A ROLE FOR INSULIN IN THE CENTRAL CONTROL OF SYMPATHETIC NERVE ACTIVITY IN HUMANS

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by

COLIN NEAL YOUNG

Dr. Paul J. Fadel, Dissertation Advisor

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The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled

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presented by Colin N. Young,

a candidate for the degree of Doctor of Philosophy

and hereby certify that, in their opinion, it is worthy of acceptance.

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Paul J. Fadel, PhD

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William Durante, PhD

_____

Cheryl Heesch, PhD

_____

Ronald Korthuis, PhD

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ABBREVIATIONS

AF – average fit

AU - arbitrary units

$AUC_{0\text{-}120}$ – area under the curve

AV3V - anteroventral third ventricle

β – beta

CVLM - caudal ventral lateral medulla

FBF – femoral blood flow

FVC – femoral vascular conductance

Hb – heart beats

HF – high fit

Hz - hertz

IML - intermediolateral cell column of the spinal cord

MAPK - mitogen-activated protein kinase

MSNA – muscle sympathetic nerve activity

NTS – nucleus tractus solitaries

OVLT - organum vasculosum lamina terminalis

RAAS - renin-angiotensin-aldosterone system

RVLM – rostral ventral lateral medulla

PI3K - phosphoinositide 3-kinase

PVN - paraventricular hypothalamic nucleus

SFO - subfornical organ

V – volts

V_{mean} – mean blood velocity

VSM - vascular smooth muscle

VO_2peak – peak oxygen consumption

ABSTRACT

Although well recognized for the regulation of peripheral metabolism, an emerging body of literature has begun to also establish a role for insulin in neural-cardiovascular control. In this regard, direct administration of insulin into the brain of experimental animals, or acute elevations in plasma insulin in healthy humans, results in robust increases in sympathetic nerve activity. In addition to evoking increases in central sympathetic outflow, recent work in rats has reported a modulatory role for insulin in the regulation of arterial baroreflex control of sympathetic nerve activity. The experiments within this dissertation were designed to further the understanding of insulin's role in modulating central sympathetic outflow in humans. The data presented demonstrate that an enhancement in insulin sensitivity increases insulin-mediated sympatho-excitation. Furthermore, we provide evidence for the first time that insulin increases the gain (i.e. sensitivity) of arterial baroreflex control of sympathetic nerve activity in healthy humans. Lastly, results are also presented in which we have begun to translate this area of research to a condition of chronic insulin resistance; type II diabetes mellitus. Collectively, the findings further our understanding of insulin in neural-cardiovascular control by demonstrating a clear role for insulin in the regulation of central sympathetic outflow in humans.

Chapter 1. Introduction

The sympathetic nervous system, a subdivision of the autonomic nervous system, is involved in the unconscious control of almost every organ in the body. Efferent sympathetic nerves, arising from the thoraco-lumbar section of the spinal cord innervate numerous tissues and exert effector action via the release of the neurotransmitter norepinephrine. Although the sympathetic nervous system works in concert with the parasympathetic division of the autonomic nervous system, as well as local regulatory mechanisms, to determine the end organ response, the information presented within this dissertation will focus primarily on the sympathetic nervous system. As stated by Walter B. Cannon in *The Wisdom of the Body* (1932) "It is the middle or thoraco-lumbar division [sympathetic] which acts promptly and directly to prevent serious changes of the internal environment. So important for homeostasis are the uses of this division that it deserves special and detailed consideration."[1]

In this regard, it is well established that the tonic rhythmic discharge of central sympathetic outflow is crucial for the control of vasomotor tone and arterial blood pressure[2]. Indeed, stimulation of efferent sympathetic nerves leads to increases in arterial blood pressure, whereas denervation of theses nerves leads to falls in blood pressure[2]; illustrating the importance of the sympathetic nervous system in the maintenance of appropriate hemodynamic regulation. However, in addition to the control of blood pressure, the sympathetic nervous system is also intimately involved in a wide array of physiological processes, ranging from renal to metabolic function[3].

Central Sympathetic Overactivity

The importance of the sympathetic nervous system is highlighted by a growing body of literature demonstrating that a number of disease states exhibit chronically elevated sympathetic nerve activity, such as hypertension[4] and heart failure[5]. Importantly, an enhanced sympathetic outflow is not only restricted to diseases of the cardiovascular system and has been described in obesity[6], metabolic syndrome[7], renal disease[8], obstructive sleep apnea[9], pre-eclampsia[10], depression[11], ulcerative colitis[12] and cirrhosis[13]. The clinical significance of a chronically active sympathetic nervous system is further illustrated by findings demonstrating that sympathetic overactivity is associated with greater one-year cardiac mortality in heart failure patients[14], is an independent predictor for the occurrence of cardiovascular events in end-stage renal disease patients[15, 16], and is associated with reduced functionality in elderly adults[17]. In this regard, numerous detrimental physiological effects have been attributed to increased central sympathetic outflow.

An important deleterious consequence of a chronically overactive sympathetic nervous system is an elevation in arterial blood pressure. Indeed, it has been postulated that increases in sympathetic nerve activity play a significant role in both the initiation and development of hypertension[18-21]. While a hypertensive state will contribute to structural and functional abnormalities of the vasculature (i.e. vascular remodeling)[22, 23], elevated central sympathetic outflow, independent of a rise in arterial blood pressure, has been shown to elicit vascular smooth muscle cell hypertrophy and proliferation[24, 25], impair endothelial cell function[26, 27] and decrease arterial compliance[28-30]. In addition to

unfavorable effects on the vasculature, exaggerated increases in sympathetic nerve activity have also been shown to induce detrimental effects on the cardiac (e.g. increased arrythmias)[31], renal (e.g. glomerulosclerosis)[32] and metabolic (e.g. dyslipidemia) systems[33, 34]. The potential pathological consequences of elevated increases in central sympathetic outflow are highlighted in Table 1 and have been extensively described in a recent review from our laboratory[3]. Although the mechanisms contributing to sympathetic overactivity in disease states remains an area on ongoing investigation, numerous findings have implicated alterations in the central control of sympathetic outflow.

