INSULIN RESISTANCE AND CONCOMITANT MACRO- AND MICROVASCULAR DYSFUNCTION IN NORMOGLYCEMIC COLLEGE-AGE SUBJECTS WITH A FAMILY HISTORY OF TYPE 2 DIABETES

by

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B.S., Kansas State University, 1978
M.S., Kansas State University, 1984

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Anatomy and Physiology
College of Veterinary Medicine

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Abstract

The overall aims of this dissertation are to determine the incidence and magnitude of insulin resistance (IR) in a cohort of normoglycemic college-age subjects with a family history of type 2 diabetes, and to ascertain if there is early macro- and microvascular dysfunction relative to IR. Study 1 (Chapter 2) revealed a 7-fold range in IR in healthy college subjects concomitant with measures of insulin, both fasted and during an oral glucose tolerance test, but not related with any measure of plasma glucose. These results emphasize that early in the etiology of carbohydrate dysregulation, abnormalities first occur with regard to insulin sensitivity. Using brachial artery blood flow (BABF, Doppler fluxometry) and near-infrared spectroscopy (NIRS) (Chapter 3) we extended the understanding of the use of these non-invasive tools to assess forearm resting metabolic rate and to compare the parameters of both the NIRS oxy-hemoglobin signal, as a index of perfusion in the microcirculation, and BABF, as an independent measure of microvascular reactivity during post occlusive reactive hyperemia (PORH). Resting metabolic rate ranged ~ 2 fold (2.83-5.15 μMO_2/min/100g) similar to direct measures. Amplitude, but not kinetic parameters for NIRS variables correlated with comparable parameters for BABF, providing evidence for the possible utility of NIRS in examining microvascular reactivity. In study 3 (Chapter 4), utilizing our extended understanding of hemodynamics garnered from the results of study 2, we assessed the influence of IR on macro- and microvascular reactivity. We observed that i) the magnitude of IR was significantly correlated with attenuation of endothelium-dependent vasodilation of the brachial artery ($P< .01$) indicating the possibility of a reduced nitric oxide bioavailability and an enhanced atherogenic milieu. Additionally we found ii) BABF at rest and during reactive hyperemia to be strongly correlated with conductance (reduced downstream resistance—an indicator of microvascular control abnormalities) independent of forearm metabolic rate, and iii) parameters of BABF (microvascular response) were also strongly correlated with brachial artery vasoreactivity (macrovascular response). In conclusion, this body of work furthers our insight into the need for earlier identification of "disease" earlier in the progression to type 2 diabetes, and provides direction for future investigations into prevention / intervention to improve microvessel functionality and to slow the atherosclerotic process in larger vessels.

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No man is an island, entire of itself; every man is a piece of the continent, a part of the main.

John Donne

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Dedication

To my mother, who even after over 20 years of life with diabetes, still fights the manifestations and implications of this disease with courage and grace.

And to my children, who I hope and pray never will.

CHAPTER 1 - Introduction

There is recent substantive evidence to support the hypothesis that in the etiology of type 2 diabetes, insulin resistance (IR) may increase for over a decade (23) before dysglycemia, at levels which identify impaired glucose tolerance (2, 17), is measured (21). During the interim, normal glucose tolerance can be achieved when a change in insulin sensitivity is mirrored by a reciprocal change in insulin secretion (as reviewed by Bergman et al.(5)). The current clinical protocol for identifying frank "disease" occurs commensurate to IR, which is not matched by compensatory hyperinsulinemia (19, 21) and postdates the progression through IR/hyperinsulinemia. Yet, IR/hyperinsulinemia, in and of itself, is not only predictive of type 2 diabetes (39) but is inexplicably linked to the presence of dyslipidemias (as reviewed by Scott, 2006 (40)), hypertension and hypercoagulability, and attenuated endothelial function (10, 34). Thus, IR/hyperinsulinemic appears to be mechanistically (32) and epidemiologically (31) related to the risk of developing atherosclerosis and cardiovascular disease.

Therefore, the early identification of IR in the healthy young adult (8) with a family history (genetic predisposition) of type 2 diabetes (38) is relevant to both medical practice and to preventative measures to improve the quality of life. However, research in this area is minimal for people between ages late 20s-40s (as reviewed in (43)), and is virtually nonexistent in the youngest adults, in their late teens and early twenties.

In this context, Study 1 (Chapter 2) was developed to ascertain the incidence and range of IR and hyperinsulinemia in a cohort of healthy, normoglycemic, college-age subjects with a family history of type 2 diabetes. A simple and practical method of assessment was used, both in fasted and postprandial glucose stimulated states. We hypothesized that there is a high prevalence of IR, indiscernible using dysglycemia criteria alone, in these young adults, but that fasting insulin or measures of insulin during an oral glucose tolerance test are highly sensitive means of quantifying IR.

Impaired microvascular vasoreactivity can also predate disease states, such as type 2 diabetes (9). It has been hypothesized that microvascular dysfunction in skeletal muscle may be mechanistically linked to insulin resistance (42). Due to ethical considerations, assessment of the microcirculation in humans to date is limited to superficial vascular beds and most often includes laser Doppler fluxmetry (skin pulp blood flow), transcutaneous oxygen tension ($TcpO_2$),

iontophoresis (for the subdermal delivery of vasoactive drugs), and/or capillaroscopy (light microscopy) (1). However, data acquired on vessels and tissues assessed by these methods (skin, finger nail fold or conjunctiva) may not necessarily be relevant to other areas, such as skeletal muscle in which control of the microcirculation may be different than for cutaneous beds (49).

These limitations to the assessment of the microcirculation in skeletal muscle, the largest repository of glucose and potentially the most prognostic organ relative to the progression to type 2 diabetes, was the impetus for Study 2 (Chapter 3). In this study we assessed the validity and effectiveness of near infrared spectroscopy (NIRS) to estimate, non-invasively, skeletal muscle metabolic rate and skeletal muscle microcirculatory reactivity in humans.

Although the utility of NIRS as a tool in physiological and clinical investigations (11) is not new (25) and although this methodology has gained acceptance for determining muscle metabolic rate (as reviewed in (20)), results via this technique do not correlate with the established method for measurements of muscle metabolic rate by the direct Fick method using blood flow and arterio-venous oxygen content differences (7, 47). There is also wide variability in reported values for NIRS derived forearm skeletal muscle metabolic rate (14-16, 24, 47)

This variability with NIRS measures appear to be due, in part, to the fact that adipose tissue thickness (ATT) is a substantial confounding influence on in vivo NIRS measurements and must be factored into NIRS muscle studies (6). ATT has been demonstrated to quantitatively affect NIRS measures of oxygen consumption (VO_2) in forearm skeletal muscle (46). Correction methods for the influence of the fat layer on NIRS derived metabolic rate have been proposed (6, 33, 41, 45, 50, 51). However, it is unclear whether any of these methods are definitive corrections or if they are applicable and necessary to Frequency Domain Multi Distance NIRS used in this study. In Study 2 (Chapter 3) we determined quantitative measurements of forearm skeletal muscle metabolic rate, with appropriate correction for ATT, which correspond to values reported utilizing the direct Fick method. This study also formed the methodological basis for Study 3 (Chapter 4).

Post occlusive reactive hyperemia (PORH) has been used to evaluate the functional aspects of skeletal muscle microcirculation in conjunction with NIRS, but only relative to peripheral vascular disease (12, 26-28). We propose that changes in the oxy-hemoglobin/myoglobin ($[HbO_2]$) signal in the initial phase of PORH reflect changes in perfusion in the microcirculation during this time period. To test this, we compared measures of $[HbO_2]$ to

corresponding measures of brachial artery blood flow (as an independent measure of microvascular reactivity) during PORH, since upstream conduit artery flow (i.e. hyperemic response) should represent the integral of all the changes in perfusion in the downstream microvasculature (i.e. hyperemic stimulus) (13, 30, 48).

Collectively, the findings reported in Chapter 3, have extended our understanding of a non-invasive technique for examining skeletal muscle metabolic and microvascular responses in humans. NIRS has potential for examining dysfunction and to assess therapeutic interventions for efficacy in improving tissue oxygenation and hemodynamics.

Since macro- and microvascular disease are the leading causes of morbidity and mortality in patients with type 2 diabetes (18), the capstone for the series of studies in this dissertation was the desire to ascertain whether IR, in young normoglycemic adults would have an impact on vasoreactivity.

In the natural history to type 2 diabetes, metabolic and physiologic changes begin early and proceed in tandem (32). This process is exacerbated by, but not dependent on, hyperglycemia. This association is corroborated by the conclusion that insulin resistance in young adults predicts coronary heart disease risk factors (3). In this context, the question arose: How early can functional macro- and microangiopathy be detected relative to IR?

The few previous studies that have looked at endothelial-dependent macrovascular dysfunction in normoglycemic subjects included those that ranged in age between the late 20s and old age (4, 9, 22, 29, 44). The literature taken together is also unequivocal that impaired microvascular reactivity is clearly present in diabetes and prediabetes but there is sparse information that predates dysglycemia in the continuum toward frank disease. Further, when reviewing the vast majority of literature involving flow mediated dilation there are crucial methodological considerations that must, but often have not, been considered. For instance, the response is almost never standardized by the magnitude of the stimulus (shear or shear rate) that mediates the response even though there is compelling justification to do so (30, 35-37). It is our contention that this shortcoming invalidates the results and conclusions of many of these investigations.

In Chapter 4 we combined measurements of FMD, corrected for the stimulus, with the techniques developed in Chapter 3, to examine and compare conduit artery and microvascular

reactivity relative to the magnitude of IR in normoglycemic college-age students with a family history of type 2 diabetes

The studies described herein were designed to further our understanding of insulin resistance and abnormalities in vascular reactivity before the onset of “pre-diabetes” in young normoglycemic college-age adults. Each chapter is self-contained (Abstract, Introduction, Methods, Results, Discussion and Conclusions and References).

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CHAPTER 2 - Insulin Resistance/Hyperinsulinemia in Normoglycemic College-age Subjects at Risk for Type 2 Diabetes

Abstract

Little is known about insulin resistance (IR) in young adults before the onset of pre-diabetes. The goal of this study was to determine insulin sensitivity, measuring plasma insulin, in the fasting state and during an oral glucose tolerance test (OGTT), in normoglycemic college-aged subjects with a family history of type 2 diabetes. Insulin sensitivity for 26 normal glucose tolerant (NGT), normotensive subjects, age 18-26 yrs., was calculated by homeostasis model assessment (ISI_{HOMA}) and from the Matsuda and DeFronzo index [ISI_{COMP} = 10,000/square root of (fasting glucose x fasting insulin) x (mean glucose x mean insulin during an OGTT)]. Body composition was assessed by dual-energy X-ray absorptiometry. Subjects were normoglycemic in fasting (84.6 $\pm$ 6.6 mg/dl) and at 2 hours OGTT (92.8 $\pm$ 19.3 mg/dl). Subjects exhibited a 7-fold range in ISI_{COMP} (10.3 $\pm$ 4.6, range 3.6-21.8). 23% were determined IR by ISI_{HOMA} ($\leq$ 0.58) and up to 54% by ISI_{COMP} ($\leq$ 9.7). ISI_{COMP} was negatively correlated with fasting insulin (r=-0.78) as well as average insulin, insulin area under the curve, and 2-hour insulin during an OGTT (r=-0.86, -0.79, -0.70 respectively, P $\leq$ 0.0001) but not with fasting glucose (r=-0.28) or 2-h glucose (r=-0.34). Inverse correlations were present with systolic blood pressure (r=-0.46, P < 0.01) and triglyceride/HDL (r=-0.38, P < 0.05) as well as waist circumference, BMI, abdominal fat %, and total body fat % but only when obese subjects were included. We conclude that IR is prevalent in NGT college-age subjects with family history of type 2 diabetes. Fasting and OGTT measures of insulin are predictive of IR for this age group, while parallel measures of glucose are not discriminative.

Introduction

"Pre-diabetes" (1) and Metabolic Syndrome X (2) include insulin resistance (IR), as identified by impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) as diagnostic criteria (3,4). Normal glucose tolerance (NGT) can be achieved when a change in insulin sensitivity is mirrored by a reciprocal change in insulin secretion (as reviewed by Bergman et al.(5)). "Disease" occurs when an increased IR is not matched by compensatory hyperinsulinemia (6,7).

For individuals born in 2000, the estimated lifetime risk for type 2 diabetes, viewed by some as end-stage IR (8), is ~33% for males and ~39% for females (9). The hypothesis that IR may increase for over a decade before dysglycemia is detected, at levels that identify IGT (10), has gained recent support (7).

IR/hyperinsulinemia, in and of itself, is not only predictive of type 2 diabetes (11) but there is a plethora of information linking IR to the presence of dyslipidemias (as reviewed by Scott, 2006(12)), hypertension and a hypercoagulable state, and attenuated endothelial function (13,14). Thus, IR is mechanistically (8) and epidemiologically (15) related to the risk of developing atherosclerosis and cardiovascular disease.

Given that the current clinical protocol for identifying "disease" postdates the progression through IR/hyperinsulinemia there is need for research on the seemingly healthy young adult (16) with a family history (FH) of type 2 diabetes (17). Research in this area is nominal for people between ages late 20s-40s (as reviewed in (18)), and is virtually nonexistent in the youngest adults. This is justified as there is growing evidence that the progression to type 2 diabetes could be reduced with lifestyle intervention (9,19,20) including diet and exercise (21) and by pharmacological intervention (22). Thus, the focus for diabetes prevention should be on reducing IR (23) before insulin secretion begins to fail and blood glucose rises (24) in order to avoid macrovascular and microvascular complications.

The focus of the present paper was to ascertain the prevalence and range of IR and hyperinsulinemia in a cohort of healthy, normoglycemic, college-age subjects with a family history of type 2 diabetes using a simple and practical method of assessment, both in fasted and postprandial glucose stimulated states. Our hypotheses were (1) there is a high prevalence of IR and hyperinsulinemia present in college-age individuals with a family history of type 2 diabetes, (2) current clinical criteria for IR for pre-diabetes or the metabolic syndrome, using dysglycemia

alone, fail to identify IR even at levels associated with frank disease, and (3) fasting insulin or measures of insulin during an oral glucose tolerance test represent a highly sensitive means of quantifying IR in these young adults.

Materials and Methods

Participants

Twenty-six healthy, sedentary, college-age subjects (13 men, 13 women), age 18-26 yrs (21.6 $\pm$ 2), participated in this study. Twenty individuals were self-reported first-or second-degree relatives of patients with type 2 diabetes (had FH) and 6 were of similar BMI and gender distribution with no known family history of type 2 diabetes (no FH). Three of the FH subjects were siblings (M, 1F). All were recruited from the general population of students at Kansas State University. Informed consent was obtained after written and verbal explanation about the possible risks and discomforts of the experimental protocol were given. The study was approved by the Institutional Review Board for Research Involving Human Subjects at Kansas State University.

Exclusion Criteria

To minimize confounding factors for insulin sensitivity, potential subjects were excluded if they had a history of smoking, had performed regular exercise in the last 6 months of >3x20 min. sessions/week of at least moderate intensity aerobic exercise, or had self-reported medical conditions of Acanthosis Nigricans, polycystic ovarian syndrome, or irregular menses with hirsutis. Exclusion criteria also included current weight loss or dietary modifications, antioxidant therapies, or a diet that included a combination of >4 servings fruit and vegetables/day. Subjects were also excluded if they were taking hormone therapy or hormonal contraception (except low dose combination ethinyl estradiol/progestin pills), antihypertensives, lipid lowering agents, drugs to control blood sugar (metformin, sulfurylureas), bronchial dilators, or psychoactive medications. On the day of screening subjects were excluded if 12 hr. fasting glucose levels were $\geq$100 mg/dl, as measured by a hand held glucometer (FreeStyle Flash, TheraSense, Alameda, CA) or if blood pressure was $\geq$140/95 mmHg. Data were rejected if measurements on subsequent days included results above the initial screening cut-off for exclusion or if biochemical analysis indicated triglycerides levels $\geq$300 mg/dl, cholesterol levels $\geq$210 mg/dl or 2 hr. post OGTT $\geq$140 mg/dl. Four subjects, from an initial 30 screened, were excluded based on the above criteria. Three overweight (OW) subjects (BMI, 25-29.9 kg/m^2), three obesity (O) stage one subjects (BMI, 30-34.9 kg/m^2), and one obesity stage two subject (BMI$\geq$35 kg/m^2), were included and distributed proportionately within FH and no FH subjects to

better represent the current prevalence of overweight and obese in this age group. Nineteen lean (L) subjects (BMI<25) were included.

Oral Glucose Tolerance Test (OGTT) and analytical procedures

Subjects reported between 0700-0800 after a 10-12 hour fast and 24 hour abstinence from any exercise, alcohol and caffeine for a 2-hour oral glucose tolerance test (OGTT). An indwelling antecubital venous catheter was inserted and kept patent with a continuous isotonic saline drip, and subjects rested at least 15 min. before the initiation of the test. Baseline blood samples were collected for fasting serum glucose (FG) and fasting serum insulin (FI) at -15, -10, and -5 min. and the mean was used as the baseline blood sample. At t=0, ingestion of 1.75 g of glucose (SUN-DEX 10 g. glucose/fl. oz.) per kg body weight commenced and was completed in <5 minutes. Samples were then drawn at t=30, 45, 60, 75, 90 and 120 min. All samples were collected in serum separating tubes and the serum separated using refrigerated centrifugation at 3,000 rpm for 10 min. Samples were immediately analyzed for serum glucose (mg/dl) (YSI 2300 glucose oxidase analyzer) and serum insulin (μU/ml) or stored in liquid nitrogen and processed later. Serum insulin was measured by monoclonal, two-site, immuno-radiometric assay (Mercodia Insulin ELISA, ALPCO Diagnostics, Windham, NH) with detection limit <1 μU/ml and intra assay CVs $\leq$5%.

Anthropometry, blood analysis and criteria for the Metabolic Syndrome

Height (cm), weight (kg), waist and hip circumference (cm), BMI, blood pressure and resting heart rate were measured. Body composition was assessed by dual-energy X-ray absorptiometry (Prodogy, General Electric v. 5.6) and a region of interest was specified between the 7^{th} rib and the iliac crest for measurement of visceral adiposity (abdominal fat %). From the baseline blood samples, total cholesterol, HDL-cholesterol, C-reactive protein (CRP) and triglyceride levels were determined. LDL was calculated from Friedewald's formula (25).

Criteria for the metabolic syndrome were defined from the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III, ATP III)(4).

Calculations

Positive incremental areas under the curve (AUC-baseline) (26) during the OGTT were calculated with the trapezoidal method for glucose (Glu_{AUC}OGTT) and insulin (I_{AUC}OGTT). AUC values were divided by the 120 min. and added back to fasting values to determine average glucose (Glu_{Av}OGTT) and average insulin (I_{Av}OGTT).

Hepatic insulin sensitivity, $ISI_{HOMA,}$ was calculated as an inverse of the homeostatic model assessment, (27), as described by Radziuk (28):

$$\mathbf{ISI_{HOMA}} = \frac{22.5 \times 18}{(FI \times FG)} \qquad (1)$$

Whole body insulin sensitivity, representing a composite of hepatic and peripheral tissues was calculated as ISI_{COMP} (29):

$$\mathbf{ISI_{COMP}} = \frac{10{,}000}{\sqrt{(FG \times FI) \times (Glu_{AV}OGTT \times I_{AV}OGTT)}} \qquad (2)$$

Statistical Analysis

A Tukey-Kramer Multiple-Comparison Test was used to compare means between all subgroups of the data set (OW/O vs. L; FH vs. no FH) for each variable of interest (NCSS 2000, NCSS Statistical Software, Kaysville, UT, USA). Significance was declared when $P<0.05$. All numerical values are presented as mean $\pm$ SD unless otherwise indicated.

Results

Participant Characteristics

Subject characteristics are shown in Table 2.1. Seven individuals were OW/O and 19 were L. Twenty subjects had FH and 6 had no FH. The male-female ratio for all subjects was evenly divided (13 females/13males) as were the ratio for all subsets of subjects. The mean age, height, and resting heart rate were not significantly different ($P>0.05$) between any of the subgroups. Neither weight, systolic blood pressure (BP) nor diastolic BP was significantly different between FH and no FH. Weight was significantly different between OW/O and L ($P<0.05$) as was systolic BP ($P<0.03$).

Anthropometry, measures of adiposity, and blood profile characteristics

Table 2.1 shows that neither waist-hip ratio nor fat-free mass was significantly different between OW/O and L, but waist circumference, BMI, as well as all measurements of adiposity (total fat mass (%) and abdominal fat (%)) were significantly different ($P<0.001$). There were no significant differences for any of the above stated anthropometric and body composition variables between FH and no FH. Table 2.1 shows lipid profiles and CRP for the subcategories of subjects. No significant differences were seen for any serum lipid measurements when comparing OW/O and L. CRP was marginally not significantly different between OW/O and L ($P =0.059$). No significant differences in any serum measurements were seen between FH and no FH except for triglycerides ($P<0.01$). No subject met the clinical criteria for the metabolic syndrome. Five subjects exhibited one of the criteria for the metabolic syndrome (two no FH) and four additional subjects had two of the criteria (one OW/three O). Low HDL was the most common metabolic syndrome trait in these nine subjects (40.7 ± 8.7, range 26-53 mg/dl). Seven subjects had high levels (>3mg/dl) of CRP and of these, five had an additional metabolic syndrome marker.

Metabolic characteristics in a fasted state and during an OGTT

Metabolic characteristics, both in the fasted state and during the 2 hour postprandial OGTT, are shown in Table 2.1 and graphically represented for 2 subjects in Fig. 2.1. FI for all subjects (5.12 ± 2.44 μU/ml, range 2.43-12.51 μU/ml) were within the normal values listed for the immunoassay used (average 9.2, range 2-25 μU/ml). FI, I_{AUC}OGTT and I_{AV}OGTT were

significantly different (P<0.05) between OW/O and L but not between FH and no FH. For all subjects FI was significantly correlated with I_{AUC}OGTT (r=0.59, P<0.005) and I_{AV}OGTT (r=0.67, P<0.0005).

FG (84.6±6.6 mg/dl, range 69.2-94.6 mg/dl) and Glu_{2H}OGTT (92.8±19.3mg/dl, range 61.3-124 mg/dl) indicated normoglycemia. There were no significant differences in any fasting or OGTT glucose measurements between OW/O and L or between FH and no FH. For all subjects, FG was not significantly correlated with Glu_{AUC}OGTT (r=0.04) or Glu_{AV}OGTT (r=0.28).

There were no significant differences between any of the subcategories of subjects for ISI_{HOMA} (1.02±0.47, range 0.37-2.07). During the OGTT, there was a large range of insulin responses. Fig. 2.1 compares an insulin sensitive (IS) subject with a more insulin resistant (IR) one. The IR subject secreted almost 10 times the amount of insulin over baseline as the IS subject during the OGTT yet, even with this level of hyperinsulinemia, the increase in serum glucose for the IR subject was 5.5 fold greater than that of the IS subject. For all subjects there was over a 7-fold range of ISI_{COMP} (10.3±4.6, range 2.95-21.8). There was no difference in ISI_{COMP} between FH and no FH. Lean subjects were more insulin sensitive (P<0.05) than OW/O subjects (ISI_{COMP}= 11.7±4.5 and 6.54±1.92 respectively, Fig. 2.2). ISI_{HOMA} was significantly correlated with ISI_{COMP} for all subjects (r=0.68, P<0.0005) and for each subcategory of subjects. CRP was not significantly correlated with ISI_{COMP} either in the group with high CRP (>3 mg/dl) or for the pool of all subjects.

Measurements of glucose and insulin relative to insulin sensitivity

FG showed no correlation with ISI_{COMP} for all subjects (r=-0.28) (Fig. 2.3) or for lean subjects (r=-0.41), while FI was highly correlated with ISI_{COMP} (P<0.0001) for all subjects (r=-0.78) (Fig. 2.3) as well as lean subjects (4.49±1.93 μU/ml range 2.43 – 9.25 μU/ml) (r=-0.80).

2-h glucose ($Glu_{2\text{-}H}$OGTT) was not significantly correlated with ISI_{COMP} either for all subjects (r=-0.34) or lean subjects only (r=-0.24). Glu_{AV}OGTT is currently not used to clinically assess insulin sensitivity; however, in this study, this measurement was negatively correlated with ISI_{COMP} (r=-0.45, P<0.03) (Fig. 2.3). I_{AV}OGTT (Fig. 2.3) as well as I_{AUC}OGTT and 2-h insulin ($I_{2\text{-}H}$OGTT) (data not shown) were all highly sensitive correlates (r=-0.86,-0.79, -0.70, all

P<0.0001 respectively) with ISI_{COMP} for all subjects as well the subset of lean subjects. Both of these measurements of insulin during the OGTT were better predictors of ISI_{COMP} than was FI.

