothelial response to stressors should be different. Prolonged high free radicals will probably cause permanent endothelial dysfunction. Also, with different kinds and doses of vitamins on different statuses of diseases such as DM and CVD, the recovery effects of vitamins on reduced endothelial response to stressors should be different.

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APPENDIX A

INFORMED CONSENT FORM

LOMA LINDA UNIVERSITY
School of Allied Health Professions

Informed Consent to Participate in Research

"Vascular endothelial function and diet in Koreans and Caucasians"

PURPOSE

You are invited to participate in a study which will examine if there is a difference in the blood vessels and the blood flow response to local heat and reduction of circulation in the skin after ingestion of a single high-fat meal in people who are either Caucasians or Koreans. Since these two ethnic groups have different genetic backgrounds which alter their skin blood flow, we want to see the difference in how these groups respond to heat, high-fat meals, and reduction of circulation.

PROCEDURES

We are looking for young healthy subjects in the age range of 20-35 years old who are either Caucasians or Koreans. There will be 20 subjects in each group. The experiment will be conducted in a controlled temperature room (72°F) and you will be resting comfortably for 20 minutes. On the first day, before beginning the experiments, physical characteristics will be measured including height, weight, blood pressure, body mass index, fat thickness under the skin, and skin thickness. The experiments will be divided into three basic sections and require 20 days consecutively.

The first set of experiments

Day 1 - 100°F heat will be applied to forearm for six minutes; the skin temperature and blood flow will be measured with a temperature sensor and a Laser beam.

- A blood sample (two tsp) will be taken.

Day 2 - 104°F heat will be applied to forearm for six minutes; the skin temperature and blood flow will be measured.

Day 3 - 108°F heat will be applied to forearm for six minutes; the skin temperature and blood flow will be measured.

The Second set of experiments

Day 4 - After 12 hours overnight fast, you will be given a high-fat meal (breakfast).

_____Initial
_____Date

Loma Linda University
Adventist Health Sciences Center
Institutional Review Board
Approved 7/25/11 Void after 4/28/2012
5110058 Chair R L Rigsby

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- Before ingestion of the high-fat meal, you will be exposed to heat at 108°F on forearm for six minutes with measuring the skin temperature and blood flow. Also, reduction of circulation will be evoked by applying a blood pressure cuff on your upper arm for four minutes followed by release and measurement of skin blood flow for two minutes.

- After two hours following the high-fat meal, same measurements will be conducted as above.

- A blood sample (two tsp) will be taken after two hours following the high-fat meal.

Day 5 - Day 11 - No study activities for a wash out period

Day 12 - After 12 hours overnight fast, you will be given a low-fat meal (breakfast).

- Before ingestion of the low-fat meal, same measurements will be conducted as Day 4.

- After two hours following the low-fat meal, same measurements will be conducted as Day 4.

- A blood sample (two tsp) will be taken after two hours following the low-fat meal.

The third set of experiments

Day 13 - Day 26 - Ingestion daily of 1000mg of vitamin C, 800IU of vitamin E and 300mg of Coenzyme Q-10 at home

Day 27 - After 12 hours overnight fast, you will be given a high-fat meal.

- Before ingestion of the high-fat meal, same measurements will be conducted as Day 4

- After two hours following the high-fat meal, same measurements will be conducted as Day 4.

- A blood sample (two tsp) will be taken before and after two hours following the high-fat meal.

_____Initial
_____Date

RISKS

Thermodes may feel warm but since the thermode is water jacket controlled through a water bath that is controlled by a computer control system, there is very little chance of skin irritation or getting a burn. The Institutional Review Board of Loma Linda University has determined the risks for this study are minimal.

BENEFITS

There will probably be no benefits to you personally. However, the study may provide a better understanding of racial differences in the response to anoxia and heat of the skin. It may also show the interaction of diet and skin blood flow.

PARTICIPANTS RIGHTS

Participation is voluntary. You may leave the study at any time. If at any time during a procedure you experience tiredness or discomfort beyond what you are willing to endure, just tell the person conducting the procedure you want to stop. This decision will NOT affect your standing with those conducting the study or loss of benefits that you are entitled to.

CONFIDENTIALITY

All records will be confidential. We will not disclose your participation without your written permission. Any publication resulting from this study will refer to you by ID number and not by your name. See attached Authorization for Use of Protected Health Information (PHI) form regarding your privacy rights.

COSTS/COMPENSATION

There is no cost for participating in these studies; you will receive monetary compensation of $100 for participation.

IMPARTIAL THIRD PARTY

If you wish to contact a third party not associated with the study for any questions or a complaint, you may contact the Office of Patient Relations at Loma Linda University, Loma Linda University Medical Center, Loma Linda, California 92354. Phone (909) 558-4647.

INFORMED CONSENT STATEMENT

I have read the contents of the consent form and the California Experimental Subject's Bill of Rights and have listened to the verbal explanation given by the investigator. My questions regarding the study have been answered to my satisfaction. I hereby give voluntary consent to participate in the study described here. Signing this form does not waive my rights nor does it release the responsibilities of the principal investigator, Jerrold Petrofsky Ph. D. or Loma Linda University of their responsibilities. I may call Dr. Jerrold Petrofsky during routine office hours at (909) 558 4300 ex 82186 or leave a voice mail message at this number during non office hours.