Central Control of Sympathetic Outflow

The central regulation of sympathetic outflow is controlled by distinct nuclei within brainstem and hypothalamic regions which project directly to sympathetic preganglionic neurons located in the intermediolateral cell column of the spinal cord[35, 36]. Using retrograde transneuronal cell labeling, Strack et al[37] identified that sympathetic preganglionic neurons receive inputs from five key areas of the brain; the paraventricular hypothalamic nucleus, A5 noradrenergic cell group, caudal raphe regions, rostral ventrolateral medulla and the ventromedial medulla. Excitatory drive from these central sympathetic areas may be intrinsically generated or chemically mediated (e.g. glutamate)[35, 36]. Nuclei involved in the control of sympathetic outflow may also be modulated by excitatory and inhibitory inputs from other regions within the central nervous system; such as the circumventricular organs which lack a blood brain barrier

Table 1.1 Potential pathological consequences of elevated central sympathetic nerve activity

Vascular effects	*Cardiac effects*
VSM cell hypertrophy and proliferation	Cardiac myocyte hypertrophy
Medial thickening	Left ventricular hypertrophy
Endothelial cell damage	↑ Incidence of arrhythmia
Endothelial dysfunction	Tachycardia
Arterial stiffness	
↑ Blood pressure variability	*Renal effects*
↑ Peripheral vascular resistance	Renal vasoconstriction
Hypertension	Sodium and fluid retention
Atherosclerosis	Glomerulosclerosis
	Microalbumineria
Metabolic effects	RAAS activation
Insulin resistance	
Dyslipidemia	

VSM, vascular smooth muscle; RAAS, Renin-angiotensin-aldosterone system

and are therefore responsive to blood-borne factors[35, 38]. In addition, numerous peripheral afferent inputs, including but not limited to the arterial and cardiopulmonary baroreceptors, chemosensitive receptors, and visceral and gastrointestinal inputs, contribute to reflexive alterations in sympathetic outflow, via brainstem pathways[35, 39]. As such, complex neuroanatomical interactions exist in which descending excitatory input from central neuronal regions, afferent reflex modulation and/or reciprocal interactions among brain regions, ultimately determines efferent sympathetic outflow.

Arterial Baroreflex Control of Sympathetic Nerve Activity

Although a number of afferent reflex pathways are involved in the modulation of the autonomic nervous system, due to the importance of sympathetic nerve activity in blood pressure homeostasis, the arterial baroreflex has received a considerable amount of attention. Arterial baroreceptors are comprised of free nerve endings located within the carotid sinus and aortic arch. Afferent neural signals from the carotid and aortic baroreceptors are relayed via cranial nerves IX (glossopharyngeal) and X (vagus), respectively[40-42]. Arterial baroreceptor afferents terminate at the nucleus tractus solitarius (NTS) in the medulla oblongata[35, 40]. The NTS exerts a tonic sympathoinhibitory influence on the rostral ventrolateral medulla (one of the central sympathoexcitatory regions), by relay projections to the caudal ventrolateral medulla (Figure 1)[35, 36, 40]. Through this central pathway, when arterial blood pressure is increased, the carotid and aortic baroreceptors are deformed, causing an increase in afferent neuronal firing and a reflex mediated decrease in sympathetic nerve activity. Conversely, when arterial blood pressure is lowered, afferent neuronal firing is reduced, resulting in an increase in

sympathetic nerve activity[40-42]. In both scenarios, the neural mediated changes in sympathetic outflow will function in the appropriate fashion to return arterial blood pressure to its original level.

The necessity of the arterial baroreflex for appropriate regulation of beat-to-beat blood pressure is illustrated in studies examining humans with baroreflex failure. Patients with denervation of the carotid baroreflex, due to complications of carotid tumor resection[43], neck irradiation[44, 45] or carotid endartectomy[46], demonstrate excessive rises and falls in blood pressure to physiological stressors, such as orthostasis, cold stress or mental tasks. Moreover, baroreflex denervation in humans and animal leads to large increases in blood pressure variability[47]; further highlighting the importance of the arterial baroreflex in the short term control of arterial blood pressure. Interestingly, early and more recent findings have demonstrated that chronic carotid baroreceptor stimulation in hypertensive dogs[48-50] and humans[51-53] leads to sustained and clinically relevant decreases in arterial blood pressure and sympathetic nerve activity. As such, these findings lend support for the arterial baroreflex in the long term control of sympathetic nerve activity and blood pressure; although, to date, this concept is not universally accepted[54].

Insulin and the Insulin Receptor within the Brain

Since the first description in 1889 that removal of the pancreas in dogs resulted in high levels of sugar in the urine[55], and the subsequent discovery that a pancreatic extract, insulin, was capable of lowering blood glucose[56], a plethora of research has been dedicated to understanding the metabolic actions of insulin. Indeed, it is well established

that insulin has profound metabolic effects, including stimulation of glucose uptake, glycogen storage control, and inhibition of lypolysis[57]. However, an emerging body of literature has indicated that, in addition to a role in metabolic control, insulin is a pleiotropic hormone with widespread mechanisms of action, including functions within the central nervous system.

Insulin is synthesized within the beta (β) cells of the Islets of Langerhans in the pancreas. The β cells produce insulin from a proinsulin precursor, via the action of several proteolytic enzymes, ultimately resulting in a 51 amino acid polypeptide. The primary stimulus for insulin release from the β-cells is an increase in plasma glucose. In addition, amino and fatty acids, as well as acetylcholine stimulation of β2 adrenergic receptors can induce insulin release, although to a lesser extent[57, 58]. Insulin exerts it actions via binding to the insulin receptor, a tyrosine kinase transmembrane receptor[58, 59]. The insulin receptor is composed of two ligand binding α subunits, linked by disulfide bonds to two membrane-spanning β subunits. Binding of insulin to the α subunits stimulates intrinsic tyrosine kinase activity within the β subunits, resulting in phosphorylation of downstream proteins and initiation of numerous signaling cascades, including the phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways[58, 59].

Insulin receptors are widely dispersed throughout the body, including numerous, yet discrete sites within the central nervous system[59-63]. In vitro work from rats has indicated that brain insulin receptor density is highest in the choroid plexus, olfactory bulb and hypothalamus, with moderate to low levels in other regions such as the cerebral cortex, brainstem and circumventricular organs[61, 62]. Although insulin is not produced in

significant amounts within the central nervous system, increases in circulating insulin (via pancreatic release) gain access to the brain via transport mediated uptake across the blood brain barrier[61, 63]. In this context, insulin has been shown to be involved in many divergent functions in the central nervous system, including effects on appetite regulation, peripheral metabolism, cognition, and neurotrophic effects[60, 61]. Moreover, the finding that the ligand and the receptor are present in central neuro-cardiovascular control areas, including hypothalamic regions, circumventricular organs and the brainstem, has led to the concept that insulin may be involved in the control of sympathetic outflow[64].