Adiposity and blood profile characteristics with insulin sensitivity

Most measurements of adiposity such as waist circumference (r=-0.52, P<0.005), BMI (r=-0.49, P<0.01), and abdominal fat % (r=-0.43, P<0.02) were correlated or almost significantly correlated (total body fat %, r=-0.36, P<0.06) with ISI_{COMP}, while waist/hip ratio was not. However, when L alone or L combined with OW subjects were analyzed; none of these markers of adiposity correlated with ISI_{COMP}. All subjects were normotensive yet systolic BP (120$\pm$9 mmHg, range 96-130 mmHg) was still significantly correlated with ISI_{COMP} for all subjects (r=-0.46, P<0.01) as well as for lean subjects (r=-0.40, P<0.01). Neither diastolic BP, nor blood lipids associated with the clinical criteria of the metabolic syndrome were correlated with $ISI_{COMP,}$ either for the subjects as a whole or any subsets, while triglyceride/HDL ratio was (r=-0.38, P<0.05).

Discussion

Our major findings are consistent with our hypotheses and demonstrate that (1) there is significant prevalence of IR and hyperinsulinemia in a cohort of lean, apparently healthy, college-age individuals with a family history of type 2 diabetes and that (2) current clinical criteria for either pre-diabetes or the metabolic syndrome, as measured by dysglycemia alone (fasting or postprandial), fail to identify IR even at levels associated with frank disease. We also determined that (3) fasting insulin and measures of insulin during an OGTT are highly sensitive means of quantifying IR in these young adults.

Our data revealed, in NGT college-age subjects, a 7-fold range in insulin sensitivity (ISI_{COMP}=2.95-21.8), similar to the 6-7-fold range found in older NGT subjects (30). In conjunction with the wide range of IR in our subjects, I_{AUC}OGTT ranged between 445-8960 μU/ml-120 min. above baseline, a 20-fold difference among subjects. As can be seen in Fig. 2.1, there was a ~10-fold difference in insulin during the OGTT between the most IR and the most IS subject yet both were clinically normoglycemic in a fasted state (<100 mg/dl), during the OGTT (<200 mg/dl) and at 120 min. OGTT (<140 mg/dl) (31).

A large proportion of our subjects exhibited ISI_{COMP} values similar to levels associated with metabolic disorder and frank disease reported in other studies. 58% of our subjects, including all OW/O subjects, had $ISI_{COMP} \leq 9.7$, the cut-off that correlated with IFG reported by Piche et al. (32). What is noteworthy is that IR was present in our subjects irrespective of the confounding influence of increased BMI since 37% of our lean subjects were also IR by these criteria. Tripathy et al. (33) determined even lower values for ISI_{COMP} (4.6 ± 1.6) associated with IFG yet 19% of our subjects were still IR by the latter criteria ($ISI_{COMP} \leq 6.2$) and two of these were lean. A review of threshold values for HOMA-IR (34) determined an equivalent ISI_{HOMA} range of cut-off to be between 0.36-0.58 and, more recently defined as ≤ 0.31 (35) and ≤ 0.30 for the same measure (36). 19% of our subjects were below the cut-off for IR based on the above studies.

The prevalence of IR has been previously reported in "young adults" between the ages of 17 and 39 years but only as defined by IFG or IGT (37-39). It is estimated that the prevalence of IFG is only 2.8% for adults aged 20-39 (38). However, the relative paucity of "pre-diabetes" in this age group may partially reflect our present clinical focus on dysglycemia as the diagnostic

marker. To this point, our subjects were all normoglycemic, even though there was prevalent IR of the magnitude associated with disease in older adults.

Direct quantification of IR in the natural history of type 2 diabetes is currently not clinically recommended (31). However, FI may prove to be a useful diagnostic tool (40), as our data, and those of others (41) demonstrate that IR can be detected from this simple measurement. We found that measures of insulin, both fasting and during the OGTT, were highly sensitive markers of IR, even in lean individuals, while parallel measures of glucose were not.

To our knowledge this is the first study to verify the presence of IR in college–age adults with an easily applicable indirect measure. IR has previously been studied in normoglycemic "young" adults. Some of these studies included overweight subjects (42-47) and others included only lean subjects (48-51) but all of these studies focused on subjects in their late 20s to mid 30s. It is known that ISI_{COMP} is attenuated by age (52). In one study markedly higher FI and increased IR have been reported in subjects age ~24 years (18). However, these findings were obtained with a clinically impractical direct method of measuring IR (euglycemic hyperinsulinemic clamp).

There is growing evidence that acquired factors, such as increased adiposity, are concomitant and mechanistically linked with IR (as reviewed in (9,53))**.** Strong correlations have been found between ISI_{COMP} and BMI (52,54) as well as waist-to-hip ratio (55). It should be noted from Fig. 2.2, that even though OW/O subjects had lower insulin sensitivity than lean there were 7 lean subjects with values equally low as OW/O, suggesting that while increased BMI is associated with IR, lower BMI does not prevent the occurrence. Nonetheless, in the present study, none of the measures of adiposity were good predictors of IR until clinical obesity.

High blood pressure and blood lipid levels are associated with IR in the metabolic syndrome. Even though all subjects were normotensive in the present study, systolic BP was found to be a prognostic marker of IR, even for just the lean individuals. Triglyceride/HDL ratio, the best lipid derived IR predictor (56), was also correlated with ISI_{COMP} for all subjects.

There is a lack of standardization of the plasma insulin assay (56) and insulin values in the literature are variable. Insulin values, obtained by radioimmunoassay, are 20-40% higher than the newer, more widely used, two-site immuno-radiometric assay (57) utilized in the present study. ISI_{COMP} was originally derived from plasma insulin values obtained with radioimmunoassay and formulated to produce a number between 0 and 12. This, undoubtedly, is

the reason that 10 of the 26 subjects from the present study had ISI_{COMP} values above 12 and why interpretation of this study in the context of previous literature is limited.

There is a wide discrepancy in ISI_{COMP} values reported in studies with NGT subjects (32,33) even when the insulin assay protocols were similar (52,54,55). Differences in subject age, BMI and ethnicity likely contributed to the variability. Thus, these factors, as well as gender, were taken into account in the present study design.

Correlations between insulin levels and calculations of insulin sensitivity were not independently derived in the present study. Both FI and FG are equally weighted in the computation for ISI_{COMP} (Eq. 2). However, FG was not significantly correlated with ISI_{COMP} while FI was strongly correlated (Fig. 2.3) even though latter values were numerically much smaller and thus had much less influence on the calculation. This was true even within the subset of lean individuals who had even lower FI.

There is a plethora of data relating the development of IR, diabetes and the metabolic syndrome with visceral and subcutaneous fat, BMI, and waist circumference (as reviewed by (53)). Since the purpose of the present study was not to confirm the previously determined affect of adiposity on ISI_{COMP}, the distribution of BMI was purposefully determined (both for FH and no FH) to represent an approximation of the prevalence of a BMI for OW/O in the U.S. population for a college-age (18-26 yrs.) cohort (as reported in the National Center for Health Statistics, unpublished tabulations).

In summary, we demonstrated that a) IR could be detected in college-aged individuals by an easily applied clinical method measuring serum insulin, b) substantial levels of IR and hyperinsulinemia are prevalent in college-age subjects preceding the deterioration of glucose tolerance and c) this metabolic profile is present despite the absence of adiposity and no family history of type 2 diabetes.

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Tables

Table 2.1 Subject Characteristics

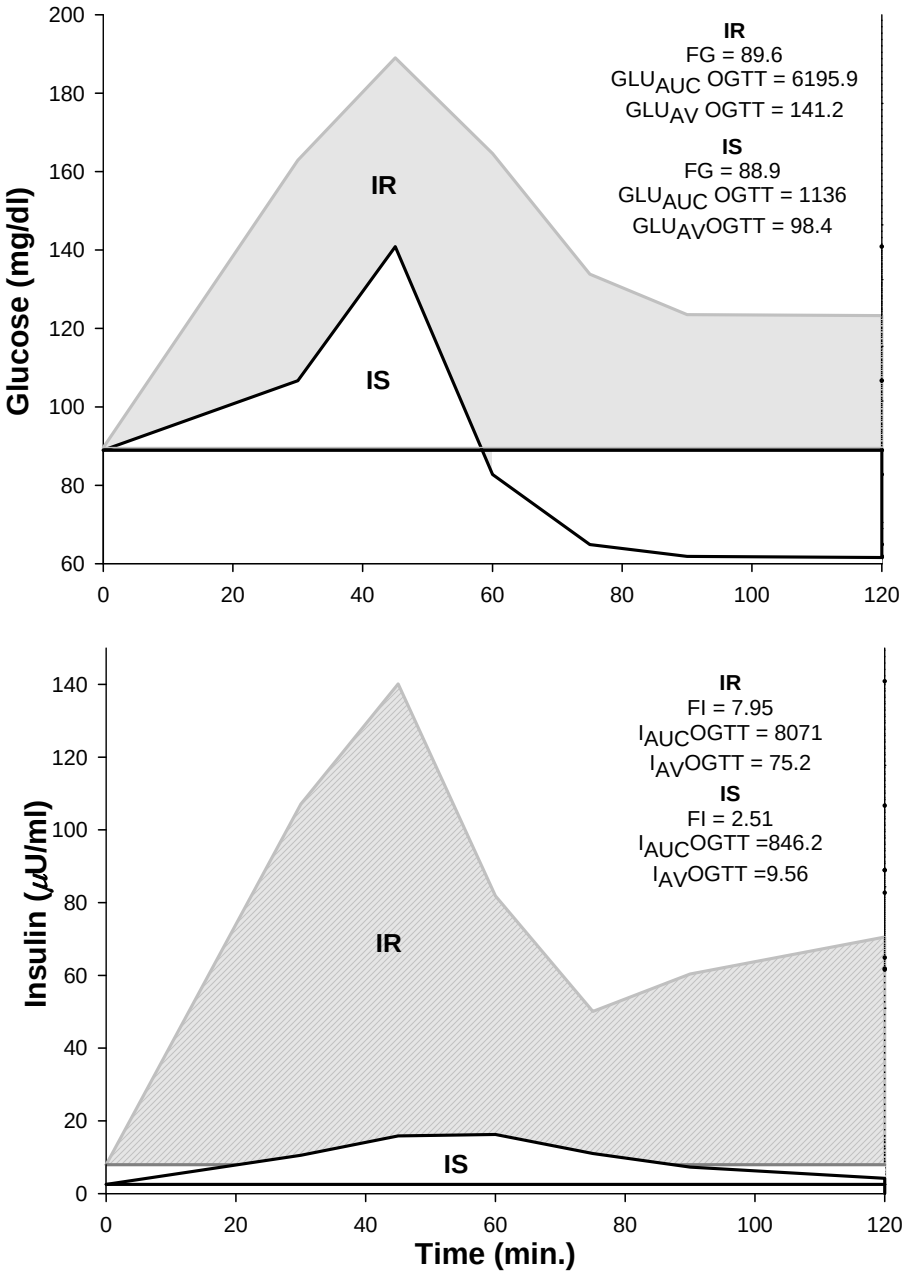
Subject Characteristics	All subjects	OW / O	Lean	FH	noFH
No. of subjects	n=26	n=7	n=19	n=20	n=6
Gender (female / male)	13/13	4 / 3	9 / 10	10 / 10	3 / 3
FH type 2 diabetes / no FH	20 / 6	5 / 2	15 / 4	N/A	N/A
Age (yr)	21.6 ± 2.0	22.9 ± 2.0	21.2 ± 1.8	21.3 ± 1.7	22.8 ± 2.6
Height (cm)	175 ± 9	176 ± 11	175 ± 9	175 ± 10	177 ± 8
Weight (kg)	75.5 ± 18.2	94.8 ± 21.1*	68.4 ± 10.7	75.6 ± 18.9	74.9 ± 17.5
Resting heart rate (bpm)	65.9 ± 8.4	67.7 ± 6.3	65.3 ± 9.2	65.4 ± 9.4	67.9 ± 3.0
Systolic BP (mmHg)	120 ± 9	126 ± 8*	117 ± 8	119 ± 10	121 ± 7
Diastolic BP (mmHg)	72.6 ± 9.0	74.6 ± 12.4	71.9 ± 7.8	71.6 ± 9.4	76.2 ± 7.1
Anthropometry					
Waist circumference (cm)	81.9 ± 12.6	93.8 ± 15.5†	77.2 ± 7.7	81.3 ± 13.1	83.6 ± 12.0
Waist-hip ratio	0.79 ± 0.08	0.81 ± 0.10	0.78 ± 0.07	0.79 ± 0.08	0.80 ± 0.06
BMI (kg/m^2)	24.4 ± 4.4	30.2 ± 4.0†	22.2 ± 2.0	24.6 ± 4.6	23.7 ± 4.1
Total fat mass (%)	27.8 ± 9.6	38.6 ± 8.6†	23.9 ± 6.5	28.0 ± 9.9	27.3 ± 9.6
Abdominal fat (%)	35.1 ± 11.0	48.1 ± 7.4†	30.3 ± 7.7	35.4 ± 11.1	33.9 ± 11.7
Fat free mass (kg x 10^2)	516 ± 117	562 ±161	500 ± 96	516 ± 121	517 ± 108
Blood lipids and CRP					
Cholesterol (mg/dl)	155 ±29	163 ± 35	153 ± 27	152 ± 29	168 ± 29
Triglycerides (mg/dl)	84.9 ± 42.5	106 ± 57	77.2 ± 34.6	74.0± 26.2	121.5± 65.9‡
HDL (mg/dl)	51.9 ±13.2	47.0 ± 14.6	53.7 ± 12.6	50.9 ± 13.8	55.2 ± 11.4
LDL_{calc} (mg/dl)	86.6 ± 23.9	95.2 ± 19.3	83.4 ± 25.1	86.1 ± 25.4	88.2 ± 19.8
TC/HDL	3.20 ± 0.91	3.61 ± 0.73	2.99 ± 0.93	3.17 ± 0.97	3.13 ± 0.77
LDL/HDL	1.81 ± 0.80	2.16 ± 0.67	1.68 ± 0.82	1.85 ± 0.86	1.66 ± 0.55
Triglycerides / HDL	1.74 ± 0.96	2.28 ± 0.91	1.54 ± 0.92	1.57 ± 0.70	2.33 ± 1.49
C-reactive protein	3.56 ± 5.61	6.95 ± 8.69	2.30 ± 3.52	4.16± 6.16	1.53 ± 2.56
Metabolic Characteristics					
FI (µU/ml)	5.12 ± 2.44	6.83 ± 3.02§	4.49 ± 1.93	5.12± 2.67	5.12 ± 1.69
I_{AUC}OGTT (μU/ml)	2968 ± 2115	4400 ± 2550§	2441 ±	2952 ± 2053	3024 ± 2520
I_{AV}OGTT (μU/ml)	29.9 ± 19.2	43.5 ± 23.0§	24.8 ±15.3	29.7 ± 18.7	30.3 ± 22.4
I_{2H}OGTT (μU/ml)	21.7 ± 16.1	30.1 ± 15.0	18.6 ± 15.7	21.1 ± 14.1	24.0 ± 23.2
FG (mg/dl)	84.6 ± 6.6	86.5 ± 9.4	83.9 ± 5.4	83.8 ± 6.5	87.3 ± 6.6
Glu_{AUC}OGTT (μU/ml)	3782 ± 2304	4534 ± 2828	3505 ±	4052 ± 2253	2883 ± 2449
Glu_{AV}OGTT (mg/dl)	116 ± 21	124 ± 24	113 ± 19	117 ± 20	111 ± 23
Glu_{2H}OGTT (mg/dl)	92.8 ± 19.3	103 ± 17	89.2 ± 19.1	93.5 ± 18.6	90.6 ± 23.2
ISI_{HOMA}	1.02 ± 0.47	0.84 ± 0.45	1.17 ± 0.44	1.10 ± 0.50	1.00 ± 0.34
ISI_{COMP}	10.3 ± 4.6	6.54 ± 1.92§	11.7 ± 4.5	10.4 ± 4.8	9.97 ± 3.91

Table 2.1 values are mean ± SD; OW / O-Overweight/Obese; FH-family history of type 2 diabetes; no FH-no family history; FI-fasting insulin; I_{AUC}OGTT, I_{AV}OGTT, I_{2H}OGTT- insulin area under the curve

above fasting, average insulin, and insulin at two hours respectively, during an oral glucose tolerance test; FG-fasting glucose; Glu_{AUC}OGTT, Glu_{AV}OGTT, Glu_{2H}OGTT-glucose area under the curve above fasting, average glucose and glucose at two hours respectively, during an oral glucose tolerance test. ISI_{HOMA}-insulin sensitivity index from fasting measures. ISI_{COMP} –composite insulin sensitivity index.
*Significantly different from lean ($P < 0.05$); † Significantly different from lean ($P < 0.001$); ‡Significantly different from FH ($P < 0.01$); § Significantly different from lean ($P = 0.03$).

Figures

Figure 2.1 Comparison of changes in serum glucose and serum insulin in one insulin sensitive and one insulin resistant normoglycemic subject during an oral glucose tolerance test.



insulin sensitive (IS-white, ISI_{COMP} = 21.82), insulin resistant (IR-gray, ISI_{COMP} = 3.54) . FG-fasting glucose; GLU_{AUC}OGTT, GLU_{AV}OGTT - glucose area under the curve above fasting and average level of glucose respectively, during an oral glucose tolerance test; FI-fasting insulin, I_{AUC}OGTT, I_{AV}OGTT - insulin area under the curve above fasting and average level of insulin respectively, during an oral glucose tolerance test.

Figure 2.2 Mean, SD and range for ISI_{COMP} for each subgroup and for all subjects.

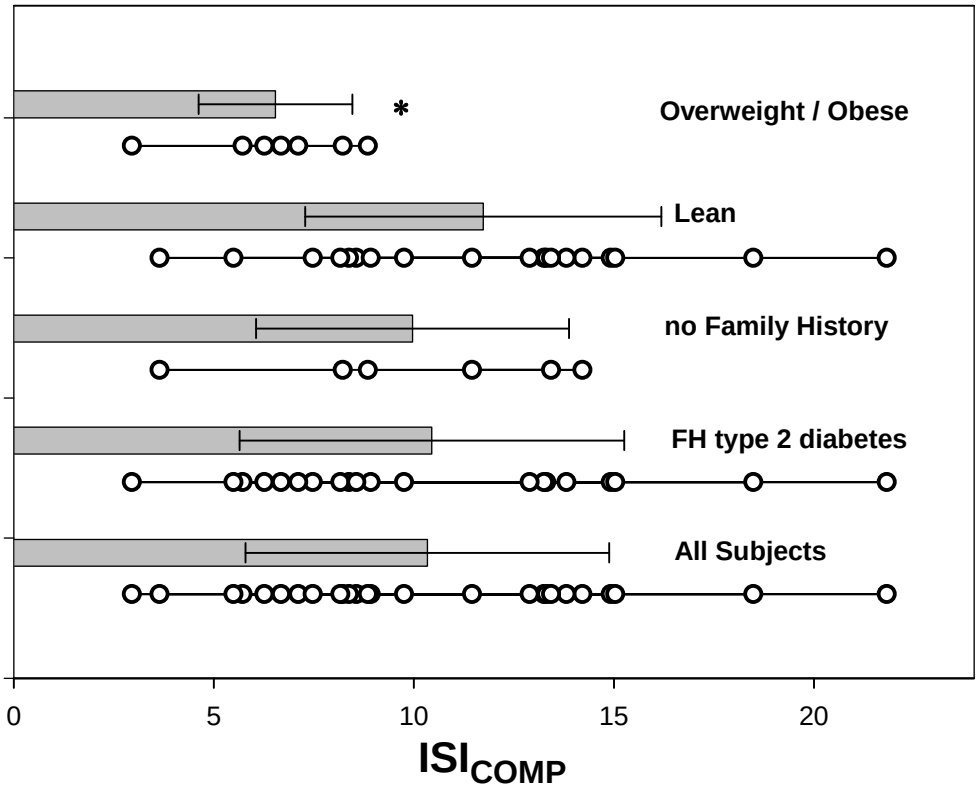
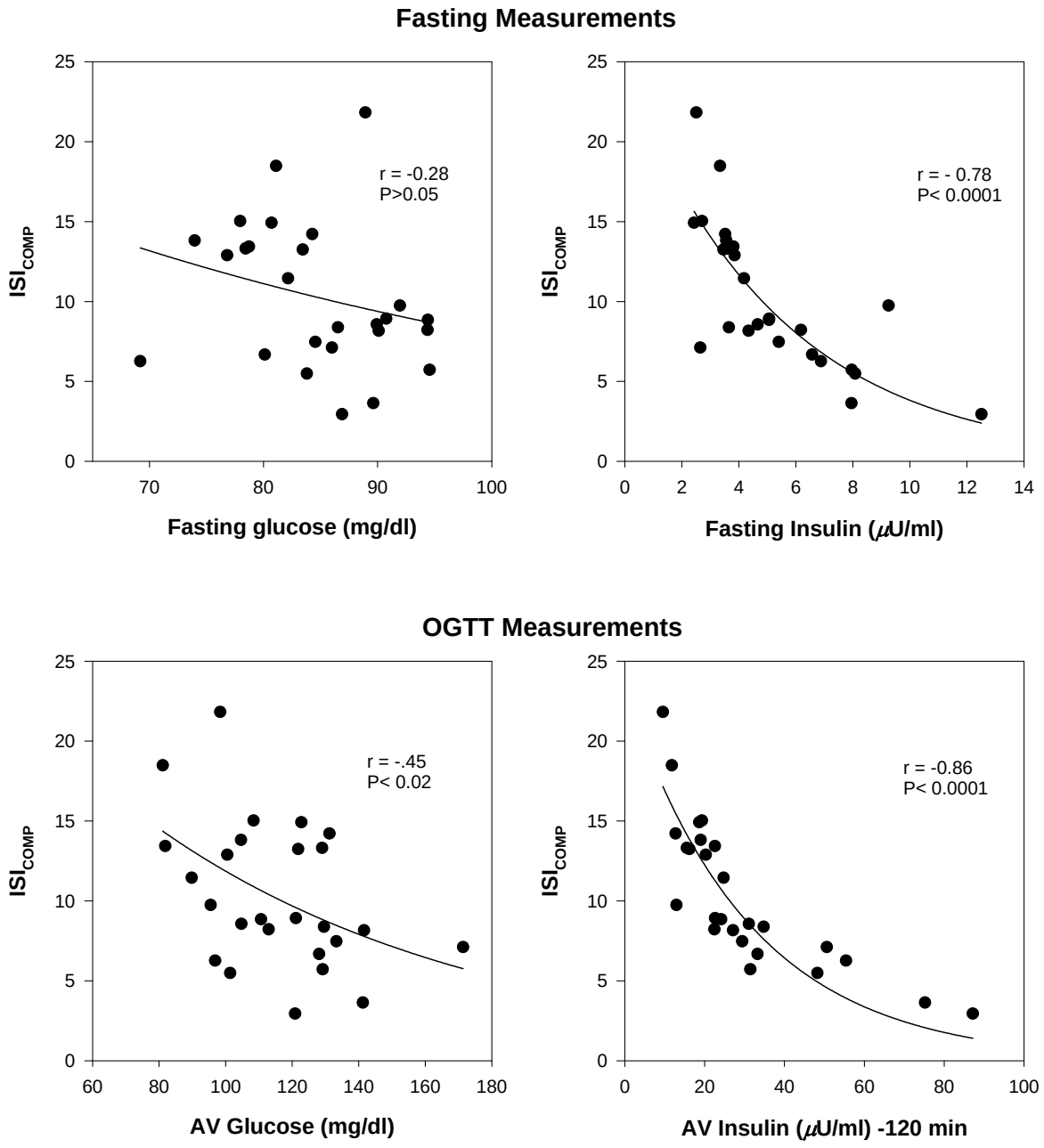
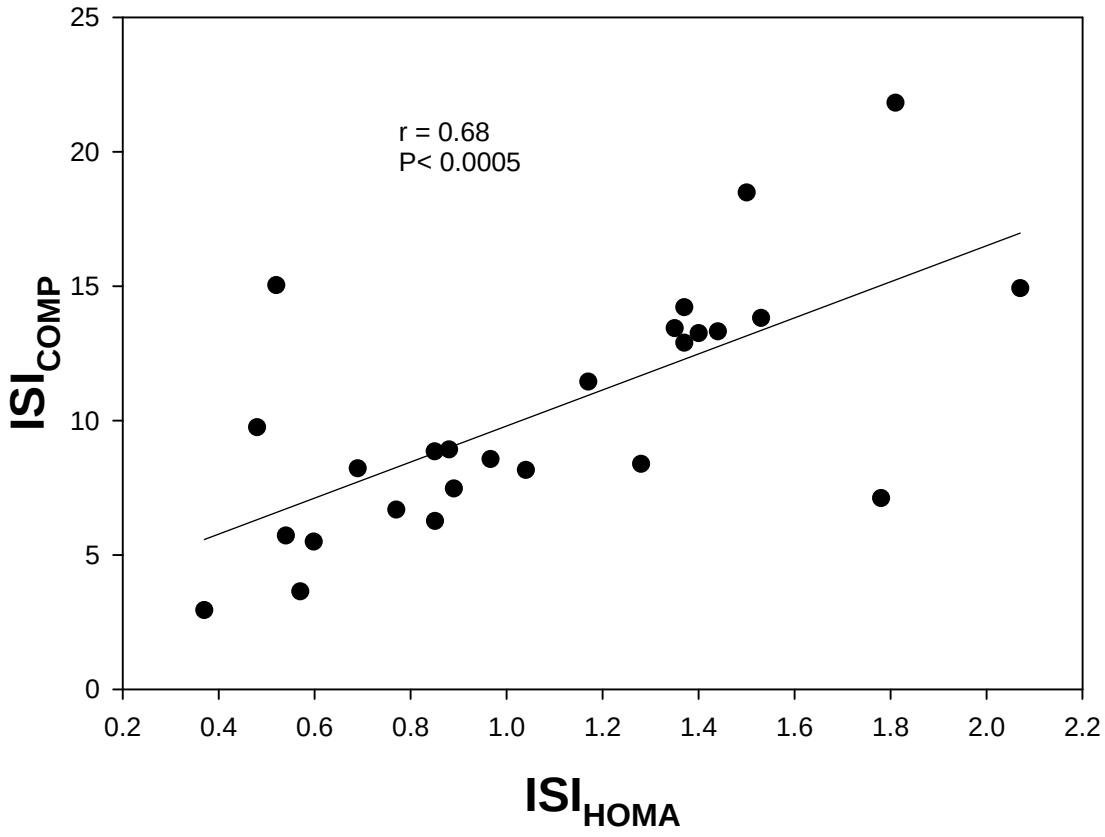


Figure 2.3 Fasting and average measurements of glucose and insulin during an oral glucose tolerance test relative to ISI_{COMP}.