_____Initial
_____Date

"Vascular endothelial function and diet in Koreans and Caucasians"

_____ _____

Signature of subject Date

INVESTIGATOR'S STATEMENT

I have reviewed the contents of the consent form and the California Experimental Subject's Bill of Rights with the person signing above. I have explained potential risks and benefits of the study.

Signature of investigator _____

Phone Number _____ Date _____

APPENDIX B

AUTORIZATION FOR USE OF PROTECTED HEALTH INFORMATION FORM

INSTITUTIONAL REVIEW BOARD
Authorization for Use of Protected Health Information (PHI)

OSR#

Per 45 CFR §164.508(b)

OFFICE OF SPONSORED RESEARCH
Loma Linda University • 11188 Anderson Street • Loma Linda, CA 92350
(909) 558-4531 (voice) / (909) 558-0131 (fax)

TITLE OF STUDY:	Vascular endothelial function and diet in Koreans and Caucasians
PRINCIPAL INVESTIGATOR:	Jerrold Petrofsky, Ph D
Others who will use, collect, or share PHI:	Authorized research personnel

The study named above may be performed only by using personal information relating to your health. National and international data protection regulations give you the right to control the use of your medical information. Therefore, by signing this form, you specifically authorize your medical information to be used or shared as described below.

The following personal information, considered "Protected Health Information" (PHI) is needed to conduct this study and may include, but is not limited to: name, age, contact number, the results of all measurements and procedures performed.

The individual(s) listed above will use or share this PHI in the course of this study with the Institutional Review Board (IRB) and the Office of Research Affairs of Loma Linda University.

The main reason for sharing this information is to be able to conduct the study as described earlier in the consent form. In addition, it is shared to ensure that the study meets legal, institutional, and accreditation standards. Information may also be shared to report adverse events or situations that may help prevent placing other individuals at risk.

All reasonable efforts will be used to protect the confidentiality of your PHI, which may be shared with others to support this study, to carry out their responsibilities, to conduct public health reporting and to comply with the law as applicable. Those who receive the PHI may share with others if they are required by law, and they may share it with others who may not need to follow the federal privacy rule.

Loma Linda University
Adventist Health Sciences Center
Institutional Review Board
Approved 4/29/11 Void after, 4/28/2012
#5110058 Chair R L Rigsby MD

OSR 5/4/2011

Subject to any legal limitations, you have the right to access any protected health information created during this study. You may request this information from the Principal Investigator named above but it will only become available after the study analyses are complete. The authorization expires upon the conclusion of this research study.

You may change your mind about this authorization at any time. If this happens, you must withdraw your permission in writing. Beginning on the date you withdraw your permission, no new personal health information will be used for this study. However, study personnel may continue to use the health information that was provided before you withdrew your permission. If you sign this form and enter the study, but later change your mind and withdraw your permission, you will be removed from the study at that time. To withdraw your permission, please contact the Principal Investigator or study personnel at (909) 558-7274

You may refuse to sign this authorization. Refusing to sign will not affect the present or future care you receive at this institution and will not cause any penalty or loss of benefits to which you are entitled. However, if you do not sign this authorization form, you will not be able to take part in the study for which you are being considered. You will receive a copy of this signed and dated authorization prior to your participation in this study.

I agree that my personal health information may be used for the study purposes described in this form.

Signature of Patient *or* Patient's Legal Representative	Date
Printed Name of Legal Representative (if any)	Representative's Authority to Act for Patient
Signature of Investigator Obtaining Authorization	Date

Loma Linda University
Adventist Health Sciences Center
Institutional Review Board
Approved 4/29/11 Void after, 4/28/2012
5100058 Chair R L Rigsby MD

OSR 5/4/2011

APPENDIX C

CALIFORNIA EXPERIMENTAL SUBJECT'S BILL OF RIGHTS FORM

CALIFORNIA EXPERIMENTAL SUBJECT'S BILL OF RIGHTS

You have been asked to participate as a subject in an experimental clinical procedure. Before you decide whether you want to participate in the experimental procedure, you have a right to:

1. Be informed of the nature and purpose of the experiment.
2. Be given an explanation of the procedures to be followed in the medical experiment, and any drug or device to be utilized.
3. Be given a description of any attendant discomforts and risks reasonably to be expected from the experiment.
4. Be given an explanation of any benefits to the subject reasonably to be expected from the experiment, if applicable.
5. Be given a disclosure of any appropriate alternative procedures, drugs or devices that might be advantageous to the subject, and their relative risks and benefits.
6. Be informed of the avenues of medical treatment, if any available to the subject after the experiment if complications should arise.
7. Be given an opportunity to ask any questions concerning the experiment or the procedure involved.
8. Be instructed that consent to participate in the medical experiment may be withdrawn at any time and the subject may discontinue participation in the medical experiment without prejudice.
9. Be given a copy of any signed and dated written consent form used in relation to the experiment.
10. Be given the opportunity to decide to consent or not to consent to a medical experiment without the intervention of any element of force, fraud, deceit, duress, coercion or undue influence on the subject's decision.

I have carefully read the information contained above in the "California Experimental Subject's Bill of Rights" and I understand fully my rights as a potential subject in a medical experiment involving people as subjects.

_____	_____
Date	Patient
_____	_____
Time	Parent/Legal Guardian

If signed by other than the patient, indicate relationship:

_____	_____
Relationship	Witness