Influence of Insulin on Central Sympathetic Outflow

A role for insulin in central sympathetic control is drawn from studies in experimental animals, whereby intracerebroventricular administration of insulin elicits robust increases in peripheral sympathetic nerve activity[65, 66]. Interestingly, insulin-stimulated increases in central sympathetic outflow appear to be dose specific, as increases in lumbar and brown adipose tissue nerve activity were noted with low dose intracerebroventricular insulin infusion, whereas adrenal and renal sympathetic nerve activity were activated at higher dosages[66]. In line with these findings, acute euglycemic hyperinsulinemia[67-71] or ingestion of a mixed meal[72-74] or glucose load[75-79] (to increase plasma insulin) have been shown to stimulate increases in muscle sympathetic nerve activity in humans. Importantly, elevations in plasma insulin could increase sympathetic nerve activity via indirect mechanisms; nonetheless, several lines of evidence are consistent with the hypothesis that hyperinsulinemia induced increases in sympathetic

outflow are primarily due to a central effect. First, insulin has vasodilatory actions and therefore a drop in arterial blood pressure during peripheral hyperinsulinemia could mediate a baroreflex-mediated increase in sympathetic nerve activity. However, in humans, the increase in muscle sympathetic nerve activity is also present during low dose euglycemic hyperinsulinemia, in which peripheral vasodilation does not occur[69]; arguing against a baroreflex-mediated increase in sympathetic outflow. In addition, Vollenweider et al[71] infused insulin and fructose into normotensive humans to examine if insulin elevates sympathetic nerve activity through indirect actions of the hormone on carbohydrate metabolism and oxidation. While insulin and fructose produced comparable increases in carbohydrate metabolism, only infusion of insulin was associated with elevated muscle sympathetic nerve activity. Collectively, these studies, utilizing a variety of maneuvers to alter brain and plasma insulin concentrations, demonstrate a central sympatho-excitatory effect of insulin.

Although the precise central region(s) involved in mediating insulin-stimulated increases in sympathetic outflow remain unclear, several areas are worthy of consideration. The anteroventral third ventricle (AV3V) region, an area containing several of the circumventricular organs (organum vasculosum lamina terminalis and subfornical organ) is well recognized as a neuronal region involved in the detection of blood-borne signals. Interestingly, lesioning of the AV3V region prevents the increase in lumbar sympathetic nerve activity in response to euglycemic hyperinsulinemia[80]. Furthermore, studies demonstrating robust increases in lumbar sympathetic nerve activity in response to lateral ventricular infusion of insulin in rats[81], suggests that a hypothalamic region, such as the paraventricular nucleus, may be involved. In line with this, Rahmouni

et al[66] have shown that global hypothalamic inhibition of PI3K blocked insulin-mediated increases in lumbar sympathetic nerve activity, whereas MAPK inhibition specifically blocked insulin-induced increases in sympathetic nerve activity to brown adipose tissue. Moreover, very recent findings suggest that an excitatory glutamatergic pathway to the brainstem may be involved in insulin stimulated sympatho-excitation[82]. Indeed, microinjection of kynurenic acid, a glutamate receptor antagonist, into the rostral ventral lateral medulla significantly reduced lumbar sympathetic nerve activity during hyperinsulinemic euglycemic clamps in rats. Of note, insulin microinjection directly into the rostral ventral lateral medulla did not alter lumber sympathetic nerve activity[82]. Taken together these findings lend insight into the potential neural pathways and signaling mechanisms involved in insulin mediated increases in central sympathetic outflow (Figure 1); however, future studies are clearly warranted to further delineate the precise neuronal pathways and mechanisms of insulin action in neural-cardiovascular control.

Insulin and Arterial Baroreflex Control of Sympathetic Nerve Activity

In addition to a sympatho-excitatory role of insulin, emerging evidence from studies in experimental animals has begun to illustrate a role for insulin in the central modulation of the arterial baroreflex. Recently, Pricher et al[81] demonstrated that increases in insulin within the brain, via lateral ventricular infusion, enhanced the gain (i.e. sensitivity) of arterial baroreflex control of lumbar sympathetic nerve activity in rats. Other studies have indicated that neurons in central baroreflex regulatory regions (i.e. nucleus tractus solitarius) are responsive to insulin[83]. Collectively, these studies indicate

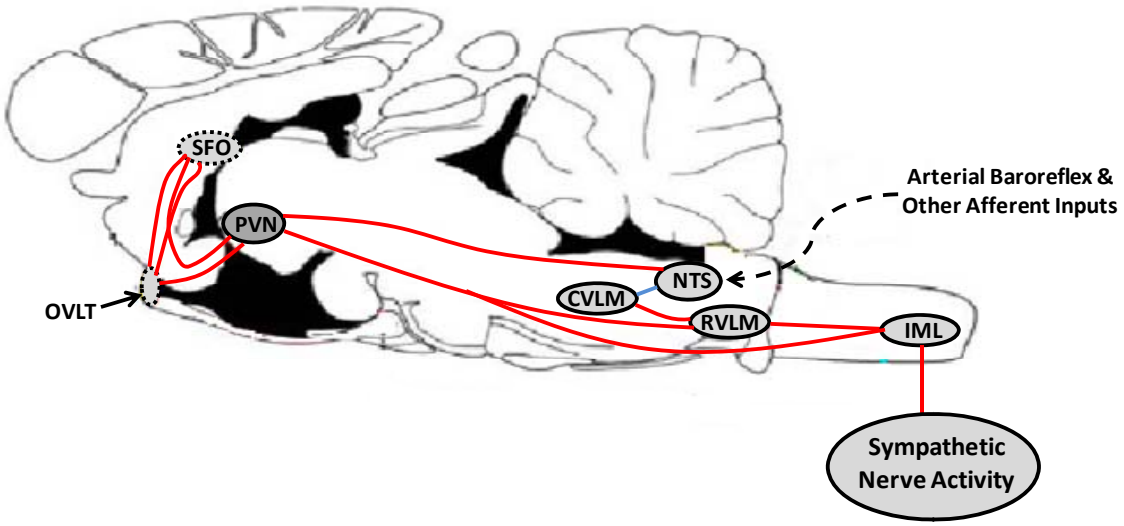

Figure 1.1 Schematic summarizing neural pathways that may play a role in insulin mediated control of sympathetic outflow. Cardiovascular regulatory regions in the forebrain, hypothalamus and brainstem may all contribute. Circumventricular organs within the anteroventral third ventricle region (outlined with dashed lines) may sense increases in circulating insulin and project directly or indirectly to hypothalamic regions. In addition, the paraventricular hypothalamic nucleus (PVN) demonstrates a high binding density of insulin (dark grey) and may also directly influence sympathetic outflow via projections to the intermediolateral cell column of the spinal cord (IML) or indirectly through projections to brainstem regions. Insulin could also modulate sympathetic outflow within brainstem regions such as the nucleus tractus solitarius (NTS), caudal ventral lateral medulla (CVLM) and rostral ventral lateral medulla (RVLM). Arterial baroreflex afferents terminate within the NTS and as such insulin may modulate arterial baroreflex control of sympathetic nerve activity by directly acting within brainstem regions or via impinging neural inputs from other brain regions.