Fasting measurements (*top*) and average measurements during an oral glucose tolerance test (OGTT) *(bottom*) of glucose (mg/dl) (*left panels*) and insulin (μU/ml) (*right panels*) relative to composite insulin sensitivity index. (ISI_{COMP}).

Figure 2.4 Measures of insulin sensitivity in a fasted state (ISI_{HOMA}) and during an oral glucose tolerance test (ISI_{COMP}).



CHAPTER 3 - Metabolic Rate and Parameters of Post Occlusive Reactive Hyperemia (PORH) in Forearm Skeletal Muscle

Abstract

OBJECTIVE—Few previous studies have used near-infrared spectroscopy (NIRS) to determine forearm skeletal muscle metabolic rate (MR) or parameters of post occlusive reactive hyperemia (PORH). Additionally, these previous results are potentially inaccurate based consequent to methodological problems. The goal of this study was to use Frequency Domain Multi Distance (FDMD) NIRS to (1) determine quantitative measurements of forearm skeletal muscle MR, corrected for the effect of adipose tissue thickness (ATT) and (2) determine NIRS parameters describing PORH as a noninvasive measure of microcirculatory function.

RESEARCH DESIGN AND METHODS—Twenty six, young (age 18-26 yrs.) normotensive subjects, underwent 3 trials of upper limb PORH and were assessed with pulsed Doppler ultrasound for brachial artery (BA) velocity and diameter, and with FDMD NIRS for changes in forearm oxy-hemoglobin/myoglobin ([HbO_2]), deoxy-hemoglobin/myoglobin ([Hb]) and total hemoglobin ([THb]) before and during the PORH protocol. Forearm skeletal muscle MR was derived from the initial linear increase in [HHb] during occlusion, while parameters of the [HbO_2] PORH response (i.e. time course and peak of the initial response and measure of recovery) were described using a sigmoidal function. All NIRS measures were corrected for ATT. Parameters describing PORH brachial artery blood flow (BABF) dynamics were also determined as an independent measure of microvascular reactivity.

RESULTS—Subjects exhibited an ~ 2-fold range in ATT-corrected MR (3.70 $\pm$ 0.54, range 2.83-5.15 μMO_2/min/100g). Ignoring the influence of ATT on NIRS signals led to several false positive and negative comparisons. ATT-corrected [HbO_2] at rest and the amplitude of the PORH response [referenced either from end cuff (both $P<0.0001$) or from rest ($P<0.001$)] were positively correlated with MR. [HbO_2] at end cuff was negatively correlated with MR ($P<0.01$). Further, the amplitude of the response for [HbO_2] [scaled either to rest or end-cuff] correlated with the corresponding parameters for BABF ($P<0.01$ and $P<0.04$ respectively). However, none of the time course parameters, nor [HbO_2] 95 s into recovery, correlated with either MR or corresponding parameters for BABF.

CONCLUSIONS—These findings indicate that 1) It is imperative to correct NIRS signals for ATT. 2) The validity of the NIRS [HbO_2] response as an index of capillary perfusion and thus, a measure of microvascular reactivity, under certain conditions, is supported by the correlation between [HbO_2] parameters and metabolic rate as well as with corresponding parameters of the BABF response. 3) However, the disparity between the kinetics of adjustment of [HbO_2] and BABF during PORH suggest that the vascular compartment sampled by NIRS may not reflect the sum total of all the forearm vasculature during the response.

Introduction

In disease states, microcirculatory perfusion can be disturbed (5, 32) and the capillary density within skeletal muscle reduced (15). Impaired microvascular vasoreactivity can predate disease states, such as type 2 diabetes (4), such that microvascular dysfunction in skeletal muscle may be mechanistically linked to insulin resistance (30).

Assessment of the microcirculation in humans most often includes laser Doppler fluxmetry (skin pulp blood flow), transcutaneous oxygen tension ($TcpO_2$), iontophoresis (for the subdermal delivery of vasoactive drugs), and/or capillaroscopy (light microscopy) (1). However, data acquired on vessels and tissues assessed by these methods (skin, finger nail fold or conjunctiva) may not necessarily be relevant to other areas, such as skeletal muscle in which control of the microcirculation may be different than for cutaneous beds (39). Thus, the need exists to develop non-invasive methods for examining skeletal muscle microvascular responses in humans both as a prognostic tool and to assess therapeutic interventions for efficacy in improving tissue oxygenation and hemodynamics.

The pioneering work of Jobsis in 1977 (20), put forward near infrared spectroscopy (NIRS), as a simple, noninvasive technique, with many possible applications. Evaluation of muscle metabolic rate (MR) (or oxygen uptake (VO_2)) can be very useful in physiological and clinical investigations. Cheatle et al (6) was the first to measure VO_2 at rest in calf muscle by NIRS. Since then, the utility of the NIRS methodology for determining muscle MR has gained greater acceptance (as reviewed in (14)). NIRS determined oxygen uptake of forearm flexor muscles has been correlated with P-MRS (3, 16) but does not correlate with the established method for measurements of muscle MR by the Fick method, determined by blood flow and arterio-venous oxygen content differences (3, 36). There is also wide variability in reported values for NIRS derived forearm skeletal muscle MR (10, 11, 13, 19, 36) likely due to variable thickness of subcutaneous fat under the probe interfering with the NIRS signal.

Adipose tissue thickness (ATT) is a substantial confounding influence of in vivo NIRS measurements and must be factored into NIRS muscle studies (2). ATT has been demonstrated to quantitatively affect NIRS measures of VO_2 in forearm skeletal muscle (34). Correction methods for the influence of the adipose layer on NIRS derived MR have been proposed for continuous wave NIRS (2, 28, 29, 33, 40). Recently a method was proposed to correct for fat pad thickness by removing the spectral influence of the fat layer from the muscle spectrum

before measuring the physiological parameters (41). However, it is unclear whether any of these methods are applicable to Frequency Domain Multi Distance (FDMD) NIRS data.

Post occlusive reactive hyperemia (PORH) has been used to evaluate the functional aspects of skeletal muscle microcirculation in conjunction with NIRS, but only relative to peripheral vascular disease (PVD)(7, 22-24). These studies evaluated PORH responses using some measure of the rate of O_2 resaturation (as %Sat). However, it is unlikely that %Sat is a robust measure of the hyperemic response. While none of the NIRS signals are a true measure of blood flow, changes in %Sat reflects changes in both oxygen uptake and arterial inflow. These also are confounded by changes occurring in total hemoglobin most likely reflecting changes in tissue capillary hematocrit (21). Therefore, % Sat is likely not to be a good surrogate index for perfusion. We propose that changes in the oxy-hemoglobin/myoglobin ($[HbO_2]$) signal in the initial phase of PORH more closely reflect changes in perfusion in the microcirculation during this time period. To test this, we compared these measures of $[HbO_2]$ to corresponding measures of brachial artery (BA) blood flow (BF) during PORH, since upstream conduit artery flow (i.e. hyperemic response) should also provide information about the changes in perfusion in the downstream microvasculature (i.e., hyperemic stimulus) (9, 26, 37).

The purposes of the present study were to utilize FDMD NIRS to (1) determine quantitative measurements of forearm skeletal muscle MR, with appropriate correction for ATT, in young, healthy college-age subjects, (2) characterize PORH response of the microcirculation using $[HbO_2]$, and (3) compare $[HbO_2]$ responses with corresponding responses in the conduit (brachial) artery as an independent measure of PORH microvascular reactivity. We hypothesized that (1) characteristics of $[HbO_2]$ during occlusion will inversely correlate with MR, (2) kinetic characteristics of the PORH $[HbO_2]$ response, including time course and peak, will positively correlate with MR, and (3) characteristics of the $[HbO_2]$ (microvascular) response will correlate with corresponding characteristics of the BABF (macrovascular) response.

Methods

Subjects

Twenty-six healthy, sedentary, college-age subjects (13 men, 13 women), age 18-26 yrs., participated in this study. Exclusion criteria were employed to mitigate influences on endothelial function. These included a history of smoking, regular exercise in the last 6 months of > 3x20 min. sessions/week of at least moderate intensity aerobic exercise, or those taking antihypertensive medications, lipid lowering agents, drugs to control blood sugar (metformin, sulfurylureas), bronchial dilators, or psychoactive medications. Women were excluded if they were taking hormone therapy or hormonal contraception (except low dose combination ethinyl estradiol / progestin pills) and were studied during the early follicular phase of their menstrual cycle as verified by progesterone (P_4) levels $\leq$ 1.5 ng/ml. All subjects were recruited from the general population of students at Kansas State University. Informed consent was obtained after both written and verbal explanation about the possible risks and discomforts of the experimental protocol were given. The study was approved by the Institutional Review Board for Research Involving Human Subjects at Kansas State University.

Anthropometry

Measurements of height (cm), weight (kg), waist and hip circumference (cm), BMI, blood pressure and resting heart rate were taken. Body composition was assessed by dual-energy X-ray absorptiometry (Prodogy, General Electric v. 5.6) and a region of interest was specified distal to the olecranon process for forearm skeletal muscle mass. Progesterone (P_4) (Progesterone RIA DSL-3900, Diagnostics Systems Laboratories, Inc., Webster, TX) and estradiol (E_2) (Coat-A-Count Estradiol, Diagnostic Products Corporation, Los Angeles, CA) were measured for females.

Protocol

All vascular tests began between 0700-0830, and were performed in a quiet, temperature-controlled room. Participants refrained from alcohol and exercise for 24 hours and caffeine for 12 hours and were fasted or had consumed a very low-fat, snack prior to coming to the lab. Vascular measurements were taken during rest (1 min.), occlusion (5 min.) and during PORH (2.5 min.) (Fig. 3.1a). Complete occlusion of the brachial artery was accomplished by rapid

inflation of a pediatric blood pressure cuff to $\geq$ 250 mm Hg, just proximal to the antecubital fossa (distal to the BA imaging). Three trials were obtained for each subject with > 10 min. between for vessel recovery. Accepted trials (see below) were averaged before analysis for BA and microcirculatory responses for each subject.

Vascular Function Tests

Subjects lay supine with the right upper limb extended laterally at heart level, and slightly supinated for the duration of the trials. The brachial artery was imaged as far proximal as possible to the antecubital fossa, to avoid a potential high origin of the radial artery (a common anomaly documented in at least 12.5% of people (8) and noted in 3 of our subjects). To maintain the ultrasound image and measurement position, the arm was secured in a wooden foam lined frame and the probe was secured with a stereotactic clamp. This allowed minor adjustments to be made to produce a simultaneous optimal blood velocity signal and vessel image, yet also insured all trials for a given subject were measured from the same 10 mm section of the artery. BA blood velocity was measured with pulsed Doppler ultrasound (GE VIVID3, GE Medical Systems), with an integrated packaged ECG and a 10L linear array transducer. The BA was imaged with the same probe at 8-MHz in 2-D B mode. Discontinuous longitudinal BA images and beat-by-beat velocity tracings (three at rest, one during the last minute of occlusion, and at 5, 10,15 s post-occlusion, and an additional ten at exactly 10-15 s intervals for the remainder of post-occlusion) were obtained, coded, and stored on the ultrasound hard drive.

Skeletal muscle oxygenation was evaluated continuously during the protocol using Frequency-Domain MultiDistance Near-infrared Spectroscopy (OxiplexTS, Model 96208, ISS, Champaign, IL, USA) system operating at wavelengths of 692 and 834 nm with a modulation frequency of the light-source intensity of 110 MHz. The probe utilized source-detector separations of 2.0, 2.5, 3.0 and 3.5 cm. FDMD NIRS provides measurement of absolute concentrations (in μM) utilizing continuous dynamic measurement of reduced scattering coefficients (μ'_S, cm^{-1}) and thus can provide quantitative measures of hemoglobin (Hb) /myoglobin (Mb) oxygenation states. The signal emanates from the microvasculature since in vessels larger than ~1mm there is a large amount of absorption of light (25). Because of similar spectral characteristics it is not possible to distinguish between hemoglobin and myoglobin

contributions to the signals. Thus, even though there is myoglobin contribution to the signal the variables reported were deoxy-hemoglobin ([HHb]) and oxy-hemoglobin ([HbO_2]).

The probe was positioned longitudinally on the lateral anterior antebrachium overlying flexor digitorum superficialis, flexor carpi radialis and brachioradialis. It is generally accepted that the region of maximal sensitivity will be found between, and below the surface of the skin at a depth of roughly half the distance between the source and detector (31). Ultrasound images were taken at the same location as the NIRS probe in order to accurately access underlying ATT. The change in [HHb] during arterial occlusion was used to estimate forearm muscle oxygen consumption, while the change in [HbO_2] during post occlusive reactive hyperemia was used as an indicator of changes in perfusion, predominately of forearm skeletal muscle, and downstream compliance of the arterioles, capillaries and venules. Total hemoglobin ([THb]) was determined as the sum of [HHb] and [HbO_2]). Tissue O_2 saturation (StO_2) was calculated as StO_2 = [HbO_2] / THb (%). The NIRS data were stored at an output frequency of 31.25 Hz.

BA diameter, velocity, and blood flow

Trials were excluded for poor or missing critical BA images, weak velocity signals or venous contamination of the velocity signals. Examinations of the BA images were performed in random order, by a single sonographer blinded to the subject identification. BA diameter was measured on frozen images with electronic calipers as the anterior to posterior endothelial-lumen interface and determined from an average of 3 successive cardiac cycles, 3 measurements / cardiac cycle, both for the widest diameter (caused by the systolic pressure wave, coincident with the T wave on the electrocardiogram) and narrowest diameter (caused by diastolic pressure, coincident with the end of QRS on the electrocardiogram). One trial for one subject was randomly chosen and blindly measured on 5 separate days to determine the reproducibility of ultrasonographer assessment of BA diameters. The average coefficient of variation for 18 measurements was 0.82%, range 0.42%-1.79%. Due to the visually large fluctuations between systolic and diastolic diameters in a number of subjects, mean vessel diameter was calculated as diastolic diameter + 1/3(systolic-diastolic diameter). Average velocity per cardiac cycle was calculated by the manufacturer's software. Brachial artery blood flow (BABF), for any time point, was calculated as average velocity x π(average diameter/2)^2. Parameters to describe the BABF PORH response were: R (baseline during resting conditions), y1 (baseline for the last 30

s of the cuff occlusion), K (the amplitude of the PORH response from y1), K' (the amplitude of the PORH response from R) and 95 s (the blood flow above R, 95 seconds after cuff release).

Forearm skeletal muscle metabolic rate

Arterial occlusion produces a closed vascular compartment, where the rate of increase in [HHb] represents the rate of oxygen extraction/utilization, which is VO_2. This is true as long as [THb] remains relatively constant. To control for this, trials were excluded in which [THb] increased $\geq$ 15% of the baseline value during the 5 min. occlusion. Data for 3 of the 26 subjects were excluded based on this criteria. Muscle oxygen uptake or metabolic rate (MR) was derived from NIRS by taking the slope of the initial linear increase in HHb ([HHb]/s) (Fig 1b). Concentration changes were expressed in μM/s and converted to MR expressed as micromoles O_2 per 100 grams of tissue per minute (μmol O_2 / min / 100 g). A value of 1.06 g/ml was used for muscle density (38) and 1:4 as the molecular ratio between hemoglobin and oxygen.

Kinetics of PORH tissue perfusion

The reappearance of oxygenated blood after cuff release should mostly reflect perfusion. Thus, microcirculatory reactivity was assessed by the kinetic response of [HbO_2] during the initial phase of PORH. The 3 trials were averaged for 20 s prior to and 60 s after cuff release. The average response was modeled using best fit for the data with a single logistic model with no time delay (Fig. 3.1c).

$$Y = y1 + \frac{K}{1 + e^{(-\ln(81) \times (t-tm)/d)}}$$

Where y1= [HbO_2] at the end of cuff occlusion, tm=the time to midpoint of the PORH response and d=the time between 10% and 90% of the response. Intrasubject coefficients of variation (CV) for the curve-fitting parameters for the 3 individual trials were determined for 5 subjects. Average CV for y1 = 4.7% (range 1.2 – 10.3), K = 6.7% (range 2.4 – 13.2), tm = 9.7% (range 6.5 – 16.5), and d = 13.4% (range 3.3 – 25.5).

To test the hypothesis that the NIRS [HbO_2] signal reflects the microcirculatory response most closely reflective of perfusion during PORH, we compared the parameters describing this response with the same parameters of an independent method, BABF, which has previously been used to describe the microcirculatory response (26, 37). The reduced temporal resolution of the

BABF response precluded accurate assessment of the time based parameters determined for [HbO_2] (i.e., Tm and d); thus, only amplitude parameters (R, y1, K and 95s) were compared. In general, descriptors of the amplitude of the response (e.g. K, 95s) are referenced to y1. When these are referenced to the resting value (R) they are denoted as K' and 95s'.

Statistics

All numerical values are presented as mean $\pm$ SD unless otherwise indicated. Pearson product moment correlation analysis was performed to assess significance of relationships between variables. Statistical analysis was performed using SigmaPlot 2001 (version 7, San Jose, CA, USA). Significance was declared for relationships whose correlation coefficient exceeded that required for $P<0.05$ (Dixon and Massey, Table A.30).

Results

Subject Characteristics

Subject characteristics are presented in Table 3.1. Gender was equally distributed in this young (21.6 $\pm$ 2.1 yrs) cohort. All subjects were normotensive. Two subjects were determined to be "overweight" (BMI 25-29.9), and two subjects were "obese" (BMI 30-34.9). The remaining 19 subjects were "lean" (BMI < 25).

Importance of Correcting NIRS Parameters for ATT

All of the [HbO_2] amplitude parameters (R,y1, K [as K, K %y1, K', and K' %R], and the values at 95 s [95s, 95s %R, 95s %y1]) were significantly correlated with MR ($P< 0.05$ to 0.0001). However, the same [HbO_2] amplitude parameters were also significantly correlated with ATT. In turn, MR was also highly significantly correlated ($r=0.77$, $P<0.0001$) with ATT (Fig. 3.2). Therefore, each [HbO2] parameter was corrected for the variation in signal amplitude introduced by skin and fat pad thickness (designated by "subscript c") by utilizing the slope of the linear relationship between each parameter and ATT. The same correction was made for the influence of ATT on MR (MR_C) (Fig. 3.2). Fig. 3.3 exemplifies the importance of correcting NIRS parameters for ATT is exemplified. Both the amplitude of the initial hyperemic response (K) and the amplitude at 95 s in recovery as a % of the end cuff value (y1) were significantly correlated with the uncorrected MR. Both of these parameters were also highly significantly correlated with ATT ($P<0.0001$ and $P<0.002$ respectively). However, when MR and these parameters were corrected for ATT (K_C and $(95s\ \%y1)_C$ respectively), K_C remained highly significantly correlated with MR_C ($P<0.001$) while $(95s\ \%y1)_C$ was not correlated with MR_C ($P>0.05$). These findings emphasize the necessity to correct NIRS signals for ATT in order to accurately assess true physiological relationships between variables. When corrections were thus applied, MR_C was significantly correlated with [HbO_2].

Forearm Skeletal Muscle Metabolic Rate

As seen in Fig. 3.2, forearm skeletal muscle metabolic rate when uncorrected for ATT was 1.70 $\pm$ 0.86 μMO_2/min/100g but with an almost 7-fold range (0.52 – 3.60 μMO_2/min/100g). However when corrected for ATT, MR_C averaged 4.66 $\pm$ 0.68, with a 1.9 fold range (3.42-6.48 μMO_2/min/100g).

Comparison of two measures (NIRS [HbO_2] and BABF) of the PORH microcirculatory response

Fig. 3.4 shows [HbO_2], [THb], and BABF during PORH for two subjects with different kinetic responses while the mean responses and kinetic parameters are presented in Table 3.2. Fig 3.5 shows relationships between the amplitudes (K) of the response, for [HbO_2] and BABF, whether related to end cuff (%y1) or to rest (%R). These two measures of microcirculatory response were significantly correlated but only after correction for ATT ($r=0.52$, $P<0.01$); $r=0.39$, $P<0.04$ respectively). These results underscore once again, the importance for correcting NIRS amplitude measures for ATT.

Discussion

There are several unique aspects to the present study: To our knowledge, this is the first report (a) to model the time course of $[HbO_2]$ using NIRS in healthy subjects during PORH, (b) to demonstrate the necessity for correcting these $[HbO_2]$ responses for ATT, (c) to correlate these with forearm skeletal muscle metabolic rate, and (d) to compare these responses with corresponding brachial artery blood flow responses as an independent assessment of microcirculatory reactivity. The major findings of the present study are: (1) $[HbO_2]$ at rest was highly correlated with MR, (2) $[HbO_2]$ at end cuff occlusion was negatively correlated with MR, (3) the amplitude of the initial $[HbO_2]$ response, but not the time course, was significantly related to MR, and (5) the scaled amplitudes of the initial $[HbO_2]$ response, but not the time course nor the amplitude in recovery, were correlated with the corresponding BABF parameters.

Importance of Correcting NIRS signals for ATT

Adipose tissue influences NIRS light propagation such that true [Hb] and $[HbO_2]$ will be underestimated if ATT is not taken into account (14). ATT in the present study was variable (range 2.48-7.30 mm) and generally low for these predominantly lean (83%) subjects. Even with this low range of ATT, however, muscle MR, as well as the amplitude parameters for $[HbO_2]$, were negatively correlated with (i.e. impacted by) ATT. Yang et al. in (2005) found that optical coefficients attributed to fat could be ignored if the fat thickness was less than 5 mm (41). Van Beekvelt et al. (35) found no correlation between ATT and forearm muscle MR but their subjects had a very narrow range of ATT, well below the 5 mm cut-off (mean 2.2 $\pm$ 0.08mm). Consistent with these observations was that forearm MR was not significantly correlated with ATT for subjects with ATT < 5mm ($r = 0.06$, $P > 0.05$) but was for subjects with ATT $\geq$ 5 mm ($r = 0.79$, $P < 0.003$) (Fig. 3.2).