OVLT, organum vasculosum lamina terminalis; SFO, subfornical organ. Sagital brain section adapted from Zimmerman & Davisson, Prog Biophys Mol Biol, 2004.

that insulin may directly influence arterial baroreflex function within central neural cardiovascular control pathways. However, studies in this area are limited and, to date, no work has been performed in humans evaluating the potential influence of insulin on arterial baroreflex control of sympathetic nerve activity.

Insulin Mediated Sympatho-excitation and Arterial Baroreflex Control in Insulin Resistant Conditions

Numerous physiological and pathophysiological conditions are characterized by a decreased sensitivity to the actions of insulin (i.e. insulin resistance), including type II diabetes mellitus, hypertension, obesity, metabolic syndrome and pregnancy. Interestingly, recent studies have suggested that insulin-mediated sympatho-excitation may be impaired in insulin resistant conditions[76, 78, 79, 84]. Indeed, obese individuals[84], insulin resistant elderly subjects[76] and metabolic syndrome patients[78, 79] all demonstrate attenuated increases in muscle sympathetic nerve activity in response to hyperinsulinemia. As such, in addition to peripheral insulin resistance, insulin resistant conditions may also exhibit a central resistance to the actions of insulin. In line with this concept, recent studies indicate that insulin resistant states are accompanied by reduced cerebrospinal fluid insulin concentrations[63, 85-87]. This reduction in insulin likely emanates from an attenuated transport of insulin into the central nervous system, as pharmacologically and diet induced insulin resistance in animal models has been shown to reduce central nervous system insulin uptake[85, 86], whereas brain endothelial cells of obese rats demonstrate impaired insulin binding[88].

In addition to impaired insulin-mediated sympatho-excitation, insulin resistant conditions are commonly characterized by impairments in arterial baroreflex control of sympathetic nerve activity[7, 89-92]. Although not causative, the concomitant occurrence of insulin resistance with reductions in arterial baroreflex function clearly suggests a link between the two. Given that cerebral spinal fluid levels of insulin are reduced in insulin resistant conditions[63, 85-87] and that insulin in the brain enhances arterial baroreflex control of sympathetic outflow[81]; Brooks and colleagues have advanced the hypothesis that a certain level of insulin within the brain is essential for normal arterial baroreflex function[81, 93, 94]. However, while arterial baroreflex-sympathetic control has been shown to be reduced in hypertension[89, 90, 92, 95], obesity[91] and metabolic syndrome[7], information on other insulin resistant conditions, such as type II diabetes mellitus, is currently lacking.

Summary

Although once thought to only be crucial for the regulation of peripheral metabolism, an emerging body of literature has established a role for insulin in neural-cardiovascular control. In healthy humans, elevations in plasma insulin during physiological[72-79] (i.e. meal intake) or experimental[67-71] perturbations results in robust increases in muscle sympathetic nerve activity; a response that has been primarily attributed to a central effect of insulin. Furthermore, direct administration of insulin into the brain of experimental animals also evokes increases in peripheral nerve activity[65, 66, 81], although the precise neural regions and/or mechanisms remain incompletely defined. In addition to evoking increases in sympathetic outflow, recent work in rats has reported

a role for insulin in the modulation of the arterial baroreflex[81]. The following projects were designed to extend this growing area of research and to examine a role for insulin in the control of central sympathetic outflow in humans. Specifically, the influence of insulin sensitivity on insulin-mediated sympatho-excitation, a role for insulin in the modulation of arterial baroreflex control of muscle sympathetic nerve activity, and translation of these findings to an insulin resistant disease state, type II diabetes mellitus, will be presented.

Specific Aims

Aim 1: Previous studies have reported that insulin resistant conditions are associated with impaired insulin-mediated sympatho-excitation[76, 78, 79, 84]. Importantly, in these previous investigations, the populations studied were all characterized by resting sympathetic overactivity. Therefore, it is possible that the sympathetic responses to insulin may have been influenced by higher basal sympathetic activity (i.e. a ceiling effect). Thus, the focus of Aim 1 was to determine if differences in insulin-stimulated sympatho-excitation occur without confounding factors such as elevated resting sympathetic activity, body mass, or age. To examine this, we studied two healthy subject groups, with distinct differences in insulin sensitivity, to investigate how insulin sensitivity influences insulin-mediated changes in central sympathetic outflow.

Aim 2: Recent findings in rats have demonstrated that insulin in the brain enhances arterial baroreflex control of sympathetic nerve activity[81]; however, the extent to which these findings can be translated to humans remains unknown. Therefore, Aim 2 was

designed to examine the influence of insulin on arterial baroreflex control of muscle sympathetic nerve activity in healthy humans.

Aim 3: Although numerous insulin resistant conditions are characterized by a reduction in arterial baroreflex control of sympathetic nerve activity, surprisingly an examination of the sympathetic arterial baroreflex has not been performed in type II diabetes mellitus. Thus, Aim 3 was designed to begin to investigate arterial baroreflex control of muscle sympathetic nerve activity in patients with type II diabetes.

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Chapter 2.

Influence of endurance training on central sympathetic outflow to skeletal muscle in response to a mixed meal

Colin N. Young[1, 5], Shekhar H. Deo[1, 5], Areum Kim[1, 5], Masahiro Horiuchi[1, 5], Catherine R. Mikus[2, 5], Grace M. Uptergrove[3, 5], John P. Thyfault[2, 3, 5] & Paul J. Fadel[1, 4, 5]

Departments of Medical Pharmacology & Physiology[1], Nutrition and Exercise Physiology[2], Internal Medicine-Division of Gastroenterology and Hepatology[3], Dalton Cardiovascular Research Center[4], University of Missouri, Columbia, MO, and Research Service, Harry S. Truman VA Medical Center[5], Columbia, MO.

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ABSTRACT

Nutrient intake is accompanied by increases in central sympathetic outflow; a response that has been mainly attributed to insulin. Insulin-mediated sympatho-excitation appears to be blunted in insulin resistant conditions suggesting that aside from peripheral insulin insensitivity, such conditions may also impair the central action of insulin in mediating sympathetic activation. What remains unclear is whether an insulin sensitive state, such as that induced by chronic endurance training, alters the central sympathetic effects of insulin during postprandial conditions. To examine this question plasma insulin and glucose, muscle sympathetic nerve activity (MSNA), heart rate, and arterial blood pressure were measured in 11 high fit (HF; VO_2 65.9±1.4 ml•kg^{-1}•min^{-1}) and 9 average fit (AF; VO_2 43.6±1.3 ml•kg^{-1}•min^{-1}) male subjects before and for 120 minutes after ingestion of a mixed meal drink. As expected, the insulin response to meal ingestion was lower in HF than AF participants (insulin area under the curve$_{0\text{-}120}$: 2314±171 vs. 4028±460 uIU•ml^{-1}•120^{-1}, HF vs. AF, $P<0.05$), with similar plasma glucose responses between groups. Importantly, following consumption of the meal, the HF subjects demonstrated a greater rise in MSNA, when compared to the AF subjects (e.g. 120 minutes: Δ21±1 vs. 8±3 bursts•100 heart beats^{-1}, HF vs. AF, $P<0.05$). Furthermore, when expressed relative to plasma insulin, HF subjects exhibited a greater change in MSNA for any given change in insulin. Arterial blood pressure responses following meal intake were similar between groups. Collectively, these data suggest that, in addition to improved peripheral insulin sensitivity, endurance training may enhance the central sympathetic effect of insulin to increase MSNA following consumption of a mixed meal.

INTRODUCTION

It is well recognized that acute nutrient intake is accompanied by robust increases in central sympathetic outflow[1-3]. Presumably, the physiological relevance of an increase in sympathetic neural activity is to facilitate blood flow redistribution and for the overall maintenance of arterial blood pressure[1-4]. In addition, increases in sympathetic outflow will stimulate facultative thermogenesis (e.g. diet-induced thermogenesis)[5-7] and may enhance glucose uptake in skeletal muscle and adipose tissue[8-12]. While a variety of factors may be implicated, numerous findings have indicated that the large rise in plasma insulin concentrations following meal intake is an important mediator of the postprandial increase in central sympathetic outflow[3, 13].

A central sympatho-excitatory action of insulin is drawn from studies in experimental animals, whereby intracerebroventricular administration of insulin elicits robust increases in peripheral sympathetic nerve activity[14, 15]. In line with these findings, a mixed meal[13, 16], glucose load[17-21] and acute euglycemic hyperinsulinemia[4, 22-25] have been shown to stimulate increases in muscle sympathetic nerve activity (MSNA) in humans. Importantly, the increase in MSNA is also present during low dose euglycemic hyperinsulinemia, in which peripheral vasodilation does not occur [24]; consistent with the hypothesis that hyperinsulinemia induced increases in sympathetic outflow are primarily due to a central effect (i.e. non baroreflex-mediated). Overall, these studies, utilizing a variety of maneuvers to alter plasma insulin concentrations, demonstrate that elevations in insulin stimulate increases in central sympathetic outflow.

Recent studies have suggested that insulin-mediated sympatho-excitation may be impaired in insulin resistant conditions. Indeed, Vollenweider et al[26] demonstrated that obese individuals exhibited attenuated MSNA responses to euglycemic hyperinsulinemia, despite

higher insulin concentrations, when compared to lean subjects. Findings in insulin resistant elderly subjects[18] and insulin resistant metabolic syndrome patients[20, 21] have similarly reported a blunted sympathetic response to increases in plasma insulin following a glucose load. As such, in addition to peripheral insulin resistance, insulin resistant conditions may also exhibit a central resistance to the actions of insulin. In line with this concept, recent studies indicate that insulin resistant states are accompanied by reduced cerebrospinal fluid insulin concentrations, likely emanating from an attenuated transport of insulin into the central nervous system[27-30]. However, while an insulin resistant condition appears to impair insulin-mediated sympatho-excitation, the influence of enhanced insulin sensitivity on insulin-induced increases in central sympathetic outflow remains unclear.

To begin to examine this question we recruited healthy endurance trained (high fit, HF) and normally active (average fit, AF) subjects. It is well characterized that chronic endurance training results in enhanced peripheral insulin sensitivity[31-35]. Therefore, our rationale was that inclusion of two healthy subject groups, with distinct differences in insulin sensitivity, would allow us to investigate how enhanced insulin sensitivity influences insulin-mediated changes in central sympathetic outflow. Direct measurements of central sympathetic outflow to skeletal muscle (i.e., MSNA) were recorded and a mixed meal was used as a physiological method to evoke robust and sustained increases in MSNA, which have been primarily attributed to insulin[3, 13]. We hypothesized that HF subjects would have a greater MSNA response, for a given plasma insulin concentration, following consumption of a mixed meal (i.e., greater central insulin sensitivity).

METHODS

Twenty healthy men volunteered for participation in the study. Subjects were asymptomatic for cardiovascular, respiratory or metabolic disease and were not taking any medications. The subject population consisted of eleven HF and nine AF men. HF subjects were all competitive endurance athletes (i.e. marathon runners and triathletes) and had been competing in endurance events for 9 ± 1 years with weekly exercise regimens including 12 ± 2 hours per week of training. In contrast, AF subjects were only recreationally active, engaging in aerobic activities for less than 30 minutes, ≤3 days per week reporting physical activities amounting to 45 ± 23 minutes per week. When recruiting HF subjects a peak oxygen uptake (VO_{2peak}) of ≥ 60ml/kg/min was used as the cutoff for inclusion. For the AF subjects we initially attempted to enroll subjects with a VO_{2peak} ≤ 45 ml/kg/min, however, this proved difficult for young healthy lean subjects and therefore, we had two subjects with a VO_{2peak} of 47 ml/kg/min in this group. A greater number of HF compared to AF subjects were recruited due to difficulty with MSNA measurements in this group (see MSNA section below). All experimental procedures and protocols were approved by the University of Missouri Health Sciences Institutional Review Board. After receiving a detailed verbal and written explanation of the intended experimental protocol and measurements, each subject provided written informed consent prior to participation.

Experimental Measurements

General measurements: Heart rate was continuously monitored using a lead II electrocardiogram (Quinton Q710, Bothell, WA, USA). An automated sphygmomanometer (Welch Allyn, Skaneatles Falls, NY, USA) was used to measure arterial blood pressure by

auscultation of the brachial artery of the right arm. Respiratory movements were monitored using a strain-gauge pneumograph placed in a stable position around the abdomen (Pneumotrace, UFI, Morro Bay, CA, USA).

Plasma Insulin and Glucose: Venous blood samples were drawn from an antecubital intravenous catheter for the measurements of plasma glucose and insulin. Glucose was analyzed using the glucose oxidase method (Thermo, Waltham, MA, USA) and insulin was determined by chemiluminescent enzyme immunoassay (Immulite 1000 Analyzer, Diagnostic Products Corp., Los Angeles, CA, USA). The areas under the glucose and insulin curves ($AUC_{0\text{-}120}$) were calculated from values measured at baseline, using the trapezoidal method[36].