Van Beekvelt et al. (33), utilizing continuous wave NIRS and skin caliper ATT measurements, determined upper and lower values for forearm skeletal muscle MR relative to ATT and proposed that subjects with different ATT could be compared by normalizing the data to ATT. Following the same rationale, MR and all $[HbO_2]$ amplitude parameters in the present study were corrected for ATT. As seen in Fig. 3.2, without correction there was a significant decrease in apparent muscle MR with increasing ATT, and accurate physiologic measures of forearm skeletal muscle MR were suspect as there was ~7-fold range in the data, similar to the

10-fold range of MR seen by Van Beekvelt et al.(33). In comparison, when corrected for ATT, there was a ~ 2-fold range in forearm skeletal muscle metabolic rate in the present study (Fig. 3.2), which is less than the that associated with direct Fick method determination of forearm MR (3, 36).

The variability imposed by ATT on MR, as well as on the [HbO_2] signal, also resulted in erroneous correlations between MR and parameters of the [HbO_2] response (Fig. 3.3). The validity of using NIRS to assess the microcirculatory response is also contingent on corrections for ATT. When uncorrected, none of the NIRS [HbO_2] parameters were significantly correlated with the BABF parameters (Fig. 3.5 top panels) but the corrected amplitudes for [HbO_2] were significantly correlated with the corresponding BABF parameters (Fig. 3.5 bottom panels). Thus, both false significant correlations (Fig. 3.3) as well as missed significant correlations (Fig. 3.5) were observed in the present study when ATT was not considered.

Forearm Skeletal Muscle Metabolic Rate

The wide range of forearm metabolic rates reported in the literature utilizing NIRS technology were most likely influenced by the range of ATT in the subjects, but there are a number of other reasons why comparison of the values in the present study with those reported in the literature may be untenable.

In the present study [THb] provided verification whether or not blood was not accumulating under the probe during cuff occlusion. Even at cuff pressures of 250-300 mm Hg, 9 of the 75 trials (12%) had $\geq$ 15% increase in [THb] during cuff inflation and were thus excluded. Few studies have reported excluding trials in which [THb] did change (10, 35). In contrast, two studies have reported 30-60 s for cuff inflation to achieve arterial occlusion (23, 24), which likely were associated with large increases in [THb].

MR at rest has been measured in the forearm using NIRS with either venous or arterial occlusion protocols. One advantage of venous occlusion is that forearm blood flow can be estimated concomitantly (10, 13, 19, 36). MR obtained during venous occlusion has been found to agree with (10) or underestimate by half (13) values obtained without occlusion. Poor reproducibility (CV of 33%) led Van Beekvelt, M. C. et al. (35) to conclude that the venous occlusion method does not provide a reliable quantitative value for MR.

On the other hand, estimating MR from NIRS using the arterial occlusion method has shown to be reproducible (8). Supra-systolic tourniquet-induced ischemia was first utilized by De Blasi, R.A. et al. (12). They derived MR by calculating the rate of conversion of HbO_2 to HHb (△[Hb diff]) during the first 60 s of the linear decrement during the cuff occlusion. Subsequent reports have used the same method (10, 11, 13, 36). However, the △[Hb diff] calculation of De Blasi et al. (12) in reality sums the rates of change of both [HbO_2] and [HHb] with the net effect of an overestimation of MR by a factor of two. In contrast, the present study defined MR as the rate of appearance of HHb (△[Hb]) (Fig. 3.1b) during the initial linear increase following the cuff occlusion/inflation. The Hb desaturation rate under these conditions seems a more appropriate measure of MR. While there is wide variability in reported values for the studies above for NIRS arterial-occlusion-derived MR, the averages are indeed over twice as high [(4.96 $\pm$ 0.76 (12), 2.7 $\pm$ 0.04 (11), 3.45 $\pm$ 0.98 (13), 2.9 $\pm$ 0.09 (10), 4.91 $\pm$ 1.34 (36), overall AV 3.78 $\pm$ 0.64 μMO_2/min/100 g)] when compared to the present study (mean 1.70 $\pm$ 0.86 μMO_2/min/100g) (all values uncorrected for ATT). If corrected for ATT, presumably all of the previous measurements would remain ~ twice as high as the present data.

Previous studies of human forearm reported VO_2 at rest to be 4.4 $\pm$ 1.3 μMO_2/min/100g (27), 5.8 $\pm$ 3.9 μMO_2/min/100g (18) and 6.7 $\pm$ 2.7 μMO_2/min/100g (35) based on direct Fick method (Av 5.6, range of averages 4.4 – 6.7 μMO_2/min/100g). The MR reported in the present study (4.66 $\pm$ 0.68, range 3.42 – 6.48 μMO_2/min/100g) is close to these previous findings based on direct measurement of muscle oxygen uptake (N.B. In two studies, NIRS-determined oxygen uptake of forearm flexor muscles has not correlated with measurements of muscle MR by direct Fick method (3, 36)).

Influence of metabolic rate on [HbO_2] responses

The NIRS [HbO_2] signal is a measure of oxygenated hemoglobin within a tissue volume and changes in the signal are presumably influenced by both arterial inflow and MR ([HbO_2] = [THb] – [HHb]). At rest with intact circulatory flow, we found that [HbO_2] at rest was significantly correlated to MR. During occlusion, with cessation of blood flow, the forearm becomes a closed vascular compartment and the [HbO_2] signal should only reflect O_2 utilization (MR) (2 in Fig. 3.1 panel b). Indeed, the amount of O_2 remaining in HbO_2 at the end of 5 min. of ischemia inversely corresponded to the metabolic rate of the tissue ($P<0.01$).

During the initial PORH response, we envisioned that [HbO_2] would be primarily influenced by changes in perfusion (3 in Fig. 3.1 panels b and c) and that parameters describing the magnitude of the response should correspond to the putative quantity of vasodilators released by the ischemic tissue which, in turn, would reflect the metabolic rate of that tissue. Indeed, we found the amplitude of the hyperemic response scaled either from end cuff or from rest, was highly significantly correlated with MR_C ($P<0.0001$ and $P<0.001$ respectively), as hypothesized. However, none of the parameters describing the time course, either of the initial response or in recovery, were correlated with MR despite a large range for the PORH [HbO_2] time responses for subjects (Table 3.2). As an example, Fig. 3.4 shows the PORH response of two subjects where the time to midpoint of the response (tm) for one is ~1/3 that of the other. We appreciate that the [HbO_2] signal is not a measure of absolute blood flow, but we wondered if the temporal profile of this signal was related to that of flow. We presume that there is a physiologic reason for intersubject differences in the time course of responses, but in light of the lack of correlation it remains unclear as to the mechanistic determinants of the [HbO_2] kinetics during PORH. Finally, the mechanism(s) responsible for restoring vascular tone to precuff conditions in the recovery phase do not seem to be determined by metabolic rate, as evidenced by the lack of correlation between the 95 s value and MR.

Comparison of NIRS [HbO2] with an independent measure of microcirculatory PORH

To validate [HbO_2] as a measure of microvascular reactivity during PORH, comparisons were made with corresponding parameters describing BABF. The significant correlations between the [HbO_2] amplitude parameters and the corresponding BABF parameters gives credence that the amplitude of the [HbO_2] response is an indicator of microvascular reactivity and thus, the potential utility of NIRS to noninvasively measure microvascular dysfunction. However, later in recovery, [HbO_2] at 95 s does not correspond to conduit artery blood flow. Disparity between the kinetics of adjustment of [HbO_2] and BABF during PORH suggest that the downstream distribution of flow may be multi-compartmental. BABF is the integral of all flow while the vascular compartment sampled by NIRS may not reflect the sum total of all the forearm vasculature during the response. Similar disparity between conduit artery and estimated capillary blood flow kinetics following the onset of exercise has been reported (17).

Study Limitations

A number of the limitations of this study are related to the general assumptions of NIRS. It is assumed that Hb is the only absorbing chromophore that changes in the oxygenation signal, which will then reflect a change in [HbO_2]. In fact, there is an unknown contribution from myoglobin concentration [Mb] under different conditions. Thus, total [Mb] has the potential to be a confounding factor to NIRS parameters measured during PORH. However, the presence of myoglobin does not influence the MR calculation because changes in the light absorption characteristics depend on the total amount of chromophore present and whether or not each is associated with O_2 (i.e. the utilization of four molecules of O_2, either from one molecule of HbO_2 or 4 molecules of oxy-myoglobin) are equivalent from the standpoint of their effect on photon absorption, the [HbO_2/MbO] signal, and the calculation of oxygen uptake. Constant tissue water content is also assumed with NIRS measurements. During PORH there can be simultaneous changes in blood flow and volume and blood volume changes affect the tissue pathlength (14). In the present study, the FDMD NIRS instrument dynamically calculated the scattering coefficient for each wavelength, thus providing a dynamic correction for any changes in the pathlength. Capillary hematocrit increases significantly in exercise (21) and presumably may change as well during PORH, producing a subsequent decrease in water content and increase in [Hb] in the microvascular volume from which the signal is derived. Contributions from skin blood flow to the signal also cannot be ruled out. However, in the present PORH protocol, changes in NIRS responses due to whole body or skin heating should be nominal since subjects were supine, at rest, participating in a non-stressful protocol and were in a thermally controlled environment. Further, source-detector separations of up to 3.5 cm should insure that a large proportion of the signal emanated from deeper tissue (31).

In the present study, BABF and NIRS were used concurrently during the same protocol in the present study to assess skeletal muscle microvascular reactivity, with the goal of validating NIRS as a noninvasive tool to assess the microcirculatory function in health and disease. The lack of correlation for the kinetic parameters of these two methodologies may well reflect comparisons that were not identical. BABF during PORH is the total blood flow to all downstream tissues after a period of ischemia. NIRS derived [HbO_2] during PORH reflects the response of primarily one vascular bed (volume of skeletal muscle immediately under the probe) during the same time period. There was a correlation between the two methodologies for the

amplitude of the initial hyperemic response, which may reflect the effect of hydraulics on conduit and local hemodynamics following cuff release. However, it is possible that the distribution of blood flow in the forearm during recovery may have influenced the lack of agreement between BABF and [HbO_2] recovery amplitude (95 s values) and kinetic parameters (tm, d).

Conclusion

The main purposes of this study were to determine a robust measure of forearm skeletal muscle metabolic rate in resting conditions and to determine parameters of the post-occlusive reactive hyperemia test utilizing NIRS. Comparisons of [HbO_2] parameters were made with brachial artery blood flow as an independent methodology to access microcirculatory reactivity. From the results of this study it can be concluded that NIRS is a useful noninvasive tool for investigating tissue metabolism and microvascular reactivity. Strong evidence was provided for the imperative to correct NIRS signals for the effects of subcutaneous fat. We found that end-cuff values for [HbO_2] were inversely correlated with metabolic rate. Further, the amplitude of the PORH response for [HbO_2] was correlated with metabolic rate (i.e. the higher the metabolic rate, the greater the PORH [HbO_2] response). The [HbO_2] amplitude also correlated with that of brachial artery blood flow, suggesting that the amplitude of the PORH response in the microcirculation was similar to that in the conduit artery. However, neither the [HbO_2] parameters describing the time course of the initial response nor in recovery were correlated with metabolic rate or with brachial artery blood flow. Further studies are warranted to evaluate the potential use of NIRS to monitor tissue microvascular responses during PORH in assessing disease states, the affects of disease on oxygen transport, and utilization and the efficacy of therapies for microvascular pathology.

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Tables

Table 3.1 Subject Characteristics

Subject Characteristics	n=23
Gender (female / male)	12/11
Age (yr)	21.6 ± 2.1
Height (cm)	175 ± 10
Weight (kg)	73.3 ± 17.6
Resting heart rate (bpm)	65.9 ± 8.4
Systolic BP (mmHg)	119 ± 8.5
Diastolic BP (mmHg)	71.9 ± 7.3
Waist circumference (cm)	81.0 ± 13.0
Waist-hip ratio	0.79 ± 0.08
BMI (kg/m^2)	23.6 ± 3.7
Total fat mass (%)	27.6 ± 8.3
Abdominal fat (%)	34.7 ± 9.9
Fat free mass ($kg \times 10^2$)	505 ± 11.6

Values are absolute numbers or mean ± S.D.

Table 3.2 Values for amplitude and kinetic parameters of [HbO_2] and brachial artery blood flow prior to and during post occlusive reactive hyperemia.

	[HbO2] amplitude parameters	**BA blood flow**
	n = 21	n = 23
$y1_C$	54.3 ± 4.5	3.28 ± 2.58
K_C	68.9 ± 6.6	79.7 ± 32.0
$(K\ \%y1)_C$	152 ± 18	315 ± 167**
R_C	94.1 ± 7.3	10.3 ± 7.3
K'_C	24.6 ± 2.5	67.5 ± 24.2
$(K'\ \%R)_C$	30.7 ± 3.6	88.2 ± 55.2*
$(95s)_C$	16.3 ± 2.6	17.0 ± 10.3
$(95s\ \%R)_C$	19.1 ± 3.9	18.2 ± 66.0
$(95s\ \%y1)_C$	33.9 ± 5.7	74.9 ± 48.2
	[HbO_2] kinetic parameters	
tm (s)	11.7 ± 4.0	----
d (s)	22.0 ± 8.0	----
tm + 0.5d	22.7 ± 7.5	----

Amplitude parameters of [HbO_2] (μM) corrected for adipose tissue thickness, and brachial artery (BA) blood flow (cm^3/s). Values at y1=end cuff, K=amplitude from end cuff, R=rest, K'= amplitude from rest, and 95s= amplitude from rest at 95 s. Values for [HbO_2] kinetic parameters (s) for tm (time to midpoint of the post occlusive reactive hyperemia (PORH) response), d (time between 10% and 90% of the PORH response). Significant correlations occurred between BA blood flow and corrected [HbO_2] amplitude parameters at *P<0.03 and **P<0.01.

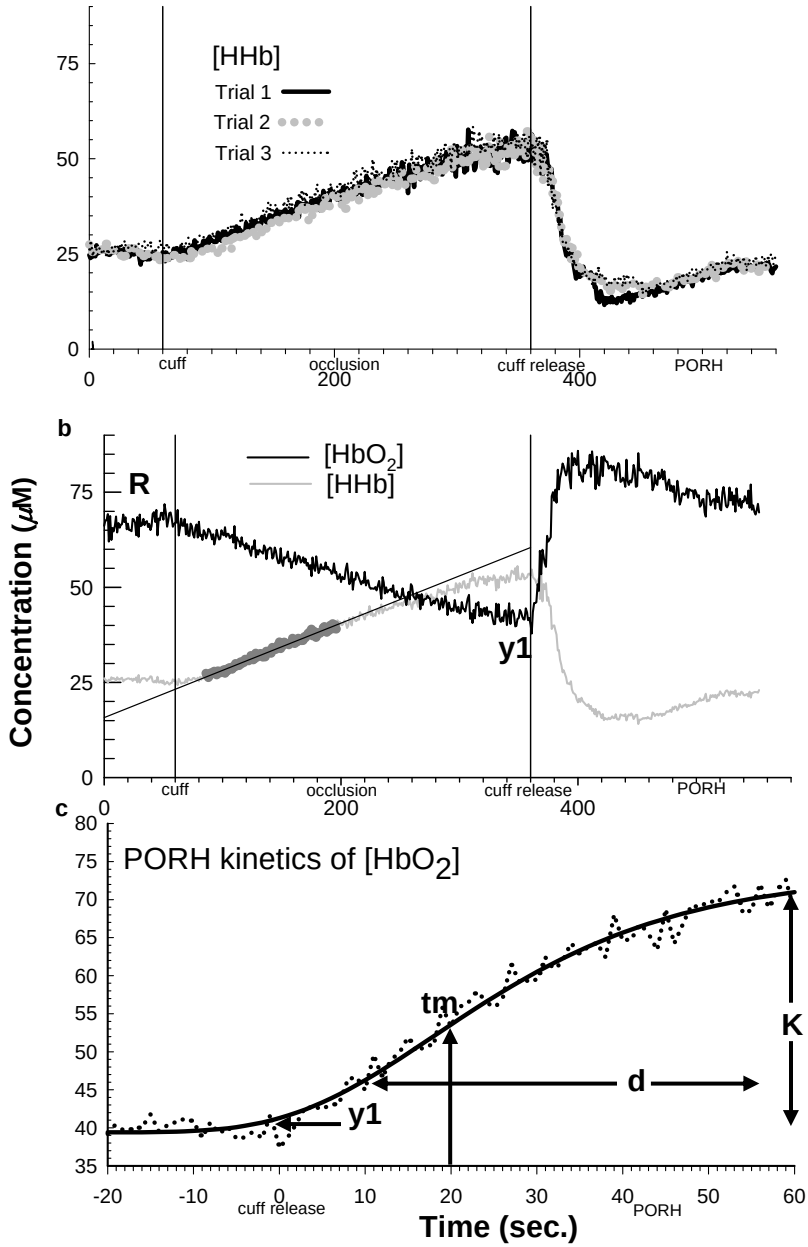
Table 3.3 Correlations between either [HbO_2] amplitude or kinetic parameters prior to and during post occlusive reactive hyperemia and metabolic rate and adipose tissue thickness.

[HbO_2] amplitude parameters	MR	ATT	$[HbO_2]_C$ with MR_C
y1	P<0.0001	P<0.0001	P<0.01
K	P<0.0001	P<0.0001	P<0.0001
K %y1	P<0.0001	P<0.0001	ns
R	P<0.0001	P<0.0001	P<.0001
K’	P<0.0001	P<0.0002	P<0.001
K’ %R	P<0.004	P<0.005	Ns
95s	P<0.0001	P<0.0002	Ns
95s %R	P<0.05	P<0.05	Ns
95s %y1	P<0.004	P<0.002	Ns
[HbO_2] kinetic parameters			
tm	ns	ns	Ns
d	ns	ns	Ns
tm + 0.5d	ns	ns	Ns

[HbO_2] amplitude (μM) or kinetic parameters (s) (*column 1*) prior to and during PORH and metabolic rate (MR) (μMO_2/min/100g) (*column 2*) and adipose tissue thickness (mm) (ATT) (column 3). Values at y1=end cuff, K=amplitude from end cuff, R=rest, K’= amplitude from rest, and 95s= amplitude from rest at 95 s. Correlation between [HbO_2] parameters corrected for ATT (*subscript c*) and MR corrected for ATT (*subscript c*) (column 4). ns = non-significant.

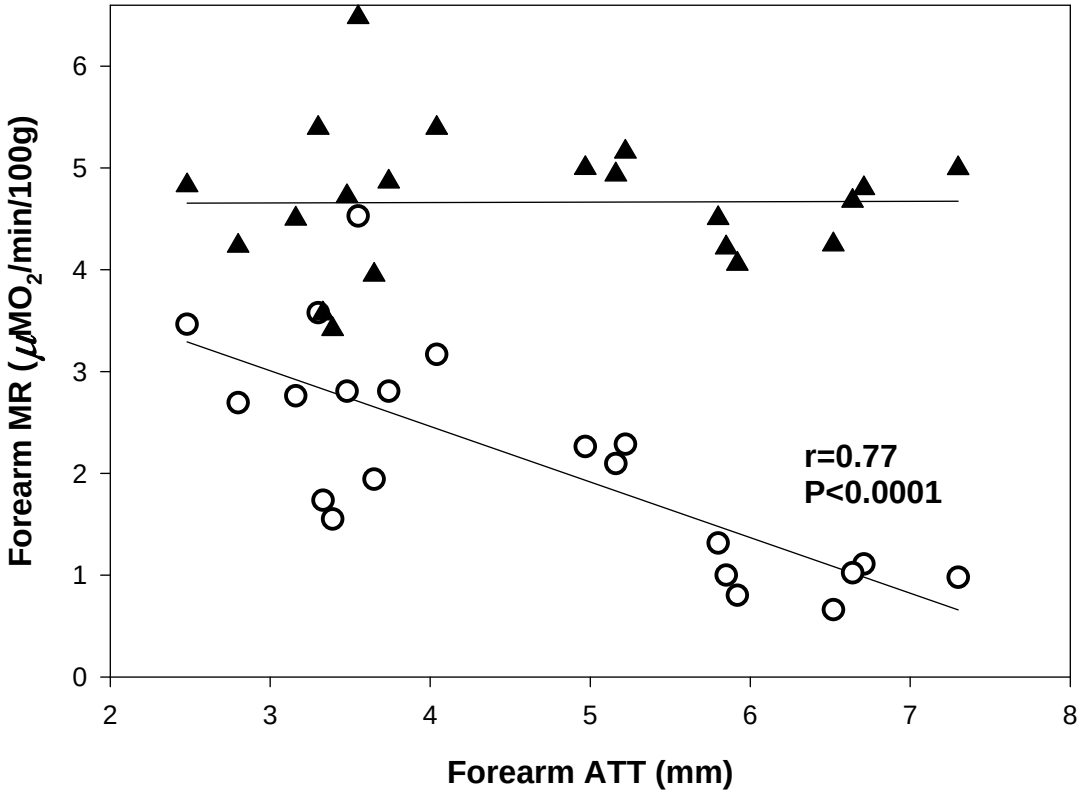
Figures

Figure 3.1 [HHb] and [HbO_2] at rest, during occlusion, and during post occlusive reactive hyperemia in one subject.



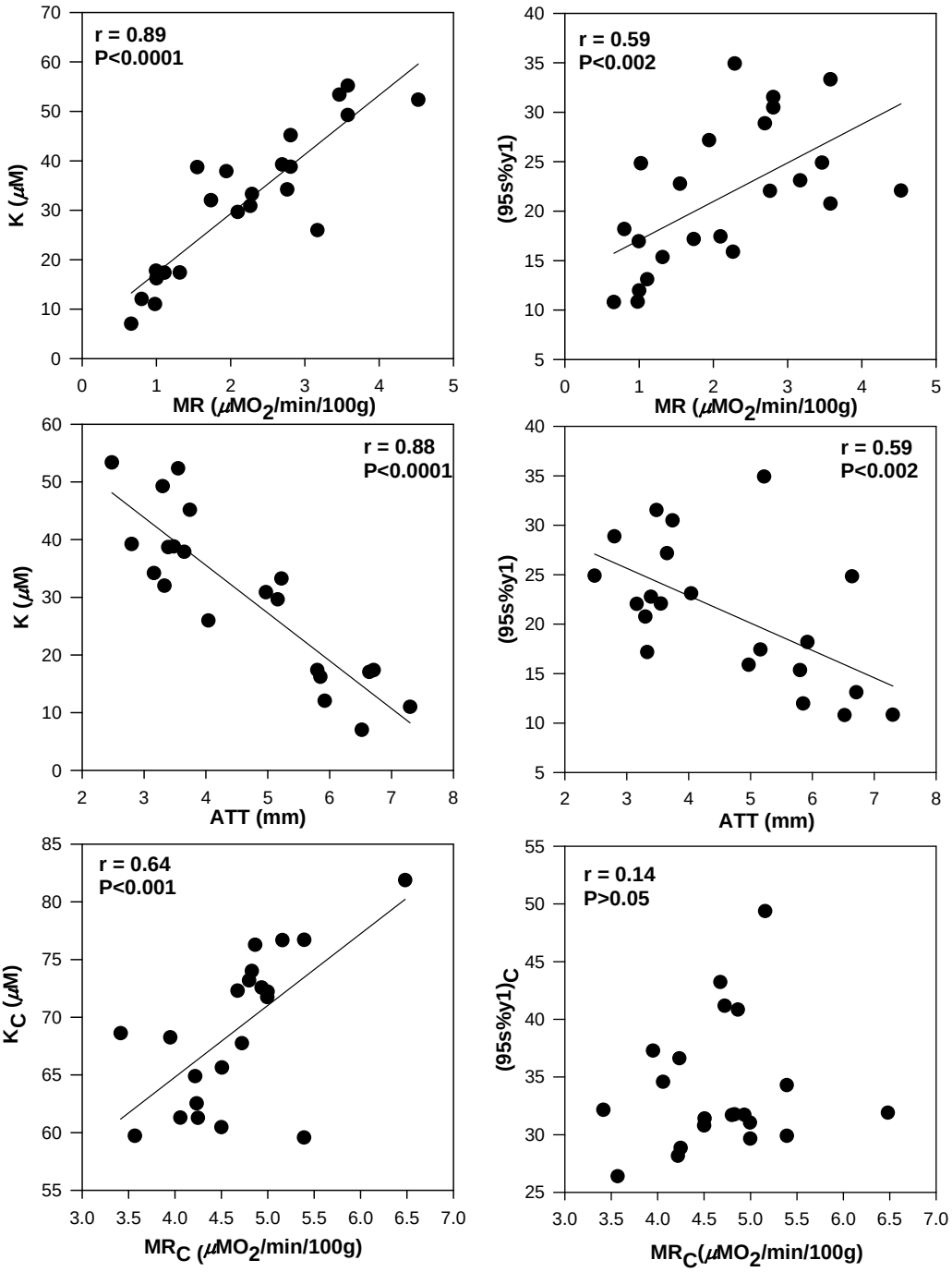
(*Panel a*) Three individual trials in one subject showing high reproducibility of [HHb] at rest (1 min), during occlusion (5 min) and during post occlusive reactive hyperemia (PORH) (2.5 min). (*Panel b*) Average of three trials of [HHb] (gray line) and [HbO_2] (black line). Initial linear increase in [HHb] (dark gray) used to determine forearm skeletal muscle metabolic rate (MR). (*Panel c*) Single logistic model of the average of three trials for [HbO_2], 30 sec prior to end cuff and 60 sec during PORH. Parameters of the response are R (rest), y1 (end cuff), K (amplitude from y1), K' (amplitude from R), tm (time to midpoint), d (time between 10% and 90% of the response) and tm + 0.5d (estimate of the peak of the response). See text for further details.