Muscle Sympathetic Nerve Activity (MSNA): Multiunit recordings of postganglionic MSNA were obtained as described previously[37-41]. Briefly, a unipolar tungsten microelectrode was inserted percutaneously through the intact, unanaesthetized skin and positioned into muscle nerve fascicles of the peroneal nerve near the fibular head of the left leg. Postganglionic sympathetic action potentials were amplified, filtered (bandwidth 700 – 2000 Hz), rectified, and integrated (time constant, 0.1 s) to obtain a mean voltage neurogram. MSNA recordings were identified by their characteristic pulse-synchronous burst pattern and increased neural activity in response to an end-expiratory apnea or Valsalva maneuver, without any response to arousal stimuli or stroking of the skin[37, 38, 41]. MSNA was not obtained in 4 HF subjects because an acceptable recording was not found in 2 subjects and recordings could not be maintained for the duration of the protocol in 2 others.

MSNA was first identified by visual inspection, independently by 2 investigators, and was then analyzed using custom designed software (MatLab, The Math Works, Natick, MA, USA)[42]. This program first detects peaks in the integrated neurogram based on a latency window

centered on each R wave from the electrocardiogram. After the peaks are chosen, the the nearest minimum value on either side of the peak is detected, which is then denoted as the start and end of a sympathetic burst. An algorithm is applied to validate each burst, which takes into account the slope of the rise and fall of the burst, as well as the magnitude. The amplitude of the largest burst at baseline was assigned a value of 1000 (arbitrary units; AU) and all other bursts within a trial were normalized with respect to this value. Sympathetic activity was quantified using standard measures, including burst frequency (bursts/min), burst incidence (bursts/100 heart beats), burst strength (burst area), and total activity (product of burst frequency and mean burst area). However, because the absolute area of a burst is dependent on the location of the microelectrode in relation to the nerve fibers that are being recorded, which cannot be determined, direct comparisons of burst strength, and total activity between groups is typically not performed. Although various normalization procedures have previously been applied, these cannot completely account for the limitation associated with potential differences in electrode placement between individuals and, ultimately, subject groups[43-45]. For example, calculating a percentage change in total activity from baseline will be affected by differences in resting total activity (i.e. microelectrode placement) and not necessarily reflect a difference in sympathetic responsiveness between two groups. In this manuscript, multiple MSNA data are included in an effort to be complete; however we focused our results on measures of sympathetic bursts, which have been shown to be reproducible over time in the same subject and comparable between groups (50). Furthermore, because of group differences in heart rate and the inherent cardiac synchronicity of MSNA[37, 38, 41], we used MSNA burst incidence for our main comparison between groups. In addition, the area under the MSNA burst incidence curve (AUC_{0-120}) was

calculated for each subject using the trapezoidal method[36] and related to the insulin $AUC_{0\text{-}120}$ as described previously[21].

Femoral artery blood flow: Femoral blood flow (FBF) was obtained in the right leg using a duplex Doppler ultrasound unit (Logiq 7, GE Medical Systems, Milwaukee, WI) equipped with a linear array transducer operating at a frequency of 10 MHz. The common femoral artery was imaged 2-3 cm proximal to the bifurcation of the superficial and deep branches. Femoral blood velocity was obtained using the same probe in pulsed-wave mode, operating at a linear frequency of 5 Hz and at an insonation angle of 60°. Arterial diameter and mean blood velocity (V_{mean}) were calculated using commercially available software (Logiq 7, GE Medical Systems, Milwaukee, WI, USA). Using femoral artery diameter and V_{mean}, FBF was calculated as: $FBF = V_{mean} \bullet \pi \bullet (\text{femoral artery diameter}/2)^2 \bullet 60$. FBF was normalized to right leg lean mass for group comparisons. Femoral vascular conductance (FVC) was calculated using the formula: FVC = FBF / mean arterial blood pressure.

Experimental Protocols

Visit 1: Subjects were familiarized with the experimental protocols and procedures, after which a whole body dual-energy X-ray absorptometry scan (Hologic Delphi A, Waltham, MA) was performed to obtain right leg lean muscle mass, used to normalize blood flow measures between groups. In addition, in order to ascertain VO_{2peak}, all subjects underwent a graded treadmill exercise test (Bruce Protocol) to exhaustion and maximal effort was determined according to established criteria[46].

Visit 2: Subjects reported to the laboratory on a separate occasion at least 5 days after completion of the first visit and were instructed to abstain from caffeinated beverages and food

for 12 hours, alcohol for 24 hours, and physical activity for 48 hours prior to the experimental session. The latter instruction was used to minimize the influence of acute physical activity effects on insulin sensitivity[47, 48]. Subjects were placed in the supine position on a medical examination table and were instrumented for measures of heart rate, arterial blood pressure, MSNA, FBF and an intravenous catheter was placed in an antecubital vein. Baseline variables were collected for a period of 20 minutes and a pre-meal blood draw was taken, after which subjects ingested a mixed meal drink (Ensure Plus, Abbott Laboratories, Columbus, Ohio, USA; 57% CHO, 28% Fat, 15% Protein) corresponding to 20% of their estimated energy expenditure calculated from body weight[49]. The calories consumed were similar between groups (521 ± 9 vs. 533 ± 14 KCals, HF vs. AF, $P>0.05$). All variables were measured for a 5 minute period, every 15 minutes, for 120 minutes following consumption of the mixed meal. Venous blood samples were also drawn every 15 minutes and the resulting plasma was stored at -80° C for later analysis of plasma glucose and insulin concentrations.

Data Analysis

Heart rate and MSNA were sampled at 1000Hz and stored for off-line analysis (Chart v5.2 and Powerlab, ADInstruments, Bella Vista, NSW, Australia). Baseline heart rate, arterial blood pressure, MSNA and FBF were calculated as mean values over a 6 minute period. Following consumption of the mixed meal, 3 minute averages were calculated from the 5 min data segments collected every 15 minutes. The ratio of a change in MSNA burst incidence to a change in insulin was evaluated at each time point for individual subjects as an index of the sympathetic response for a given concentration of plasma insulin.

Statistical analysis

Statistical comparisons of physiological variables were conducted using a 2-way analyses of variance (fitness•time) with repeated measures and a Tukey test was employed *post hoc* when significant main effects were found. Statistical significance was set at $P < 0.05$ and analyses were conducted using SigmaStat (Jandel Scientific Software, SPSS Inc., Chicago, IL, USA) for Windows. Statistical comparisons were performed using all time points following meal intake. However, for presentation purposes, only 30 minute time points are reported in some cases. Results are presented as means ± standard error (SE).

RESULTS

Subject characteristics: Age, height, weight and body mass index did not differ between groups (Table 2.1). Similarly, fasting plasma glucose and insulin were not different between groups (Table 2.1 and Figure 2.1). As anticipated, the HF subjects had a higher VO_{2peak} than the AF subjects, with a mean group difference greater than 20 ml/kg/min (Range: HF 60-73 ml/kg/min, AF 36-47 ml/kg/min). HF subjects exhibited a significantly lower heart rate at baseline, whereas resting systolic (SBP), diastolic (DBP) and mean arterial blood pressure (MBP) were similar between groups (Table 2.2).

Metabolic responses to a mixed meal: Mixed meal intake induced a significant rise in plasma glucose, with similar responses between groups at each time point (Figure 2.1A) and when quantified as glucose AUC (Figure 2.1C). In contrast, HF subjects demonstrated much less of an increase in plasma insulin, compared to the AF subjects (Figure 2.1B). This enhanced insulin sensitivity in the HF subjects was illustrated by an approximately 45% lower insulin $AUC_{0\text{-}120}$ (Figure 2.1D).

MSNA responses to a mixed meal: At rest, MSNA burst incidence and burst frequency was comparable between groups (Figure 2.2, Figure 2.3, and Table 2.3). Following ingestion of the mixed meal, the HF subjects demonstrated a greater rise in MSNA burst incidence, as well as burst frequency, compared to AF subjects. Results obtained for MSNA total activity and burst strength are also provided in Table 2.3.

MSNA : insulin relationships: Figure 2.4 illustrates the ratios of a change in MSNA burst incidence to a change in plasma insulin, indicating that for a given change in insulin HF subjects had a greater change in MSNA burst incidence. Similar results were found when relating the MSNA burst incidence $AUC_{0\text{-}120}$ to the insulin $AUC_{0\text{-}120}$ (Figure 2.4B).

FBF responses to a mixed meal: Resting FBF was not different between groups. In response to the mixed meal, FBF was increased above baseline values, with similar increases in the HF and AF subjects. Likewise, resting FVC was the same between groups and increased similarly in response to mixed meal ingestion (all Table 2.2).

Cardiovascular responses to a mixed meal: Overall there were no differences in the arterial blood pressure responses to mixed meal intake between groups. Following the mixed meal, SBP, DBP and MAP were slightly, but significantly increased in both groups. Heart rate responses to the mixed meal were not different between groups and therefore heart rate was lower throughout the entire protocol in the HF compared to the AF subjects owing primarily to baseline differences (all Table 2.2).

DISCUSSION

The major finding of the current study was that HF subjects demonstrated a greater increase in MSNA burst incidence compared to AF subjects following consumption of a mixed meal. Importantly this greater sympathetic activation occurred despite lower plasma insulin concentrations, with comparable plasma glucose between groups. Furthermore, when MSNA responses were expressed relative to plasma insulin, HF subjects exhibited a greater change in MSNA for any given change in insulin. Collectively, these data suggest that, in addition to improved peripheral insulin sensitivity, endurance training may also enhance the central actions of insulin to increase MSNA following consumption of a mixed meal.

Nutrient intake is accompanied by increases in central sympathetic outflow; a response that has been mainly attributed to insulin[3, 13]. In the current study we used chronic endurance training as a model to establish two distinct subject groups. An enhancement in the peripheral sensitivity to the actions of insulin (i.e. glucose disposal) is well characterized with chronic endurance training[31-35] and therefore, we rationalized that endurance trained individuals would exhibit a lower insulin response with similar glucose concentrations following the mixed meal. Furthermore, if enhanced central insulin sensitivity was present, we reasoned that the HF subjects would exhibit a similar or greater increase in MSNA despite the lower plasma insulin. Indeed, following consumption of the mixed meal, the HF subjects demonstrated a significantly larger increase in MSNA when compared to the AF subjects.

From our direct sympathetic recordings, an increase in central sympathetic outflow to skeletal muscle can be characterized by either an increase in efferent sympathetic impulses (i.e. burst incidence) or an increase in the strength of the sympathetic bursts (i.e. burst area) or a combination of the two[50]. Interestingly, the rise in MSNA following nutrient intake was evident

when examining both burst occurrence (i.e. burst incidence), as well as burst strength in both groups. These data suggest that the increase in central sympathetic outflow to skeletal muscle following a mixed meal is due to an enhanced sympathetic firing rate as well as enhanced neuronal recruitment[50]. The larger increase in MSNA burst incidence in the HF compared to AF subjects clearly demonstrates a greater postprandial central sympatho-excitation. In addition, the greater increases in burst strength in the HF group suggests that this fitness effect on MSNA may include greater neuronal recruitment[51]; however, group comparisons of burst strength need to be interpreted cautiously because the area of a burst is dependent on the location of the recording microelectrode, which cannot be determined[38, 52]. Therefore, we focused our results on measures of sympathetic bursts, which have been shown to be reproducible over time in the same subject and comparable between groups[52]. Furthermore, the use of MSNA burst incidence is warranted because of group differences in heart rate and the inherent cardiac synchronicity of MSNA[37, 38, 41].

Following food intake, coordinated cardiovascular, metabolic and neural responses occur in order to distribute, absorb and store nutrients. Postprandial activation of the sympathetic nervous system is crucial for blood flow redistribution and overall maintenance of arterial blood pressure[1-3, 7]. Although a number of factors have been suggested[3], several lines of evidence indicate that the elevation in plasma insulin following a meal plays a prominent role in stimulating the increases in central sympathetic outflow[3, 13]. First, a strong positive correlation has been reported between plasma insulin and the increases in MSNA after a glucose meal in healthy subjects[17, 53]. Second, a mixed meal and glucose load have been shown to elicit the greatest changes in MSNA when compared to fat or protein intake alone, in which insulin secretion is minimal[13]. In addition, euglycemic hyperinsulinemia within the postprandial range

increases MSNA in healthy humans to a similar extent as that following a mixed meal[24]. Third, recent animal studies have provided clear evidence indicating a direct action of insulin to increase central sympathetic outflow[14, 15]. Lastly, alterations in the sympathetic response to a mixed meal and glucose load have been identified in insulin resistant conditions[18, 20, 21, 26]. Overall, although insulin independent mechanisms cannot be completely discounted, these data indicate that a mixed meal represents a physiological method to investigate insulin-mediated stimulation of central sympathetic outflow.

In the current study, when the MSNA burst incidence responses to the mixed meal were expressed relative to insulin, a greater change in sympathetic outflow for a given change in plasma insulin concentrations was noted in the HF group. This held true whether the values were expressed at each time point or as a ratio of the MSNA $AUC_{0\text{-}120}$ to the insulin $AUC_{0\text{-}120}$. These data suggest that an enhancement in insulin sensitivity, even in otherwise healthy individuals, improves insulin stimulated sympatho-excitation to skeletal muscle (i.e. enhanced central insulin sensitivity). Of note, MSNA burst frequency data also suggests that HF subjects exhibit greater central insulin sensitivity. Although the physiological consequence of a greater increase in central sympathetic outflow in the HF subjects is beyond the scope of the current project, it may be that an augmentation in sympathetic outflow following mixed meal intake allows for greater control over redistribution of blood flow and absorption of nutrients; thus contributing to the enhanced peripheral insulin sensitivity.