Figure 3.2 Forearm metabolic rate as a function of adipose tissue thickness.



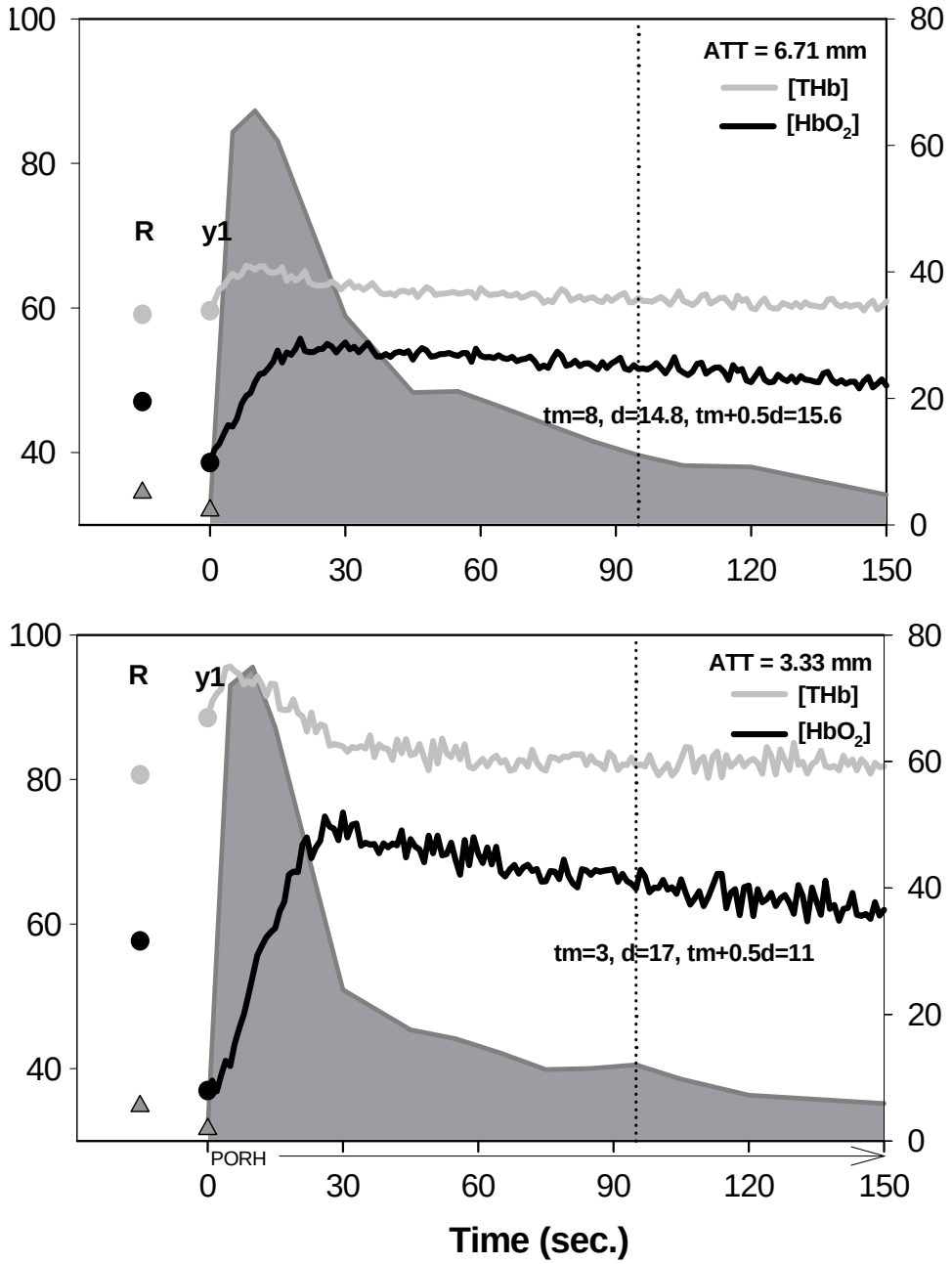
Forearm metabolic rate (MR) as a function of adipose tissue thickness ATT uncorrected (*open circles*) and corrected (*filled triangles*) for adipose tissue thickness (ATT). Uncorrected MR (Av = 1.70 $\pm$ 0.86, Range 0.52 – 3.60 μMO_2/min/100g) (6.9x range). Corrected MR (MR_C) (Av = 4.66 $\pm$ 0.68, Range 3.42 – 6.48 μMO_2/min/100g) (1.9x range).

Figure 3.3 Amplitude Amplitude of the [HbO_2] post occlusive reactive hyperemia response and value at 95 s as a % of end cuff as functions of the metabolic rate and adipose tissue thickness



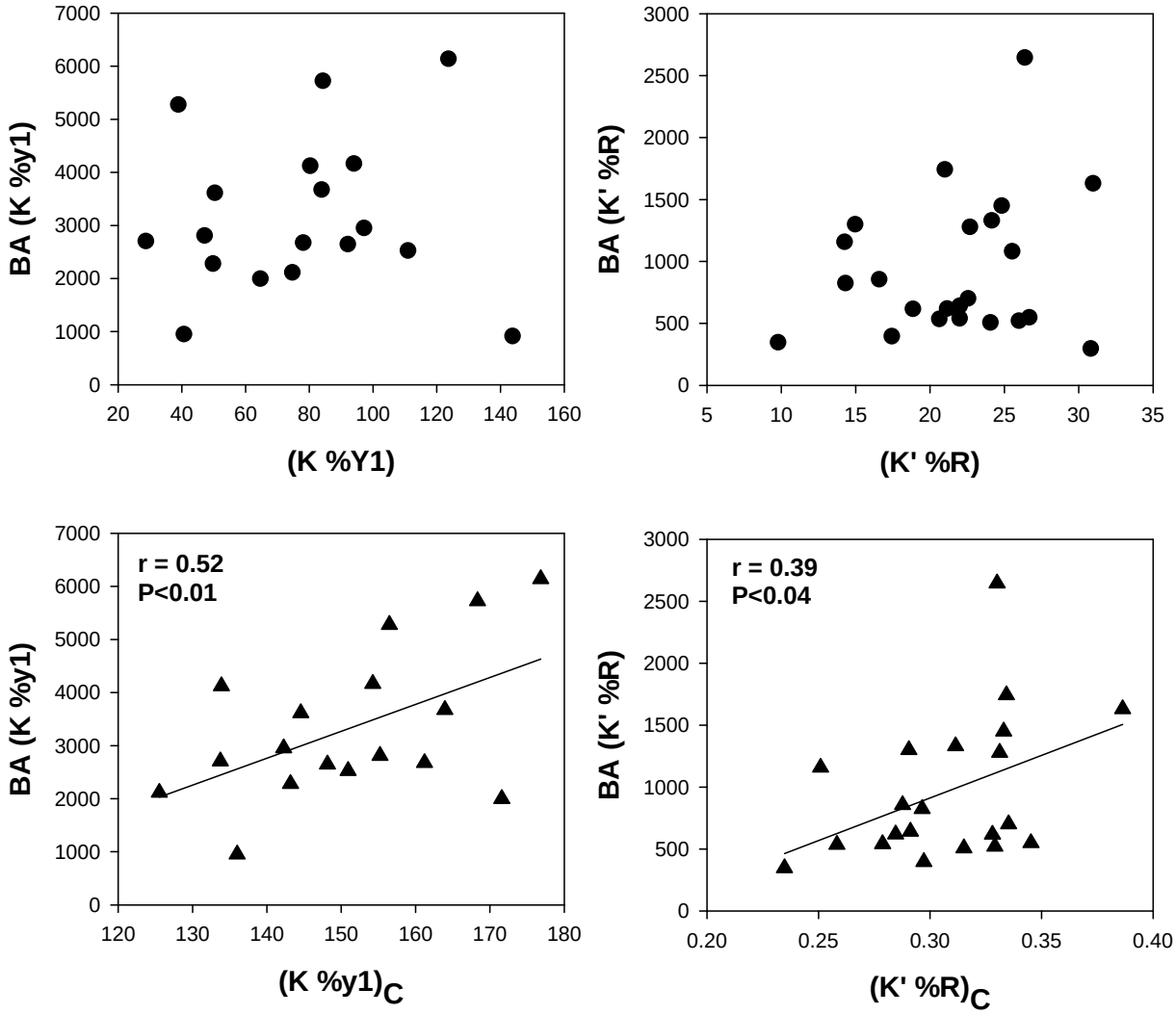
Amplitude of the [HbO_2] post occlusive reactive hyperemia response (K) and value at 95 s as a % of end cuff (95s %y1) as functions of the metabolic rate (MR) (*top panels*) and adipose tissue thickness (ATT) (*middle panels*) showing significant relationships. However, when K and 95s %y1 are corrected for ATT (*subscript c, lower panels*), only K_C remains significantly related to MR corrected for ATT (MR_C).

Figure 3.4 te[HbO_2], [THb] and brachial artery blood flow at rest, end cuff and for 2.5 min of post occlusive reactive hyperemia in two subjects.



[HbO_2] (μM) (black line), [THb] (μM) (lt gray line) and brachial artery blood flow (dk gray fill) (cm^3/s) for 2.5 min of post occlusive reactive hyperemia (PORH) after cuff occlusion release (Time = 0) in two subjects. Parameters at rest (R) and end cuff (y1) are shown for [THb] (lt gray circle), [HbO_2] (black circle) and brachial artery blood flow (dk gray triangle).

Figure 3.5 Comparison of two measures of the post occlusive reactive hyperemia microcirculatory response.



Comparison of brachial artery (BA) amplitudes from end cuff (K) and from rest (K') of initial post occlusive reactive hyperemia response (PORH) with that of [HbO_2] in the forearm. Uncorrected (*upper panels*) and corrected (*lower panels*) for adipose tissue thickness (ATT). %y1, scaled to end cuff value. %R, scaled to rest value. Only the corrected [HbO_2] parameters are significantly correlated with the corresponding brachial artery blood flow parameters.

CHAPTER 4 - Reduced Macrovascular Reactivity and Microvascular Control Abnormalities Relative to Insulin Resistance in Normoglycemic College-age Subjects with a Family History of Type 2 Diabetes

Abstract

Objective—There are virtually no data regarding the prevalence of endothelial and microvascular dysfunction relative to the magnitude of early insulin resistance (IR) in normoglycemic, college-age adults with a genetic susceptibility for type 2 diabetes. Thus, the goals of this study were to determine for a range of IR in normoglycemic, college-age adults with a family history of type 2 diabetes; (1) post-occlusive reactive hyperemia (PORH) flow-mediated dilation (FMD) of the brachial artery (BA) as a measure of macrovascular vasoreactivity, (2) parameters describing both near-infrared spectroscopy (NIRS) and BA blood flow (BF) responses to PORH as indices of skeletal muscle microvascular reactivity, and (3) the relationship between macro- and microvascular reactivity.

Research Design and Methods- Twenty-six, young (18-26 years), normoglycemic subjects were assessed for hepatic insulin sensitivity (ISI_{HOMA}) and whole body insulin sensitivity (ISI_{COMP}) by measures of plasma glucose and insulin both in fasting conditions and during an oral glucose tolerance test (OGTT). Each subject underwent three trials of upper limb PORH while BA velocity and were determined by Doppler ultrasound and changes in forearm oxy- (hemoglobin/myoglobin) ([HbO_2]) and deoxy- (hemoglobin/myoglobin) ([HHb]) were determined by NIRS. Forearm skeletal muscle metabolic rate (MR) was estimated from the initial linear decrease in [HHb] during the cuff occlusion. Endothelium-dependent macrovascular reactivity was determined by peak % change in BA diameter standardized by the area under the curve shear rate stimulus. The PORH [HbO_2] responses were modeled and parameters describing amplitude and the time course of the response were determined.. The same parameters describing the BABF response were determined, as a second independent index of microvascular reactivity.

Results—Subjects exhibited a 5-fold range in ISI_{COMP} (9.88 $\pm$ 3.98, range 3.6-18.5) and 54% were IR ($ISI_{COMP} \leq 9.7$). Decreasing ISI_{COMP} (increasing IR) was negatively correlated with % change in BA diameter ($r = -0.49$, $P < 0.01$). Microvascular reactivity, as measured by [HbO_2] parameters, was not significantly different between IR and insulin sensitive (IS) subjects while most BABF amplitude responses were significantly different between IR and IS. Additionally, increasing ISI_{COMP} was significantly negatively correlated with BABF at rest and the hyperemic

response from end cuff ($r=-0.48$, $P<0.01$ and $r=-0.43$, $P<0.03$ respectively). This enhanced BF relative to IR was not correlated with mean arterial pressure, but was correlated with an increase in conductance at rest, at peak hyperemia and at 95 s ($P<0.01$, $P<0.04$, $P<0.01$ respectively). Parameters of BABF (microvascular response) at rest, peak hyperemia from rest, at 95 s PORH and total hyperemic response were significantly negatively correlated with % change BA diameter (macrovascular response) (all $P<0.04$).

Conclusions—The present study demonstrated that IR in otherwise healthy, normoglycemic, college-age subjects with a family history of type 2 diabetes is associated with both early macro- and microvascular dysfunction.

Introduction

Macro- and microvascular disease are the leading causes of morbidity and mortality in patients with type 2 diabetes (21). Endothelial dysfunction is incidental to macrovascular disease and predictive of cardiovascular events (12, 80), and has been linked to a decrease of the bioavailability of nitric oxide (NO). Since NO is a potent antiatherogenic molecule, it is believed to be a main mechanistic link between attenuated macrovascular reactivity and concurrent macrovascular pathology. Under specific conditions, brachial artery (BA) flow-mediated dilation (FMD) is almost entirely mediated by NO (58). BA FMD is an independent predictor of future cardiac events (80), correlated with coronary vasomotor dysfunction and may be useful for early detection of coronary artery disease (5).

In the natural history of type 2 diabetes, metabolic and physiologic changes begin early and proceed in tandem (49). However, even though this process is exacerbated by, it is not dependent on, hyperglycemia. This association is corroborated by the conclusion that insulin resistance (IR) in young adults predicts coronary heart disease risk factors (4) and is an injunction that the progress of cardiovascular complications and silent atherosclerosis can hide behind a façade of "health", particularly in young adults. IR can be detected in young adult, nondiabetic offspring of type 2 diabetic parents (67, 73). The few prior studies that have examined endothelial-dependent macrovascular dysfunction in normoglycemic subjects included those that ranged in age between the late 20s and old age. However, for the vast majority of literature involving BA FMD, the response is not standardized by the magnitude of the stimulus that mediates the response even though there is compelling justification to do so (46, 56-58).

Microvascular complications are frequent in diabetes, as evidenced by the fact that ~62% of diabetic patients will have retinopathy in the course of their disease (86). Retinal and renal microangiopathy cause diabetic retinopathy and nephropathy respectively, and the microangiopathy of the vasa nervosum contributes to the pathogenesis of neuropathy. The literature taken together is unequivocal that impaired microvascular reactivity is clearly present in diabetes and prediabetes, but there is sparse information that predates dysglycemia in the continuum toward frank disease and these few studies have utilized acetylcholine or local heating to evaluate cutaneous microcirculatory responses. These studies may not necessarily be relevant to control of the microcirculation in skeletal muscle. We propose to utilize two non-invasive methods to determine changes in downstream microvascular reactivity in skeletal

muscle during post-occlusive reactive hyperemia (PORH): BA blood flow (BF) (using duplex Doppler ultrasound) and forearm tissue oxygenation (using frequency domain multi distance (FDMD) near infrared spectroscopy (NIRS)).

We hypothesize that in normoglycemic, college-age students with a family of type 2 diabetes; (1) macrovascular dysfunction, measured by BA FMD, after correction by the shear rate (SR) stimulus, will be diminished relative to the magnitude of IR, (2) parameters for BABF and for FDMD NIRS will be attenuated relative to the magnitude of IR, and (3) parameters describing macrovascular reactivity (FMD) will correlate with corresponding parameters for microvascular reactivity (BABF and NIRS).

Methods

Subjects and exclusion criteria

Twenty-six healthy, sedentary, college-age subjects (13 men, 13 women), age 18-26 yrs., participated in this study. Twenty individuals were self-reported first-or second-degree relatives of patients with type 2 diabetes (had family history (FH)) and 6 were of similar BMI and gender distribution with no known family history of type 2 diabetes (no FH). Three of the FH subjects were siblings (2M, 1F). To minimize confounding factors for insulin sensitivity and/or vasoreactivity, potential subjects were excluded if they had a history of smoking, regular exercise in the last 6 months of > 3x20 min. sessions/week of at least moderate intensity aerobic exercise, or had self-reported medical conditions of Acanthosis Nigricans, polycystic ovarian syndrome, or irregular menses with hirsutis. Exclusion criteria also included current weight loss or dietary modifications, antioxidant therapies or a diet that included a combination of > 4 servings fruit and vegetables/day or hormone therapy or hormonal contraception (except low dose combination ethinyl estradiol/progestin pills). Female subjects were studied during the early follicular phase of their menstrual cycle as verified by progesterone (P_4) levels $\leq$ 1.5 ng/ml. Potential subjects taking antihypertensives, lipid-lowering agents, drugs to control blood sugar (metformin, sulfurylureas), bronchial dilators, or psychoactive medications were excluded. On the day of screening subjects were excluded if 12 hr. fasting glucose levels were $\geq$ 100 mg/dl, as measured by a hand held glucometer (FreeStyle Flash, TheraSense, Alameda, CA) or if blood pressure was $\geq$ 140/95 mmHg. Data were rejected if measurements on subsequent days included results above the cut-off for exclusion as enumerated for the initial screening, or if biochemical analysis indicated triglycerides levels $\geq$ 300 mg/dl, cholesterol levels $\geq$ 210 mg/dl or 2 hr. post OGTT $\geq$ 140 mg/dl. Four subjects, from an initial 30 screened, were excluded. Three subjects were overweight (OW) (BMI, 25-29.9 kg/m^2), three subjects were obese (O) stage 1 (BMI, 30-34.9 kg/m^2), and one subject was obese stage 2 (BMI $\geq$ 35 kg/m^2). Nineteen lean subjects (L = BMI < 25) were included. All were recruited from the general population of students at Kansas State University. Informed consent was obtained after both written and verbal explanation about the possible risks and discomforts of the experimental protocol were given. The study was approved by the Institutional Review Board for Research Involving Human Subjects at Kansas State University.

Oral Glucose Tolerance Test (OGTT) and analytical procedures

Subjects reported to the lab between 0700-0800 after a 10-12 hour fast and 24 hour abstinence from any exercise, alcohol and caffeine for a 2-hour oral glucose tolerance test (OGTT). An indwelling antecubital catheter was inserted and kept patent with a continuous isotonic saline drip. Subjects rested at least 15 min. before the initiation of the test. Baseline blood values were collected for fasting serum glucose (FG) and fasting serum insulin (FI) at -15, -10, and -5 min. and the mean of the 3 results was used as the baseline blood sample. Subjects then ingested 1.75 g of glucose (SUN-DEX 10 g. glucose/fl. oz.) per kg body weight and timing of the test was referenced to the beginning of the drink (t=0) with subjects having <5 minutes to completely consume the drink. Additional samples were then drawn at t=30, 45, 60, 75, 90 and 120 min. All samples were collected in serum separating tubes and the serum separated using refrigerated centrifugation at 3,000 rpm for 10 min. Samples were immediately analyzed for serum glucose (mg/dl) (YSI 2300 glucose oxidase analyzer). Serum insulin (μU/ml) was stored in liquid nitrogen and measured later by monoclonal, two-site, immuno-radiometric assay (Mercodia Insulin ELISA, ALPCO Diagnostics, Windham, NH) with detection limit < 1 μU/ml and intra assay CV $\leq$ 5%.

Anthropometry

Measurements of height (cm), weight (kg), waist and hip circumference (cm), BMI, blood pressure and resting heart rate were taken. Body composition was assessed by dual-energy X-ray absorptiometry (Prodogy, General Electric v. 5.6) and regions of interest were specified between the 7^{th} rib and the iliac crest for measurement of visceral adiposity (abdominal fat %) and distal to the olecranon process for forearm skeletal muscle mass. From a resting baseline blood sample, total cholesterol, HDL-cholesterol, C-reactive protein (CRP) and triglyceride levels were determined. LDL was calculated from Friedewald's formula (23). Progesterone (P_4) (Progesterone RIA DSL-3900, Diagnostics Systems Laboratories, Inc., Webster, TX) and estradiol (E_2) (Coat-A-Count Estradiol, Diagnostic Products Corporation, Los Angeles, CA) were measured for females. Criteria for the metabolic syndrome were defined from the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III, ATP III) (20).

Metabolic Calculations

Positive incremental areas under the curve (AUC-baseline) (2) during the OGTT were calculated with the trapezoidal method, from t=0 to 120 min, for glucose (mg/dl) (Glu_{AUC}OGTT) and insulin (μU/ml) (I_{AUC} OGTT). AUC values were divided by the 120 min. and added back to fasting values to determine average glucose (Glu_{Av} OGTT) and average insulin (I_{Av} OGTT) during the OGTT.

A measurement of hepatic insulin sensitivity, $ISI_{HOMA,}$ was calculated as an inverse of the homeostatic model assessment, (44), as described by Radziuk (59), after correcting for different units of glucose than the original calculation.

$$\mathbf{ISI_{HOMA}} = \frac{22.5 \times 18}{(FI \times FG)} \qquad (1)$$

Whole body insulin sensitivity (ISI_{COMP}), representing a composite of hepatic and peripheral tissues (43), was calculated as:

$$\mathbf{ISI_{COMP}} = \frac{10{,}000}{\sqrt{(FG \times FI) \times (Glu_{AV}OGTT \times I_{AV}OGTT)}} \qquad (2)$$

Although the present study did not validate insulin resistance (IR) in our subject cohort by independent means, in order to facilitate group comparisons, subjects were considered IR if their $ISI_{COMP} \leq 9.7$, the upper limit of 95% ISI_{COMP} for IR subjects as determined by impaired fasting glucose (IFG), reported recently by Piche et al. (52).

Protocol

All vascular tests began between 0700-0830 hrs, and were performed in a quiet, temperature-controlled room. Participants refrained from alcohol and exercise for 24 hrs and caffeine for 12 hrs and were fasted or had a very low-fat snack prior to coming to the lab. All vascular measurements were taken during rest (1 min.), occlusion (5 min.) and during post-occlusive reactive hyperemia (PORH) (2.5 min.). For three of the initial subjects, blood pressure (BP) was continuously monitored noninvasively at the radial artery (Model 7000, Colin, TX) of the contralateral arm. No changes in BP occurred prior to, during occlusion or during post

occlusion. During occlusion, ischemia was induced by rapid inflation of a pediatric blood pressure cuff, placed just proximal to the antecubital fossa and distal to the brachial artery (BA) imaging, to $\geq$ 250 mm Hg. Three trials were obtained for each subject with > 10 min. between adjacent tests to permit vessel recovery. Accepted trials (see below) were averaged before analysis for BA and microcirculatory responses for each subject.

Vascular Function Tests

Standard guidelines were followed for flow-mediated dilation (FMD) of the BA in assessing endothelial function in a conduit vessel (15). The mechanism for this response is the activation of endothelial nitric oxide synthase by shear stress and the release of nitric oxide (NO) resulting in FMD. This noninvasive method was first described by Celermajer et al. in 1992 (13). Briefly, subjects lay supine with the right upper limb extended at heart level, and slightly supinated for the duration of the trials. The brachial artery was imaged as far as possible proximal to the antecubital fossa, to ascertain and avoid a potential high origin of the radial artery (a common anomaly documented in at least 12.5% of people (8) and noted in three of our subjects). To maintain the ultrasound image and measurement position, the arm was secured in a wooden foam lined frame and the probe was secured with a stereotactic clamp. This allowed minor adjustments to be made to produce a simultaneous optimal blood velocity signal and vessel image. All trials for a given subject were measured from the same 10mm section of the artery. BA blood velocity and image were measured with pulsed Doppler ultrasound (VIVID3, GE Medical Systems) with an integrated packaged ECG and a 10 L linear array transducer operating at 8-MHz in 2-D B mode. Discontinuous longitudinal BA images and ~3 beat-by-beat velocity tracings were recorded per frame. Three frames were obtained at rest, one during the last minute of occlusion, one each at 5, 10 and 15 s post-occlusion, and an additional ten frames at exactly 10-15 s intervals for the remainder of post-occlusion. A total of 17 frames per trial were obtained, coded, and stored on the ultrasound hard drive for later analysis.

Skeletal muscle oxygenation was evaluated continuously during the protocol using a Frequency-Domain Multi Distance (FDMD) Near-infrared Spectroscopy (NIRS) (OxiplexTS, Model 96208, ISS, Champaign, IL, USA) system operating at wavelengths of 692 and 834 nm with a modulation frequency of the light-source intensity of 110 MHz. The probe utilized source-detector separations of 2.0, 2.5, 3.0 and 3.5 cm. FDMD NIRS utilizes continuous dynamic

measurement of reduced scattering coefficients (μ'_S, cm^{-1}) and thus can provide quantitative measures (μM) of hemoglobin (Hb) /myoglobin (Mb) oxygenation states. These signals arise primarily from the microvasculature since in vessels larger than ~1mm there is a large amount of absorption of light (42). Because of similar spectral characteristics it is not possible to distinguish between hemoglobin and myoglobin contributions to the NIRS signal. Thus, the variables in the present study were reported as deoxy-[hemoglobin + myoglobin] ([HHb]) and oxy-[hemoglobin + myoglobin] ([HbO_2]).

The probe was positioned longitudinally on the lateral anterior antebrachium overlying the flexor digitorum superficialis, flexor carpi radialis and brachioradialis. It is generally accepted that the volume of interrogation is found between, and below the surface of the skin at a depth of roughly half the distance between, the source and detector (68). Ultrasound images were taken at the same location as the NIRS probe to accurately assess underlying adipose tissue thickness (ATT). The change in [HHb] during the initial period of arterial occlusion was used to calculate forearm muscle oxygen consumption (metabolic rate (MR)), while the change in [HbO_2] during post-occlusive reactive hyperemia (PORH) was used as an indicator of changes in perfusion, predominantly of forearm skeletal muscle, and downstream compliance of the arterioles, capillaries and venules. MR and all [HbO_2] parameters used to describe PORH were corrected for the influence of ATT as described previously (72). Total hemoglobin ([THb]) was determined as the sum of [HHb] and [HbO_2]), while tissue O_2 saturation (StO_2) was calculated as StO_2 = [HbO_2] / THb (%). The NIRS data were stored at an output frequency of 31.25 Hz.

BA measurements and hemodynamics

Trials were excluded for poor or missing critical BA images, or weak or venous influence on the velocity signals. Examinations of the BA images were performed in random order, by a single sonographer blinded to the subject identified. BA was measured on frozen images with electronic calipers as the anterior to posterior endothelial-lumen interface and determined from an average of 3 successive cardiac cycles, 3 measurements / cardiac cycle, both for the widest (caused by the systolic pressure wave, coincident with the T wave on the electrocardiogram) and narrowest (caused by diastolic pressure, coincident with the end of QRS on the electrocardiogram). The intraobserver coefficient of variation was 0.82% $\pm$ 0.37%, range 0.42% - 1.79%, similar to the CVs for single observer manual analysis of 0.95-1.15% obtained by

Woodman et al.(87). Due to the visually large fluctuations between systolic and diastolic diameters in a number of subjects, mean vessel (BA) per cardiac cycle was calculated as diastolic + 1/3(systolic-diastolic). Average velocity (V) per cardiac cycle was calculated by the manufacturer's software. Shear rate (SR) per cardiac cycle was calculated as V / BA for each cardiac cycle. Brachial artery blood flow (BABF) per cardiac cycle was calculated as V x π(BA /2)^2. The data for ~ 3 cardiac cycles per frame were averaged to produce measures of BA , V, SR, and BABF, for each of the 17 time frames prior to and during PORH, and plotted. Parameters to describe the BABF PORH response were: R (baseline during resting conditions), y1 (baseline for the last 30 s of the cuff occlusion), K (the amplitude of the PORH response from y1), K' (the amplitude of the PORH response from R), 95 s (the blood flow above R, 95 seconds after cuff release) and AUC (total hyperemic response above blood flow at rest).

Conduit endothelium dependent vasodilation

Macrovascular reactivity was assessed as the magnitude of FMD of the BA during PORH. This measure is contingent not only on the bioavailability of NO, but also the magnitude of the stimulus that produces the response. Accurate shear stress measurements are difficult to make in vivo because velocity and viscosity measurements must be taken close to the vessel wall and blood flow is pulsatile. Shear stress can be estimated by viscosity x velocity/. It is generally accepted that shear rate (SR) is an adequate surrogate measure (as reviewed by Pyke, K.E. et al. (58) since the FMD response appears to be independent from whole body viscosity (47). Both the peak and the duration of the SR stimulus has been determined to be important for the peak FMD response (35, 58) with the majority of the response contingent on the elevated shear stimulus over time (57). Thus, in the present study BA endothelial dependent vasoreactivity was calculated as:

$$\frac{\text{(BA peak -BA constriction) / BA constriction (\% change diameter)}}{\text{Area under the curve shear rate increase from rest for 55 s post cuff release } (SR_{AUC})}$$

i.e. BA endothelial dependent vasoreactivity = % change diameter / SR_{AUC} . BA constriction was the smallest BA , which frequently occurred 5-10 s post cuff release.

Forearm skeletal muscle metabolic rate

During arterial occlusion the rate of increase in [HHb] represents the rate of oxygen utilization, which is VO_2, so long as [THb] remains relatively constant. To control for this, trials were excluded in which [THb] increased $\geq$ 15% of the baseline value during the 5 min occlusion. Muscle oxygen uptake or metabolic rate (MR) was derived from the slope of the initial linear increase in HHb ([HHb]/s.). Concentration changes were expressed in μM/s. and converted to MR expressed as micromoles O_2 per 100 grams of tissue per min (μMO_2 / min / 100 g). A value of 1.06 g/ml was used for muscle density (37) and 1:4 for the molecular ratio between hemoglobin and oxygen.

Kinetics of PORH tissue perfusion

The reappearance of oxygenated blood after cuff release should mostly reflect perfusion. Thus, microcirculatory reactivity was assessed by the kinetic response of [HbO_2] during the initial phase of PORH. The 3 trials were averaged for 20 s prior to and 60 s after cuff release. The average response was modeled using a single logistic model with no time delay.

$$Y = y1 + \frac{K}{1 + e^{(-\ln(81) \times (t-tm)/d)}}$$

Where y1=end cuff, K= amplitude from y1, tm = the time to midpoint of the response, and d = time between 10% and 90% of the response. In addition, resting value (R), time to 90% peak (as tm + 0.5d) and amplitude from R at 95 s (95 s) were determined. Intrasubject coefficients of variation (CV) for the curve-fitting parameters for the 3 individual trials were determined for 5 subjects. Average CV for y1 = 4.7% (range 1.2 - 10.3), K = 6.7% (range 2.4 -13.2), tm = 9.7% (range 6.5 - 16.5), and d = 13.4% (range 3.3 - 25.5). Finally, similar parameters as were used for BABF were calculated for the PORH [HbO_2] response: R, y1, K, K', 95 s after cuff release as well as the total hyperemic response above resting values (AUC).

Statistics

All numerical values are presented as mean $\pm$ SD unless otherwise indicated. One-way analysis of variance (ANOVA) was performed to assess significance of relationships among the

groups. Correlations between variables were tested using univariate analysis by Pearson correlation analysis. Statistical analyses were performed using SigmaPlot 2001 (version 7, San Jose, CA, USA). Significance was declared for comparisons or relationships for $P<0.05$ (17).

Results

Subject Characteristics

Anthropometric and clinical characteristics are shown in Table 4.1. The male-female ratio for all subjects was evenly divided (13 females/13males). Fifty-eight percent of subjects demonstrated IR with more males (10 of 15) being IR than females (5 of 15). Height, weight and systolic blood pressure (BP) were significantly different between IS and IR ($P<0.01$). The mean age, resting heart rate and diastolic blood pressure (BP) were not significantly different between IS and IR subgroups. All subjects were normotensive (systolic BP=117 $\pm$ 8 mmHg, range 96-130 mmHg), yet systolic BP was still significantly correlated with ISI_{COMP} for all subjects ($r=-0.46$, $P<0.01$). Waist circumference was significantly different between IS and IR ($P<0.005$) as was hip circumference ($P<0.001$, data not shown) while waist-hip ratio was not significantly different between the two subgroups. BMI ($P<0.005$), abdominal fat % ($P<0.05$) and fat free mass ($P<0.01$) were significantly different between IS and IR, while total fat mass % was not.

Blood Chemistry Measurements

Table 4.2 shows the blood chemistry measurements for the subcategories of subjects. No significant differences were seen for any serum lipid measurements or C-reactive protein between IS and IR subjects. For all subjects, triglyceride/HDL ratio was significantly correlated with ISI_{COMP} ($r=-0.38$, $P<0.05$).

Metabolic Characteristics

Metabolic characteristics in the fasted state and during the 2-hr postprandial OGTT are shown in Table 4.2. None of the subjects met the clinical criteria for the metabolic syndrome (MS). Three of the IS subjects exhibited one of the MS criteria (low HDL) while six IR subjects had at least one MS criteria, three of these had two MS criteria. Nine of the 26 subjects had high levels (>3 mg/dl) of CRP and of these, one had an additional MS marker and two IR subjects had two additional MS markers. FI (range 2.43-12.51 μU/ml) was within the normal range of values listed for the immunoassay used (range 2-25 μU/ml) yet there was a significant difference between IS and IR subjects ($P<0.001$). There were significant differences between IS and IR for all measures of OGTT insulin: I_{AUC}OGTT ($P<0.01$), I_{AV}OGTT ($P<0.005$), and I_{2H}OGTT ($P<0.05$). All subjects were normoglycemic by FG (range 69.2 - 94.6 mg/dl) and Glu_{2H}OGTT

(range 61.3-124 mg/dl) clinical criteria. However, FG was still highly significantly different between IS and IR ($P<0.001$). IS and IR were significantly different ($P<0.001$) for both measures ISI_{HOMA} and ISI_{COMP}.

Macrovascular Stimulus and Reactivity

Macrovascular PORH stimulus and response variables are shown in Table 4.3. There was a wide range of both stimulus, SR_{AUC}, (range 260-806 s^{-1})) and response, % change diameter, (range 0.89-15.2 %). Fig 1 shows the different shear rates and similar BA responses for two subjects. However the variability in the response when standardized by the stimulus (% change diameter / SR_{AUC}) was greatly reduced (range 1.44-5.05). Fig. 4.2 shows % change diameter / SR_{AUC} for two subjects . There were no differences between IS and IR subjects for any of the resting or PORH SR variables. Resting brachial artery was significantly greater for IR compared to IS ($P<0.02$) (Table 4.3) and % change in diameter was significantly negatively correlated with resting for all subjects ($r=0.53$, $P<0.003$) (Fig. 4.3). Resting was significantly greater for male versus female subjects ($P<0.0001$) (Fig 3) but % change in diameter failed to reach a statistically significance difference between genders ($p=0.11$) because of the large variability (Fig. 4.3). However, when standardized by SR_{AUC}, % change in diameter was similar between genders ($P<0.95$) (Fig. 4.3), and was not significantly correlated with resting ($p=0.85$) (Fig. 4.3), nor was significantly different between IS and IR ($P<0.16$) (Table 4.3). ISI_{COMP} was also not correlated with resting ($r=0.21$, $p= 0.30$) (Data not shown).

Fig. 4.4 illustrates the importance of normalizing the % change in BA diameter to the stimulus. The upper panel suggests there is no significant relationship between % change in diameter and ISI_{COMP}. However, ISI_{COMP} was significantly correlated with % change in diameter normalized to SR_{AUC} ($r=0.49$, $P<0.01$) (Fig. 4.4). In addition, the normalized % change in diameter was significantly different between IS and IR ($P<0.05$) (Table 4.3).

Microvascular Reactivity

$[HbO_2]$ parameters before and during PORH are shown in Table 4.4. There were no significant differences between IS and IR for any of the values. Additionally there were no correlations between any of the blood lipid, CRP or metabolic variables and $[HbO_2]$ parameters for the subjects as a whole or any subgroup of the subjects.

The post-occlusion increase in BABF was a second measure of microvascular reactivity and parameters describing this response are also shown in Table 4.4. In contrast to [HbO_2] measures of microvascular reactivity, BABF exhibited a multitude of significant differences between IS and IR. Fig. 4.2 portrays the BABF responses for an IS and an IR subject. BABF at rest (R) was significantly greater in IR relative to IS ($P<0.05$) (Table 4.4) and significantly inversely correlated with ISI_{COMP} ($r =-0.48$, $P<0.01$) (Fig. 4.5). K and K' were significantly greater in IR relative to IS (both $P<0.03$) (Table 4.4) and the former significantly negatively correlated with ISI_{COMP} ($r =-0.43$, $P<0.03$) (Fig. 4.4) while the latter showed a trend but failed to reach significance in correlating with ISI_{COMP} ($P<0.07$) (data not shown). In addition, there were significant increases in the total hyperemic response from rest (AUC) and blood flow 95 seconds after cuff release (95 s) for IR relative to IS ($P<0.01$ and $P<0.05$ respectively) (Table 4.4) but these parameters did not correlate with ISI_{COMP}. R was highly significantly correlated with K' ($r=0.72$, $P<0.0001$).

Forearm conductance

Table 4.5 lists variables influencing conductance and differences in conductance between IS and IR subjects. There was no significant difference between IS and IR for mean arterial pressure (MAP), forearm skeletal muscle MR nor were these variables significantly correlated with ISI_{COMP}. However, all measures of conductance were significantly higher for IR relative to IS (all $P<0.03$) and conductance at rest (C_R) ($r=-0.47$, $P<0.01$) (Fig. 4.5), at peak hyperemia (C_{PH}) ($r = -0.36$, $P<0.04$) (Fig. 4.5) and at 95 s (C_{95}) ($r=-0.44$, $P<0.01$) (data not shown) were all significantly negatively correlated with ISI_{COMP}. Forearm skeletal muscle MR was not correlated with C_R or C_{PH} but was significantly correlated with C_{95} ($p=0.37$, $P<0.05$).

Macrovascular reactivity and microvascular reactivity

Normalized % change in diameter (macrovascular reactivity) was significantly negatively correlated with PORH BABF parameters for R ($P<0.04$), K' ($P<0.02$), 95 s ($P<0.03$), and AUC ($P<0.03$) (microvascular reactivity) (Fig. 4.5).

Discussion

In the present study we assessed macrovascular endothelial function of the brachial artery as well as skeletal muscle microvascular reactivity in the forearm using post-occlusive reactive hyperemia in healthy, normoglycemic, college-age subjects with a family history of type 2 diabetes, and related these results to the magnitude of IR. We have extended the findings of altered conduit vessel reactivity for those at risk for developing type 2 diabetes to include the youngest post-adolescent adult and provide evidence that the degree of vascular impairment is relative to the level of IR even in normal glucose tolerance. Specifically, as hypothesized, decreasing ISI_{COMP} (increasing IR) was significantly negatively correlated with reduced BA reactivity during post-occlusive reactive hyperemia. Our results strongly emphasize the importance of interpreting vascular reactivity data only when dilatory responses have been standardized by the stimulus (shear rate). Using a noninvasive method (NIRS), we also observed abnormalities in microvascular structure/vascular reactivity as changes in $[HbO_2]$ as well as changes in brachial artery blood flow during the 2.5 min. PORH period. As hypothesized, there were significant differences between IR and IS for BABF at rest (R), at peak hyperemia (K and K') and in the magnitude of hyperemia in recovery (AUC and at 95 s) during PORH. We found that decreasing ISI_{COMP} was positively related to R and K. Further, this enhanced hyperemic response relative to decreasing ISI_{COMP} was not correlated with a concurrent higher MAP (upstream pressure) but was strongly correlated with an increase in conductance (reduced downstream resistance) at rest (C_R), at peak hyperemia (C_{PH}) and at 95 s (C_{95}). However, conductance was not related to an increase in forearm skeletal muscle MR at any time point. Until now there has been little information regarding microcirculatory responses in first-degree relatives of patients with type 2 diabetes (6) and none, to our knowledge in skeletal muscle microcirculation.

Macrovascular Function

Endothelial dysfunction precedes the advent of cardiovascular disease and is an intermediate phenotype to, and plays a pivotal role in, the advent of diabetic vascular pathology. There is abundant evidence that frank diabetes is linked to reduced endothelial-mediated dilation (9, 16, 85). Important to the focus of the present research is that 50% of diabetic patients already have coronary artery disease at the time of diagnosis and it is not surprising that prediabetic

status of impaired fasting glucose (IFG) has been associated with a 25% decrease in endothelial vasoreactivity compared with normal glucose tolerant (NGT) subjects (61). The focus of the few previous studies documenting abnormalities in BA reactivity of normoglycemic subjects with a family history of type 2 diabetes have been largely group analysis between subjects with family history and those with no family history of type 2 diabetes. Two early studies, using forearm blood flow responses to intra-arterial infusion of acetylcholine (Ach), found no difference between IR and IS groups (51, 75). More recently, McSorley et al. found a positive association between insulin action and ACh-induced vasodilation (45). Caution should be used in comparing any of these latter studies to the present study since dilation induced by Ach infusion is incompletely blocked by L-NMMA, an inhibitor of nitric oxide synthase, indicating that it is only partially NO mediated (19). However, PORH under the specific conditions employed in the present protocol (brachial or radial artery specific, 5 minutes distal cuff occlusion without ischemic handgrip exercise, in healthy subjects), has been shown to elicit primarily a NO-dependent response (27, 58, 74) as it can be almost entirely blocked by L-NMMA (37) and PORH after 5 min of arterial occlusion is minimally affected by prostaglandin inhibition (48).

Similarly, recent reports of significant coronary vasomotor dysfunction relative to IR and FH of type 2 diabetes have also used pharmacological vasodilation and measured differences in myocardial blood flow (32, 55). Recently, Tesauro et al., using FMD, found a 23.8% lower in endothelium-dependent BA vasodilation and decreased IS in normoglycemic offspring of type 2 diabetes patients compared to controls (70). Others have also reported similar reduction of BA vasodilation during PORH for subjects with FH (10, 24) (23.4% and 39% lower respectively) with (23%, (10)) or without (39%, (24)) a concurrent reduction in insulin sensitivity. Balletshofer et al. was the first to report a weak but significant correlation ($r = 0.38$), between decreased FMD endothelial function and the magnitude of IR (as determined by euglycemic hyperinsulinemic clamp) and a large, (34.6%) reduction in FMD of IR relative to IS subgroups (6). Therefore, the 21% reduction in FMD in IR vs. IS subjects we observed is similar to previous findings (10, 70), but substantial differences in methodologies and exclusion criteria (e.g., smoking (6) and obesity (10)) make direct comparisons difficult. Additionally, in all of the previous studies subjects were either middle and old aged (10), in their late 30s or older (24, 45, 60) or “young” subjects who were in their late 20s to mid 30s (6, 70). To our knowledge, the present study is the first to report concurrent vascular and metabolic abnormalities in the

youngest of normal glucose tolerant adults (age 18-26), and significant correlation between endothelium-dependent FMD and the magnitude of IR.

Microvascular Function

Utilizing BABF during PORH we observed that the vasoreactivity of smaller arterioles may be impaired very early in individuals at risk for type 2 diabetes. The literature taken together is unequivocal that impaired microvascular reactivity is clearly present in prediabetes, but the attendant scenario is far from clear (81). Forearm blood flow response to ACh have been found to be significantly impaired (76) and cutaneous hyperemia to local heating is decreased in subjects with IFG relative to normoglycemic subjects (34). Some data have suggested that impairment occurs even earlier and may be linked to the etiology of the disease (65). Skin ACh-induced vasodilation is also impaired in normal glucose tolerant subjects at risk for type 2 diabetes (11) but the message is controversial as the antithesis was observed for microvascular hyperemia to local heating in a similar cohort (40).

The latter discussion emphasizes the difficulty of comparing this study to previous literature. In a cross section of studies there is heterogeneity of the included IR states, other concomitant metabolic syndrome characteristics, and lack of research in relevant tissues. Data acquired on vessels and tissues a (skin, finger nail fold or conjunctiva) assessed by laser Doppler fluxmetry (skin pulp blood flow), transcutaneous oxygen tension (TcpO2), iontophoresis (for the subdermal delivery of vasoactive drugs), and/or capillaroscopy (light microscopy) (1), may not necessarily be relevant to other areas, such as skeletal muscle, as determined in the current study. Further, control of the microcirculation may be different for skeletal muscle than for cutaneous beds (84). Microvascular flow is more tightly regulated locally and involves neuronal, humoral and metabolic control (54). There is growing appreciation that there are differences in vascular control dependent on the level of the vessel in the vascular tree (62). As introduced previously, the importance of FMD macrovascular vasodilation is the indication of nitric oxide (NO) bioavailability as a marker of cardiovascular protection from atherosclerotic processes. In contrast, the role of flow-induced vasodilation in the microcirculation is controversial (62). NO seems to have limited influence in small arterioles relative to larger vessels (54), which may explain the lack of difference in the contribution of NO to cutaneous microvascular dilation in patients with type 2 diabetes compared to controls (66).

The term “diabetic microangiopathy” implies a common progression but studies indicate that different organ beds (skeletal muscle vs. cutaneous) in different subgroups (young vs. old), with variability in cofactors (e.g. normotensive vs. hypertensive), can progress through various stages of altered vascular control at different rates as accentuated or retarded by different levels of glycemic control (71). Thus, it is not surprising that, contrary to the findings of attenuated microvascular dilation (63, 81), or no impairment of microvascular vasodilatory capacity (40) in pre-diabetes, a major finding of our study was that disturbances in microvascular function in normotensive, college-age adults manifest as an increase in peak, total hyperemic and recovery responses in IR subjects relative to IS. Wajcberg et al. recently observed similar increased total postischemic BABF response in IGT patients compared to lean NGT Hispanic adults but no differences in peak hyperemic response (78). Interestingly they found the opposite results (i.e., enhanced peak hyperemic but similar recovery characteristics) in Hispanic type 2 diabetic and obese children relative to lean NGT children (78).

In light of these results, though much emphasis has been placed on vascular dysfunction as defined as reduced dilation, the physiology of small arterioles and capillaries is also strongly related to vasoconstriction such that vascular dysfunction of this characteristic results in both capillary hyperperfusion and hypertension (82). The increased resting blood flow observed in the present study is a frequent observation in diabetes, particularly at early stages. In our college-age cohort not only were there significant differences between IS and IR, but resting blood flow was significantly higher and was associated with the magnitude of IR. It has been proposed that excessive vasodilation, consequent to enhanced basal blood flow, can cause reduced reactive constriction. Similarly, high circulating insulin levels, characteristic of these IR but normoglycemic subjects, act to attenuate constriction and thus, enhance skeletal muscle blood flow (82).

In the progression toward microangiopathy, our young adult IR subjects with compensatory hyperinsulinemia (as seen during the OGTT) may well be in the initial functional stage. It is a potential scenario that metabolic abnormalities coupled with oxidative stress and high sympathetic tone have produced loss of arteriole myogenic responses and lack of normal vasoconstriction in terminal arterioles (81) as evidenced in our results. Our results also show that even though systolic BP was significantly higher in concert with magnitude of IR in our normotensive cohort, when BABF was normalized for differences in mean arterial pressure, the

resulting conductance responses were highly correlated with IR. Thus, a decrease in downstream resistance (presumably associated with decrease in microvascular constrictor tone) was a main reason for the differences observed in hyperemic response. Interestingly, differences in conductance were not related to muscle MR. Increased muscle capillarization has also been found in pre-diabetic patients (18), but it is beyond the scope of this study to determine whether there were similar microvascular structural alterations in our subjects. The strong positive correlation of BABF at rest (R) with the increase in BABF above rest at peak hyperemia (K') indicates that greater microvascular conductance at rest is associated with accentuated conductance during PORH in our subjects. In turn, both are related to the degree of IR.

Macro vs. Microvasoreactivity

Several studies have shown no correlation between endothelium-dependent PORH in the macrovasculature and the degree of vasoreactivity in the microvasculature. All of these studies used cutaneous laser Doppler imaging either during reactive hyperemia- (64) or acetylcholine-induced (25, 26) microvascular vasodilation. In contrast, in the present study, we used standardized BA responses and BABF as a measure of microvascular reactivity, which has revealed a number of significant negative correlations at rest and during PORH between the reactivity of a conduit artery and the reactivity of the downstream microvasculature of that artery. We observed that the larger the percent change in of the BA, the lower was the BABF at rest, at peak hyperemia and during recovery (i.e. the smaller the endothelium-derived response of the conduit artery the larger the microcirculatory vasodilation).

Implications

Our results indicate an attenuated macrovascular endothelium-derived vasoreactivity and an abnormal response of smaller arterioles and capillaries in the youngest of adults with a family history of type 2 diabetes relative to the magnitude of their insulin resistance. This suggests, at the level of the conduit artery, that these "healthy" college-age students are already at risk for atherogenic progression, and that there may already may be structural and functional microvascular modifications that may exacerbate their progression toward type 2 diabetes and to the microangiopathy all too commonly associated with this disease. This is important given that the current clinical approach for identifying "disease" postdates the progression through IR/hyperinsulinemia. Given the plethora of evidence that the progression to type 2 diabetes

could be reduced with lifestyle intervention (29) including diet and exercise (39), and by pharmacological intervention (3), early identification of evidence of dysfunction would provide time for reversal of the etiology.

Additionally, determining microvascular function in the progression may provide better insight into the etiology of the disease, both from a vascular and metabolic perspective. It has been shown that the enhanced capillary permeability and plasma extravasation associated with hyperperfusion and capillary hypertension (53) can result in remodeling of the capillary basement membrane (22) and ultimately lead to microvascular failure (71). In light of the growing evidence of certain beneficial effects of agents in the drug arsenal, such as metformin, at the level of the smallest vessels (83), improved microvessel functionality may make it imperative to be more aggressive in identifying patients earlier in the disease process.

Study Strengths and Limitations

In the present study we found endothelial dysfunction in insulin-resistant young adults with and without a family history of type 2 diabetes. The differences we observed in FMD could not be attributed to other confounding variables, because we excluded, or did not show any difference between IR vs. IS for other cardiovascular risk factors such as hypercholesterolemia, hyperlipidemia and hypertension. Additional attention was given in the protocol to avoid vasoactive factors that impact vasomotor tone including temperature of the room, vasoactive medications, smoking, exercise, prandial state, patient anxiety and phase of the menstrual cycle (14, 38). While it is controversial what effect antioxidants have on vasoreactivity, we also controlled for diet and antioxidant use. Women were studied in the follicular phase of their menstrual cycle, as verified by progesterone (P_4) levels, to mitigate the influence of estrogen. Repeat measures (3) of FMD helped ensure a true assessment for each subject. The protocol was initiated at the same time in the morning in order to facilitate all three trials within the morning as FMD has been shown to be stable during a 2-hr morning period. Additionally, repetitive reactive hyperemia does not cause significant differences across in trials in healthy college students (28)

Gender was evenly represented in all subjects and within subgroups of FH and no FH. But an unequal gender distribution occurred in IS vs. IR subgroups (73% vs. 33% female / male

respectively) and may be the reason for the differences between these two groups in height, weight, waist circumference, fat free mass and resting brachial artery .

Elevated sympathetic activation is common in certain pathologies and has been shown to blunt the FMD response (31). Hypertension has also been documented coincident with elevated plasma ET-1 (41). The clinical importance of BA FMD testing is the link between FMD and NO bioavailability. Although we have carefully utilized a protocol that produces a stimulus that is largely NO mediated, FMD is also a function of other vasoregulatory mechanisms such as sympathetic activation and endogenous vasocontrictors. To minimize these influences, our subjects were all young and screened to include only healthy, non-hypertensive individuals and the protocol was planned to minimize environmental factors that may increase sympathetic outflow. Nevertheless, although MAP was not different between IS and IR, systolic BP was significantly different between the groups (114 $\pm$ 9 vs. 124 $\pm$ 7 mmHg), which may contribute to the decrement in the FMD response in the IR group. A recent study has reported an increased peripheral sympathetic outflow linked to a decreased endothelial-dependent FMD of healthy offspring of type 2 diabetic patients (33). An alternate explanation is that the increased systolic BP reported in the present study is just a concurrent cardiac risk factor that clusters with the IR syndrome (4). This seems highly plausible since systolic BP was not related to FMD (r=0.08).

A strength of the present study, in contrast to virtually all research involving FMD done to date, is that the data for macrovascular reactivity was standardized for the stimulus. The outcome of this is that intra-subject comparisons are equally weighted when calculating the average and, most importantly, that inter-subject differences and group comparisons are a more meaningful reflection of physiologic differences and not confounded by magnitude of the stimulus. We have included Fig. 4.1 as an example of potential error that can be introduced when variability of the SR stimulus is not accounted for. When uncorrected, the % change in diameter for these two subjects was identical and the customary inference would be that both have similar endothelium-dependent vasodilation, vascular health and vasoprotective state. In reality, when responses were corrected for the stimulus, there was actually > 2-fold difference in the response between the 2 subjects (Fig. 4.4). We have also observed that not taking stimulus variability into account can obfuscate results and subsequent conclusions from those results. A main finding of this study was that the magnitude of IR in very young adults is significantly

negatively correlated with BA vasoreactivity, a finding that would have been missed without correcting changes in BA (Fig. 4.4).

The potential affect of resting vascular dimension on the FMD of conduit arteries is not a new concept and has resulted in some studies either correcting FMD for baseline (7, 30, 36) or reporting no significant difference in resting BA (50). In some studies, flow was determined to be the same between subgroups as justification for appropriate group comparisons (as reviewed by Pyke, K.E. and Tschakovsky, M.E. (58) However, blood flow is actually not the stimulus for flow mediated dilation and should not be used to quantify the stimulus. The awareness of the need to correct for shear stress is not new (46, 56-58), but potential erroneous conclusions, such as gender differences in vascular reactivity, are common (7, 11, 70).

As shown in the present study, females, tested in the low estrogen phase of their menstrual cycle, do not have intrinsically greater BA reactivity compared to males. Further, smaller arteries are not inherently more reactive than larger arteries (Fig. 4.1). However, this could be another potential confounding influence in studies that have taken BA measurements over a wide range of distances from the antecubital fossa (2-15 cm), i.e. have a greater range of BA diameters (6, 50, 69, 79), and have not corrected for the differences in stimulus when determining the response.

In the present study we do not have a measure of endothelial- independent vasodilation, so we cannot rule out the affects of alterations in smooth muscle function in our results. While there is still some controversy regarding a difference in either nitroglycerine or sodium nitroprusside induced vasodilation between IS and IR (69), the majority of studies indicate no difference in vascular smooth muscle function between IS and IR subjects (6, 24, 33, 45, 60).

In the present study we have used 2 different methods to access post-ischemic reactivity. Some studies have utilized PORH brachial artery flow parameters as indications of impaired downstream microvascular function/structure (46, 77). With this methodology we found significant differences relative to IR. However, the NIRS [HbO_2] parameters did not indicate the same enhanced perfusion that BABF parameters did. Limitations of this technology and the use of the [HbO_2] signal has been reported previously (72).

[HbO_2] parameters for the initial hyperemic response from rest and from end cuff scaled to their respective baselines have been shown to correlate with corresponding BABF parameters (Townsend, D.K. Chapter 2).

Conclusions

In this study we found altered conduit vessel reactivity for those at risk for developing type 2 diabetes in the youngest post-adolescent adults, and provided evidence that the degree of vascular impairment is related to the level of IR even in normal glucose tolerance. Our results also strongly emphasize the importance of interpreting data only when BA responses have been standardized by the stimulus. Additionally, we observed significantly increased BABF at rest, and an enhanced peak hyperemia, and magnitude of hyperemia in recovery during PORH related to IR. We found that this enhanced hyperemic response relative to the magnitude of IR was not correlated with a concurrent increase in mean arterial pressure (upstream pressure) but strongly correlated with an increase in conductance (reduced downstream resistance), which was not related to an increase in forearm skeletal muscle MR. Parameters of BABF at rest, peak hyperemic response, and magnitude of hyperemia in recovery are also strongly correlated with the BA endothelium-derived vasoreactivity.

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Tables

Table 4.1 Anthropometric and clinical characteristics for insulin sensitive and insulin resistant subjects.

	All subjects	IS	IR
	n=26	n=11	n=15
Gender (female / male)	13/13	8 / 3	5 / 10
FH type 2 diabetes / no FH	20 / 6	8 / 3	12 / 3
Age (yr)	21.6 ± 2.0	20.9 ± 1.8	22.1 ± 2.0
Height (cm)	175 ± 9	170 ± 7	179.1 ± 9†
Weight (kg)	75.5 ± 18.2	62.0 ± 8.6	85.3 ± 17.1*
Resting heart rate (bpm)	65.9 ± 8.4	68.5 ± 7.8	63.3 ± 8.5
Systolic BP (mmHg)	120 ± 9	114 ± 9	124 ± 7*
Diastolic BP (mmHg)	72.6 ± 9.0	70.2 ± 6.9	74.4 ± 10.2
Mean BP (mm Hg)	88.3 ± 8.1	84.9 ± 2.3	90.9 ± 2.0
Waist circumference (cm)	81.9 ± 12.6	74.0 ± 7.0	88.0 ± 12.8*
Waist-hip ratio	0.79 ± 0.08	0.77 ± 0.08	0.81 ± 0.08
BMI (kg/m^2)	24.4 ± 4.4	21.5 ± 2.2	26.5 ± 4.5*
Total fat mass (%)	27.8 ± 9.6	24.4 ± 7.3	30.4 ± 10.5
Abdominal fat (%)	35.1 ± 11.0	30.2 ± 9.1	38.7 ± 11.2‡
Fat free mass (kg)	51.6 ± 11.7	45.0 ± 8.7	56.5 ± 11.4†

Significant difference between insulin sensitive (IS) and insulin resistant (IR) subjects †$P<0.01$, *$P<0.005$, ‡$P<0.05$.

Table 4.2 Biochemical measurements for insulin sensitive and insulin resistant subjects.

	All subjects	IS	IR
Blood lipids and CRP	n=26	n=11	n=15
Cholesterol (mg/dl)	161.2 ± 28.2	163 ± 35	160 ± 23
Triglycerides (mg/dl)	87.1 ± 46.9	81.2 ± 44.9	91.4 ± 49.5
HDL (mg/dl)	53.2 ±15.3	57.2 ± 16.7	50.2 ± 14.0
LDL_{calc} (mg/dl)	90.6 ± 22.0	89.4 ± 29.0	91.5 ± 16.2
TC/HDL	3.21 ± 0.91	3.02 ± 1.05	3.36 ± 0.81
LDL/HDL	1.85 ± 0.77	1.72 ± 0.96	1.95 ± 0.61
Triglycerides / HDL	1.81 ± 1.19	1.52 ± 0.88	2.02 ± 1.37
C-reactive protein	3.53 ± 5.12	2.93 ± 3.34	3.97 ± 6.20
Metabolic Characteristics	n=25	n=10	n=15
FI (µU/ml)	5.22 ± 2.43	3.45 ± 0.52*****	6.41 ± 2.50
I_{AUC}OGTT (mIU/l)	3054 ± 2113	1757 ± 476†	3918 ± 2347
I_{AV}OGTT (mIU/l)	30.7 ± 19.1	18.1 ± 4.10‡	39.1 ± 20.6
I_{2H}OGTT (mIU/l)	22.4 ± 16.0	14.1 ± 6.70§	28.0 ± 18.1
FG (mg/dl)	84.4 ± 6.7	79.7 ± 3.18‡	87.5 ± 6.6
Glu_{AUC}OGTT (mIU/l)	3888 ± 2286	3411 ± 2016	4206 ± 2465
Glu_{AV}OGTT (mg/dl)	116 ± 21	107 ± 18.8	123 ± 20
Glu_{2H}OGTT (mg/dl)	94.1 ± 18.6	91.3 ± 18.1	96.0 ± 19.3
ISI_{HOMA}	1.05 ± 0.45	1.37 ± 0.38*****	0.84 ± 0.35
ISI_{COMP}	9.88 ± 3.98	14.1 ± 1.86*****	7.1 ± 2.0

Significant difference between insulin sensitive (IS) and insulin resistant (IR) subjects *****P<0.001, †P<0.01, ‡P<0.005, §P<0.05.

Table 4.3 Macrovascular stimulus and response in the brachial artery for insulin sensitive and insulin resistant subjects.

	All subjects	**IS**	**IR**
Shear rate (SR) (s^{-1})	n=26	n=11	n=15
Resting	21.4 ± 10.2	17.3 ± 6.8	24.6 ± 11.5
Max change	159 ± 46	162 ± 34	157 ± 54
% change diameter	9.58 ± 4.37	10.71 ± 4.09	8.68 ± 4.53
AV change-55 s	43.9 ± 13.5	44.8 ± 9.4	43.2 ± 16.4
Brachial artery diameter (mm)			
Resting	3.92 ± 0.66	3.56 ± 0.44	4.18 ± 0.68*****
% change diameter	7.05 ± 3.03	8.03 ± 4.24	6.33 ± 1.49
% change diameter / SR_{AUC}	2.75 ± 0.84	3.12 ± .93	2.48 ± 0.68†

Macrovascular stimulus (shear rate (SR) (s^{-1})) and response (diameter) (mm) in the brachial artery. Significant difference between insulin sensitive (IS) and insulin resistant (IR) subjects *****P<0.02, †P<0.05.

Table 4.4 Measures of microvascular reactivity prior to and during post occlusive reactive hyperemia for insulin sensitive and insulin resistant subjects.

	All subjects	IS	IR
[HbO_2]	**n=23**	**n=11**	**n=12**
y1	54.3 ± 4.5	53.5 ± 4.8	55.1 ± 4.3
K	68.9 ± 6.6	68.7 ± 6.0	69.1 ± 7.4
K %y1	153 ± 18	155 ± 19	150 ± 18
R	94.1 ± 7.3	93.6 ± 7.2	94.5 ± 7.7
K’	24.6 ± 2.5	24.5 ± 2.4	24.7 ± 2.7
K’ %R	30.7 ± 3.6	31.0 ± 4.1	30.4 ± 3.2
tm	11.9 ± 4.0	12.1 ± 5.5	11.7 ± 2.1
d	22.0 ± 8.2	24.7 ± 10.4	19.5 ± 4.7
tm + 0.5d	22.9 ± 7.6	24.5 ± 10.2	21.4 ± 4.0
95s	16.3 ± 2.6	16.3 ± 3.0	16.3 ± 2.3
95s %y1	33.9 ± 5.7	34.9 ± 6.9	33.1 ± 4.4
95s %K	38.6 ± 10.0	35.9 ± 9.7	41.1 ± 10.1
95s %R	19.1 ± 3.9	19.6 ± 4.5	18.7 ± 3.5
95s %K’	54.4 ± 16.3	56.5 ± 17.3	52.4 ± 16.1
Forearm ATT (mm)	4.62 ± 1.45	4.08 ± 1.34	5.03 ± 1.45
BABF	**n=26**	**n=11**	**n=15**
y1	3.28 ± 2.58	2.47 ± 1.35	3.82 ± 4.00
K	79.7 ± 32.1*****	61.5 ± 16.2	91.8 ± 34.8**§**
K %y1	31.5 ± 16.7††	32.2 ± 12.5	31.0 ± 19.5
R	10.3 ± 7.3	6.63 ± 3.43	13.0 ± 8.0†
K’	67.5 ± 24.2*******	55.8 ± 15.4	75.5 ± 25.9**§**
K’ %R	8.82 ± 5.52	10.8 ± 6.1	7.28 ± 4.47
AUC	2548 ± 975 ******	2039 ± 674	2912 ± 986*****
95s	6.71 ± 5.38†	4.53 ± 3.23	8.32 ± 5.99‡
95s %y1	2.72 ± 2.08	2.55 ± 1.64	2.83 ± 2.39
95s %K	0.09 ± 0.05	0.08 ± 0.04	0.09 ± 0.06
95s %R	0.81 ± 0.66	0.79 ± 0.62	0.81 ± 0.69
95s %K’	0.10 ± 0.08	0.08 ± 0.06	0.12 ± 0.09

Measures of microvascular reactivity defined by NIRS oxy-hemoglobin ([HbO2]) and brachial artery blood flow (BABF) parameters prior to and during post occlusive reactive hyperemia. Values at y1=end cuff, K=amplitude from end cuff, R=rest, K’= amplitude from rest, AUC (area under the curve brachial artery blood flow from rest) and 95s= amplitude from rest at 95 s. Significant difference between insulin sensitive (IS) and insulin resistant (IR) subjects *****P<0.01, †P<0.02, **§**P<0.03, ‡P<0.05 (*IR column*). Significant positive correlation with systolic blood pressure (*all subjects column*) *******P<0.01, ******P<0.02, *****P<0.03. Significant positive correlation with forearm skeletal muscle metabolic rate (*all subjects column*) ††P<0.02, †P<0.05.

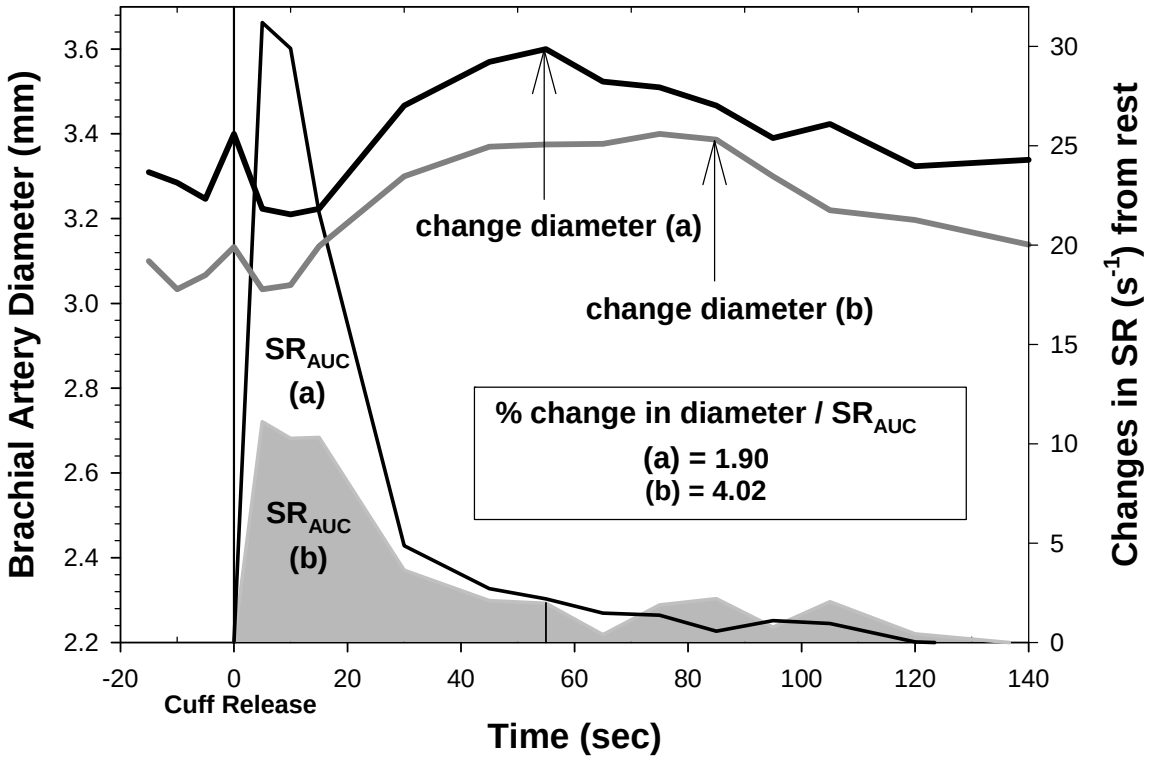
Table 4.5 Variables influencing and values for conductance for insulin sensitive and insulin resistant subjects.

	All	IS	IR
Muscle mass (g)	1087	902	1231
Metabolic rate	4.66	4.43	4.88
Mean arterial pressure	88.3	84.8	90.9
Conductance-rest (C_R)	0.12	0.07	0.15
Conductance-peak	0.88	0.73	0.99
Conductance-95 s (C_{95})	0.19	0.13	0.24

Significant difference between insulin sensitive (IS) and insulin resistant (IR) subjects *****P<0.01, †P<0.02, §P<0.03, ‡P<0.05 (*IR column*). Positively significantly correlated with metabolic rate (*all subjects column*) ‡P<0.05.

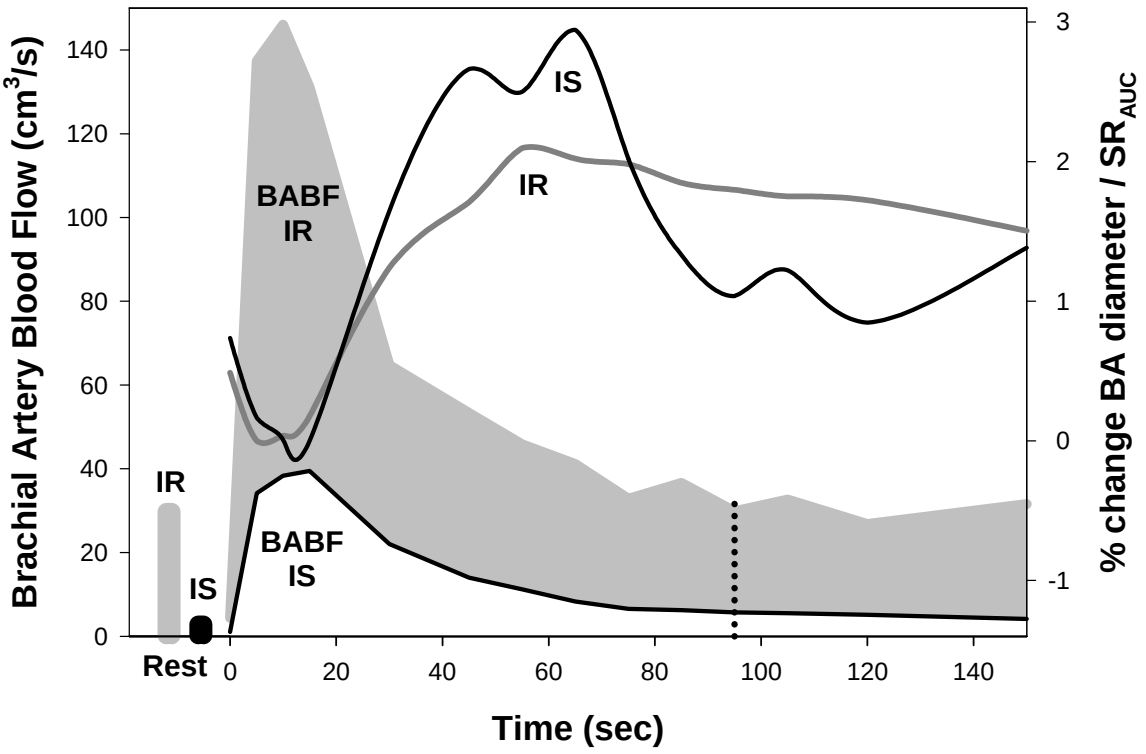
Figures

Figure 4.1 Shear rate and brachial artery diameter after post occlusive reactive hyperemia for two subjects.



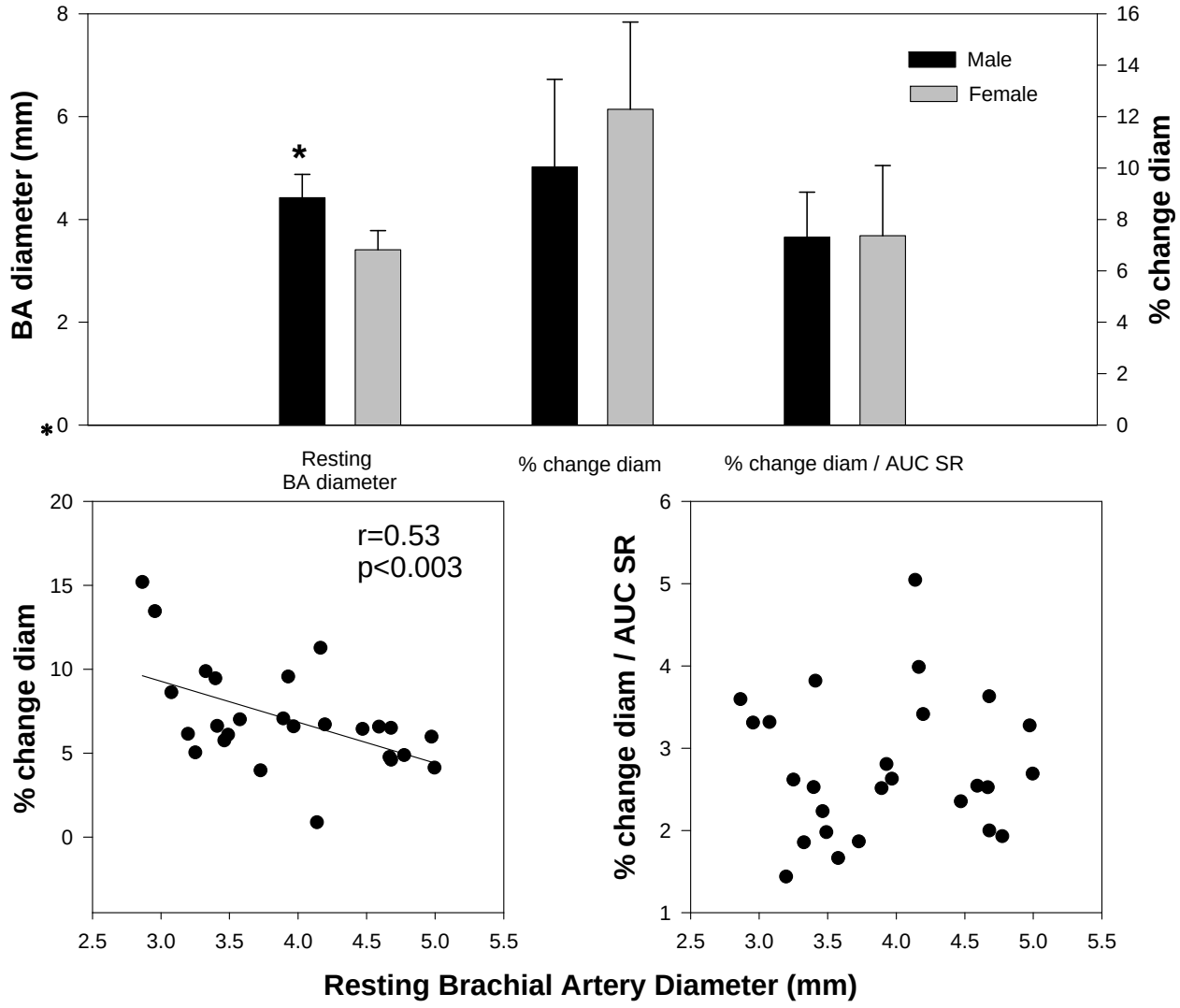
Shear rate (SR) (s^{-1}) and brachial artery (BA) diameter (mm) after post occlusive reactive hyperemia for two subjects, (a) (black) and (b) (gray). % change in diameter from the baseline is the same for (a) vs. (b) (12.15% and 12.09% respectively). When the % change in diameter is standardized by the SR stimulus (SR_{AUC}), then it is apparent that there actually is > 2 fold difference in the response between the two subjects (1.90 vs. 4.02).

Figure 4.2 Resting and post occlusive reactive hyperemia responses for brachial artery blood flow and % change in brachial artery diameter for an insulin resistant and an insulin sensitive subject.



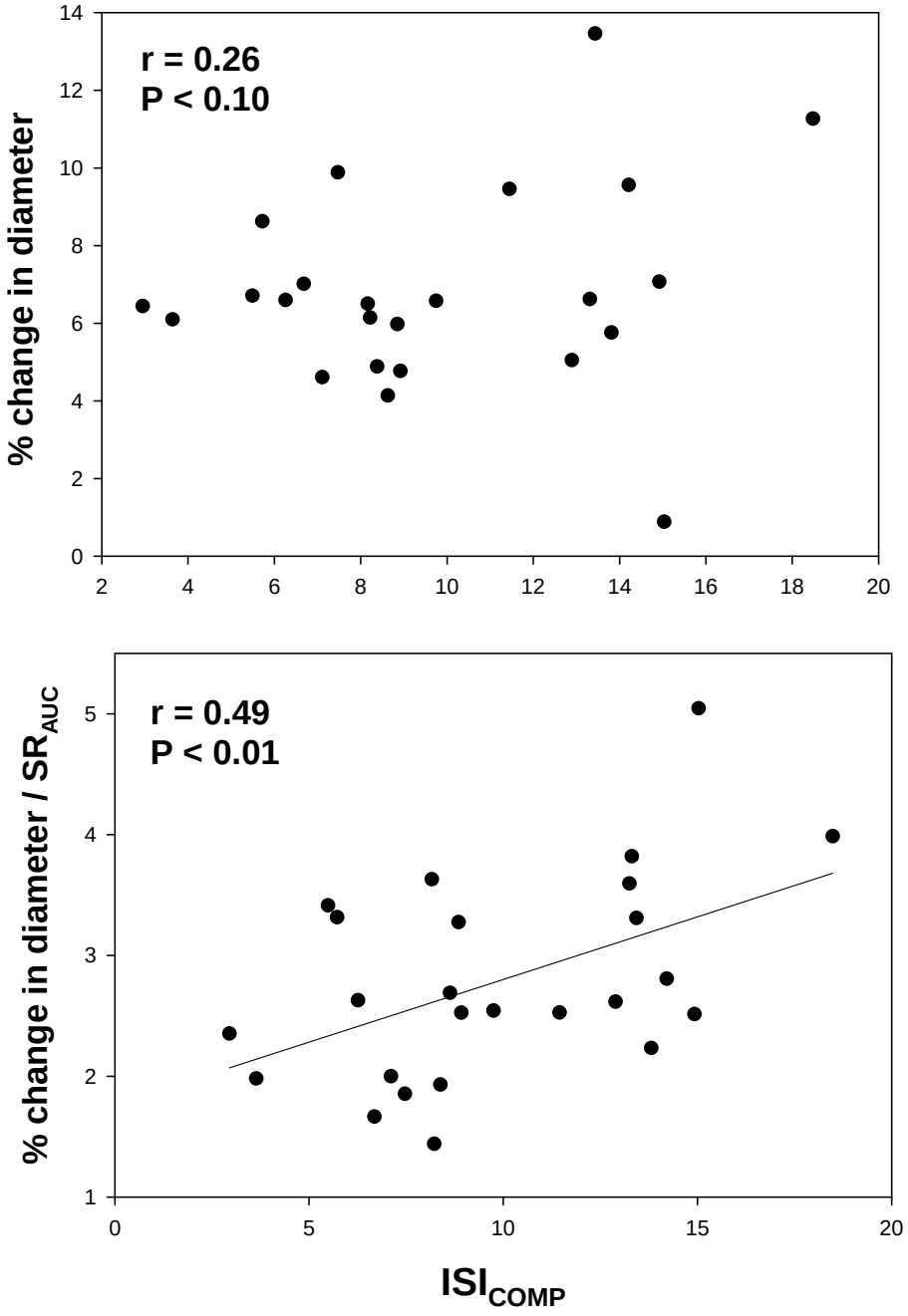
Brachial artery (BA) blood flow (BF) (cm^3/s) and % change in BA diameter standardized by shear rate (SR_{AUC}) (arbitrary units). Cuff release occurred at t=0. The insulin resistant (IR) subject (ISI_{COMP} = 2.95) is characterized by higher resting BABF (gray vs. black bar), larger initial hyperemic response, larger total hyperemic response (gray vs. white fill) and higher blood flow above rest at 95s (dotted line) relative to the insulin sensitive (IS) subject (ISI_{COMP} = 13.43). Concomitant with these characteristics is a smaller % change BA diameter / SR_{AUC} for the IR relative to the IS subject (gray vs. black line).

Figure 4.3 Affects of gender and resting brachial artery diameter on % change in brachial artery diameter during post occlusive reactive hyperemia.



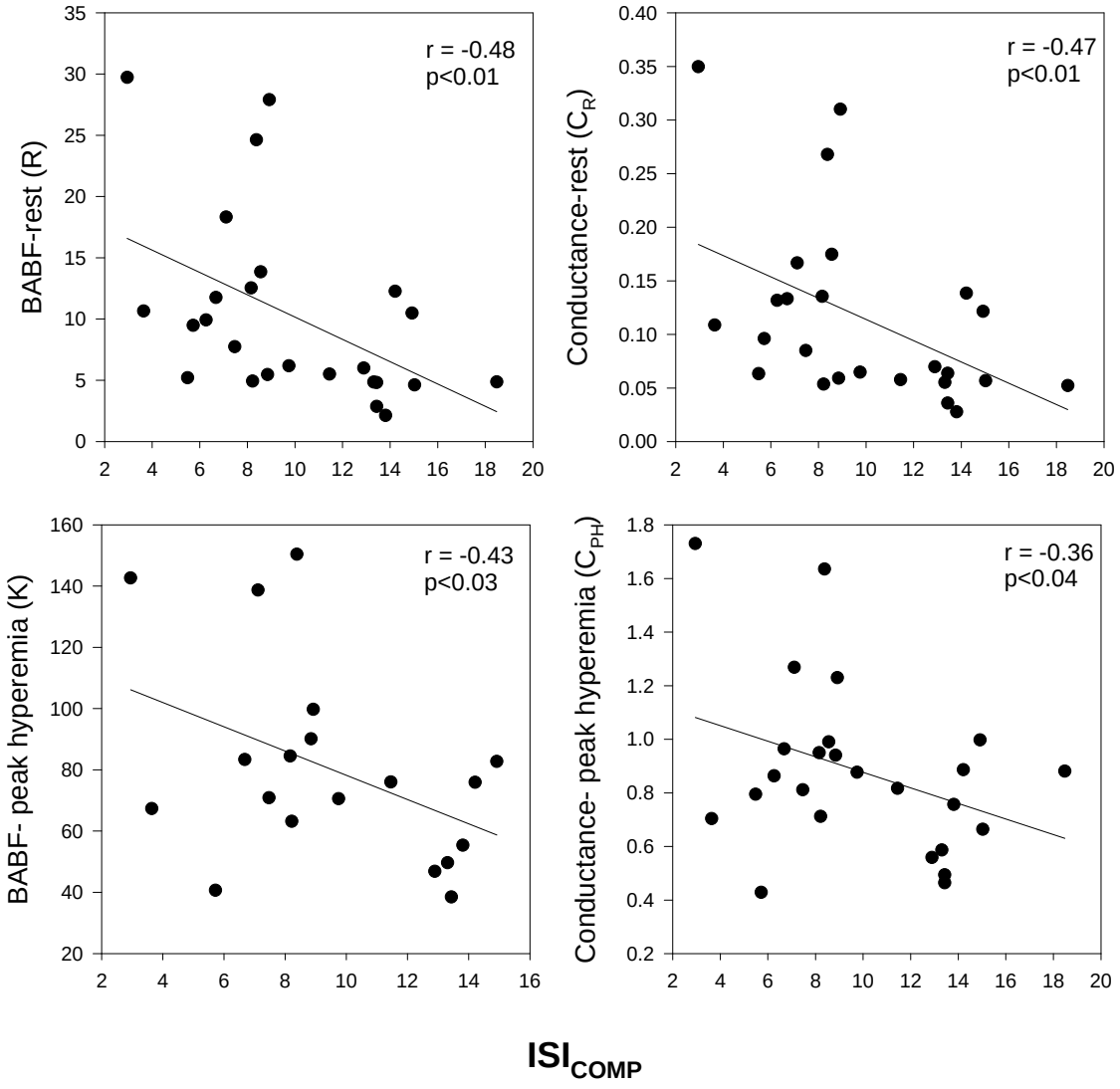
Affects of gender (*top panel*) and resting brachial artery diameter (resting diam) (*bottom panels*) on % change in brachial artery diameter (% change diameter). Men have a significantly larger resting diameter (*P<0.0001) but % change diameter standardized by the stimulus (area under the curve shear rate (AUC SR) is not significantly different between the genders (*top panel*). % change diameter is significantly correlated with resting diameter (P<0.0003) but not when the response is standardized by the stimulus (*bottom panels*).

Figure 4.4 Macrovascular reactivity relative to insulin sensitivity in normoglyncemic college-age subjects



Brachial artery reactivity (% change in diameter) not standardized by the shear rate stimulus (*top panel*) and standardized by the shear rate stimulus (% change in diameter / SR_{AUC}) (*bottom panel*) over a range of whole-body insulin sensitivities (ISI_{COMP}). There is significant correlation between conduit artery reactivity and insulin sensitivity in these normoglycemic subjects but this relationship is only discernable when the response is standardized by the stimulus (bottom panel) ($P<0.01$).

Figure 4.5 Parameters of brachial artery blood flow at rest and peak hyperemia and conductance at rest and peak hyperemia relative to insulin sensitivity.



Parameters of brachial artery blood flow (BABF) at rest (R) and peak hyperemia (K) and conductance at rest (C_R) and peak hyperemia (C_{PH}) (right figures) relative to insulin sensitivity (ISI_{COMP}). Blood flow parameters R and K are significantly negatively correlated with ISI_{COMP} ($P<0.01$ and $P<0.03$ respectively) but are significantly correlated with mean arterial pressure. Thus, differences in conductance at rest and peak hyperemia, which are also significantly negatively correlated with ISI_{COMP} ($P<0.01$ and $P<0.04$ respectively), must be the reason for the differences in BABF relative to insulin sensitivity at these times.

CHAPTER 5 - Conclusions

Integrating the studies described in this dissertation, we conclude that substantial levels of IR and hyperinsulinemia are detectable preceding the deterioration of glucose tolerance in the youngest of adults with a family history of type 2 diabetes. In addition, we observed attenuated macrovascular endothelium-derived vasoreactivity and an abnormal response of smaller arterioles and capillaries relative to the magnitude of insulin resistance. This suggests that, at the level of the conduit artery, some of these apparently "healthy" college-age students are already at risk for atherogenic progression, and that there may already may be structural and functional microvascular modifications that will exacerbate their progression toward type 2 diabetes and to the microangiopathy all too commonly associated with this disease.

In the first study (Chapter 2) we quantified a 7-fold range in insulin sensitivity with 58% of our subjects, including 37% of our lean subjects, exhibiting levels associated with metabolic disorder and frank disease reported in other studies. This was determined from measures of fasting insulin and insulin during an OGTT, which proved to be a highly sensitive means of quantifying IR in these young adults, while parallel measures of serum glucose were not. To our knowledge, this is the first study to verify the presence of IR in college–age adults with an easily applicable indirect measure.

In the Study 2 (Chapter 3) we found a ~ 2-fold range in forearm skeletal muscle metabolic rate in resting conditions, comparable with previous findings based on direct Fick method of muscle oxygen uptake. Additionally, parameters of the post-occlusive reactive hyperemia test utilizing near infrared spectroscopy were determined to access microcirculatory reactivity and verified by amplitude parameters of brachial artery blood flow (as an independent assessment of microcirculatory vasodilatory response). From the results of this study, it can be concluded that NIRS is a useful noninvasive tool for investigating tissue metabolism and microvascular reactivity. Strong evidence was provided for the imperative to correct NIRS signals for the effects of subcutaneous fat.

Finally, in Chapter 4 we assessed macrovascular endothelial function of the brachial artery as well as skeletal muscle microvascular reactivity in the forearm using post-occlusive reactive hyperemia and related these results to the magnitude of IR. To our knowledge, these studies are the first to report concurrent vascular and metabolic abnormalities in the youngest of

normal glucose tolerant adults (age 18-26). A strength of our study, in contrast to virtually all research involving flow mediated dilation conducted to date, is verification that the data for macrovascular reactivity must be standardized for the stimulus or results will be obfuscated. Moreover, a major finding of our study was that disturbances in microvascular function manifest as an increase in peak, total hyperemic and recovery responses in IR subjects relative to insulin sensitive subjects. Additionally, this enhanced hyperemic response was not correlated with a concurrent increase in MAP (upstream pressure), but was strongly correlated with an increase in conductance (reduced downstream resistance), which was not related to an increase in forearm skeletal muscle MR.

In conclusion, this body of work furthers our insight into the need for identification of "disease" earlier in the natural history of type 2 diabetes. This seems warranted given the plethora of evidence that the progression to type 2 diabetes could be reduced with lifestyle intervention. Furthermore, in light of the growing evidence of certain beneficial effects of agents in the drug arsenal, earlier detection allows for earlier intervention to slow the etiology of atherogenic progression and to improve abnormal microvessel functionality, both of which have been substantiated relative to early IR in college-age adults in this series of studies.

Appendix A - Curriculum Vitae

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1974-1976	College of William and Mary, Williamsburg, Va.	
May 1978	Kansas State University	B.S. Biology, *Cum Laude*
May 1984	Kansas State University	M.S. Biology
Dec. 2007	Kansas State University	Ph.D. Physiology

Research/Work Experience

1978-1984 Teaching Assistant, Division of Biology, Kansas State University

1997-2000 Six/tenths Instructor, Division of Biology, Kansas State University

2000-present Full time Instructor, Division of Biology, Kansas State University

- Lecture 5 days a week on human anatomy and physiology
- Administration and management of an 8 credit hour course with 160-200 students, 3 other instructors and approximately 17 practicum students.
- Instructor for the Cadaver Dissection Teams

Academic Awards

1978 Most Promising Undergraduate Award, Division of Biology, Kansas State University

1979 John C. Frazier Award for Botanical Research, Division of Biology, Kansas State University

2001 William L. Stamey Award for Excellence in Teaching, College of Arts and Science, Kansas State University

2005 Jane Westfall Award for Women in Physiology, Department of Anatomy and Physiology, Kansas State University

Society Memberships

American College of Sports Medicine

American Physiological Society

Grants

Summer 1978 Funded as an Undergraduate Research Participant, National Science Foundation

2004 University Small Research Grant (USRG) (PI-Thomas J. Barstow), Kansas State University

Research Papers

1. Downey AE, Chenoweth LM, **Townsend DK**, Ranum JD, Ferguson CS, Harms CA. *Respir Physiol Neurobiol.* 2007 May 14;156(2):137-46.

2. Harper AJ, Ferreira LF, Lutjemeier BJ, **Townsend DK**, Barstow TJ. Human femoral artery and estimated muscle capillary blood flow kinetics following the onset of exercise. *Exp Physiol.* 2006 Jul;91(4):661-71.

3. Ferreira LF, Lutjemeier BJ, **Townsend DK,** Barstow TJ. Effects of pedal frequency on estimated muscle microvascular O2 extraction. *Eur J Appl Physiol.* 2006 Mar;96(5):558-63.

4. Ferreira LF, Harper AJ, **Townsend DK**, Lutjemeier BJ, Barstow TJ. Kinetics of estimated human muscle capillary blood flow during recovery from exercise. *Exp Physiol*. 2005 Sep;90(5):715-26.

5. Ferreira LF, Lutjemeier BJ, **Townsend DK**, Barstow TJ. Dynamics of skeletal muscle oxygenation during sequential bouts of moderate exercise. *Exp Physiol.* 2005 May;90(3):393-401.

6. Ferreira LF, **Townsend DK**, Lutjemeier BJ, Barstow TJ. Muscle capillary blood flow kinetics estimated from pulmonary O2 uptake and near-infrared spectroscopy. *J Appl Physiol*. 2005 May;98(5):1820-8.

7. Lutjemeier BJ, Miura A, Scheuermann BW, Koga S, **Townsend DK**, Barstow TJ. Muscle contraction-blood flow interactions during upright knee extension exercise in humans. *J Appl Physiol*. 2005 Apr;98(4):1575-83.

Research Papers, in preparation

1. Townsend, D.K., M.D. Haub, B.J. Lutjemeier, D.A. Freeze, T.J. Barstow. Insulin resistance / hyperinsulinemia in normoglycemic college-age subject

2. Townsend, D.K. and T.J. Barstow. Metabolic rate and parameters of post occlusive reactive hyperemia (PORH) in forearm skeletal muscle.

3. Townsend, D.K., B.J. Lutjemeier, L.F. Ferreira T.J. Barstow. Reduced macrovascular reactivity and microvascular control abnormalities relative to insulin resistance in normoglycemic college-age subjects with a family history of type 2 diabetes

Abstracts

1. Ornelas, S. and Townsend, D.K. Insulin sensitivity and microvascular function in subjects with family history of type II diabetes. Developing Scholars Program, KSU, 2007.

2. Rosenkranz, S., Townsend, D.K., Steffens, S., Wright, J., Harms, C. Effects of a high-fat meal on pulmonary function in healthy subject. Central States ACSM, 2006.

3. Lutjemeier, B.J., Ferreira, L.F., Townsend, D.K., Barstow, T.J. The effect of contraction frequency on the central and peripheral blood flow / VO_2 relationship. Integrative Physiology of Exercise, ACSM 2006

4. Harper, A.J., Ferreira, L.F., Lutjemeier, B.J., Townsend, D.K., Barstow, T.J. Muscle capillary and femoral artery blood flow kinetics following the onset of exercise. ACSM 2006

5. Townsend, D.K., Haub, M.D., Ferriera, L.F., Lutjemeier, B.J., Barstow, T.J. Insulin sensitivity and endothelial function in college-age subjects with a family history of type 2 diabetes. ACSM 2006

6. Lutjemeier, B.J., Ferreira, L.F., Townsend, D.K., Barstow, T.J. Frequency analysis of muscle contractions and NIRS variables: implications for tissue gas exchange. ACSM 2006.

7. Harper, A.J., Ferreira, L.F., Lutjemeier, B.J., Townsend, D.K., Barstow, T.J. Estimated kinetics of muscle capillary blood flow during recovery from exercise. ACSM 2005.

8. Ferreira L.F., Hueber, D.M., Lutjemeier, B.J., Townsend, D.K., Barstow, T.J. Muscle oxygenation during incremental exercise and recovery: implications of assuming scattering constant. ACSM 2005.

9. Townsend, D.K., Haub, M.D., Lutjemeier, B.J., Ferreira, L.F., Harper, A.J., Barstow, T.J. Dissociation of glucose homeostasis and insulin sensitivity in college-age subjects at risk for type 2 diabetes. ACSM 2005

10. Lutjemeier, B.J., Harms, C.A., Harper, A.J., Ferreira, L.F., Townsend, D.K. Barstow, T.J. Pedal frequency does not alter the cardiac output:VO_2 relationship during cycling. ACSM 2005.

11. Barstow, T.J., Ferreira, L.F., Lutjemeier, B.J., Townsend, D.K. Tissue oxygenation by NIRS as a function of pedal rate during incremental exercise. ACSM 2004.

12. Townsend, D.K., Ferreira, L.F., Lutjemeier, B.J., Barstow, T.J. The influence of adipose tissue thickness on near-infrared spectrometry during intra-contraction knee extension exercise. ACSM 2004.

13. Ferreira, L.F., Lutjemeier, B.J., Townsend, D.K., Barstow, T.J. NIRS-derived estimate of muscle blood flow kinetics during moderate- and heavy-intensity cycling exercise. ACSM 2004.

14. Lutjemeier, B.J., Townsend, D.K., Ferreira, L.F., Barstow, T.J. Impact of muscle contraction on arterial blood flow and tissue gas exchange by NIRS. ACSM 2004.

15. Townsend, D.K., Lutjemeier, B.J., Miura, A., Scheuermann, B.W., Barstow, T.J. Skeletal muscle vascular conductance during dynamic knee extension exercise. ACSM 2003.

16. Lutjemeier, B.J., Townsend, D.K., Koga, S., Harms, C.A., Barstow, T.J. Oscillations in femoral venous blood flow during dynamic exercise do not simply reflect muscle pump. ACSM 2003.

17. Lutjemeier, B.J., Townsend, D.K., Koga, S., Harms, C.A., Barstow, T.J. Oscillations in femoral venous blood flow during dynamic exercise do not simply reflect muscle pump. CSACSM 2002

18. Lutjemeier, B.J., Townsend, D.K., Hoelting, B., Miura, A., Scheuermann, B.W., Barstow, T.J. Relationship between mean and oscillations in muscle blood flow during dynamic exercise. ACSM 2002.

19. Lutjemeier, B.J., Townsend, D.K., Hoelting, B., Miura, A., Scheuermann, B.W., Barstow, T.J. Relationship between mean and oscillations in muscle blood flow during dynamic exercise. CSACSM 2002.

Presentations

1. "Endothelial Dysfunction Relative to Insulin Resistance in College-age Individuals" Department of Anatomy and Physiology Seminar Series. Fall 2005

2. "Microvascular Responses During Reactive Hyperemia in Early Insulin Resistance". Department of Anatomy and Physiology Seminar Series. Spring 2006

3. "Relationships Between Metabolic Abnormalities and Vascular Dysfunction". Department of Anatomy and Physiology Seminar Series. Spring 2007