Recent investigations have suggested that the central stimulatory actions of insulin may be blunted in insulin resistant conditions[18, 20, 21, 26]. Indeed, the sympatho-excitation following a mixed meal, glucose load or euglycemic hyperinsulinemic clamp is blunted in elderly insulin resistant individuals[18], insulin resistant metabolic syndrome patients[20, 21] and obese subjects[26],

respectively. In addition, Straznicky et al[21] recently demonstrated that diet and exercise induced weight loss in insulin resistant metabolic syndrome subjects improved the blunted sympathetic responsiveness to a glucose load, as measured by whole body norepinephrine kinetics. Moreover, a positive correlation between maximal oxygen uptake and the insulin-induced increases in whole body norepinephrine spillover has been noted in metabolic syndrome patients[20]. Taken together these findings suggest that an insulin resistant state may blunt insulin-mediated sympathetic neural responses and that potential improvements in insulin sensitivity may restore insulin's ability to increase sympathetic outflow. The current findings are in agreement and extend this work by directly assessing MSNA in two groups of young, lean healthy individuals, who display distinct differences in insulin sensitivity (i.e., different postprandial insulin responses to a meal). Importantly, in these previous investigations, the populations studied were all characterized by resting sympathetic overactivity. Therefore, it is possible that the sympathetic responses to insulin may be influenced by higher basal sympathetic activity (i.e. a ceiling effect)[54]. The current findings allowed us to determine if differences in insulin stimulated sympatho-excitation occur without confounding factors such as elevated resting sympathetic activity, body mass index, or age.

While the exact mechanism(s) contributing to alterations in insulin stimulated changes in central sympathetic outflow to skeletal muscle remain unknown, several possibilities are worthy of consideration. First, it is possible that alterations in insulin transport across the blood brain barrier occur with changes in insulin sensitivity. In support of this concept, a reduced cerebrospinal fluid to plasma insulin ratio has been reported in obese individuals, with the ratio being highly negatively related to the degree of insulin resistance[29]. Moreover, pharmacologically induced insulin resistance in animal models has been shown to reduce central

nervous system insulin uptake[27, 28], whereas brain endothelial cells of obese rats demonstrate impaired insulin binding[30]. Thus, our findings demonstrating a more robust increase in MSNA, in the HF compared to the AF subjects, may be due to differences in insulin delivery and/or rate of transport into the brain. In addition, it is possible that central insulin-mediated signaling cascade pathways may be altered with changes in central insulin sensitivity. Findings from rodent models suggest that insulin stimulated increases in sympathetic outflow primarily occur within hypothalamic regions through both the phosphoinositide 3-kinase and mitogen-activated protein kinase pathways[14, 15, 55]. Given that both of these pathways have been shown to be modified in the periphery in insulin resistant states[48] and in response to endurance training[56], it is plausible that alterations in either of these signaling pathways within the central nervous system occurs with changes in insulin sensitivity.

A role for insulin in mediating peripheral vasodilation has been well documented[57-59]. Although not the main focus of our study, considering the known increases in peripheral insulin sensitivity with endurance training[31-35], one may have anticipated a greater insulin-mediated increase in blood flow in the HF, compared to the AF subjects, following the mixed meal. However, we did not observe any group differences in absolute FBF or FVC responses following ingestion of the mixed meal. In line with our findings, the peripheral blood flow responses following a glucose load in insulin resistant and insulin sensitive metabolic syndrome subjects have also been shown to not differ between groups with differences in insulin sensitivity[20, 21]. It may be that the majority of insulin stimulated vasodilation following meal intake occurs within the microvasculature. As discussed by Renkin and colleagues[60, 61], enhancing capillary surface area will greatly increase nutrient delivery in direct proportion to the surface area available for nutrient exchange (e.g. microvascular recruitment), whereas increasing total limb blood flow (i.e.

FBF) would minimally enhance nutrient exchange. Therefore, although we did not see a difference between the HF and AF groups in large conduit vessel blood flow, we cannot rule out differences in postprandial microvascular blood flow responses between groups.

In summary, we found that HF subjects demonstrated a greater increase in MSNA burst incidence following ingestion of a mixed meal in comparison to AF subjects. Moreover, when the MSNA responses were expressed relative to changes in plasma insulin, the HF subjects had a greater increase in MSNA for any given change in insulin. Collectively, these data suggest that, in addition to improved peripheral insulin sensitivity, endurance training may also enhance the central actions of insulin to increase MSNA following consumption of a mixed meal.

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Table 2.1 Subject Characteristics

	Average Fit	High Fit
Age (yr)	26 ± 2	29 ± 2
Height (cm)	179 ± 2	180 ± 1
Weight (kg)	75 ± 4	74 ± 2
Body Mass Index ($kg \cdot m^{2-1}$)	23 ± 1	23 ± 1
VO_{2peak} ($ml \cdot kg^{-1} \cdot min^{-1}$)	43.6 ± 1.3	65.9 ± 1.4 *
VO_{2peak} ($L \cdot min^{-1}$)	3.3 ± 0.2	4.9 ± 0.2 *
Glucose ($mg \cdot dl^{-1}$)	92.9 ± 3.7	96.7 ± 2.9
Insulin ($uIU \cdot ml^{-1}$)	3.6 ± 0.9	2.7 ± 0.3

VO2peak, peak oxygen uptake. Values are means ± SE. *P<0.05 vs. average fit.

Table 2.2 Cardiovascular and hemodynamic responses to a mixed meal

		Time					ANOVA		
		Baseline	30	60	90	120	Group	Time	Interaction
Average Fit	**SBP (mmHg)**	116 ± 3	119 ± 4	118 ± 3	118 ± 4	120 ± 3	0.824	<0.001	0.994
	DBP (mmHg)	68 ± 3	68 ± 3	68 ± 3	69 ± 3	71 ± 3	0.915	0.023	0.741
	MAP (mmHg)	84 ± 3	85 ± 2	85 ± 3	86 ± 3	87 ± 3	0.908	0.002	0.879
	HR ($b \cdot min^{-1}$)	61 ± 2	70 ± 3	68 ± 2	71 ± 2	72 ± 3	<0.001	<0.001	0.284
	Femoral blood flow ($ml \cdot min^{-1} \cdot kg^{-1}$)	29.84 ± 2.02	34.77 ± 4.07	43.30 ± 5.43	43.75 ± 6.56	39.30 ± 6.52	0.819	<0.001	0.229
	Femoral vascular conductance ($ml \cdot min^{-1} \cdot kg^{-1} \cdot mmHg^{-1}$)	0.36 ± 0.03	0.41 ± 0.05	0.52 ± 0.07	0.52 ± 0.08	0.45 ± 0.07	0.910	<0.001	0.214
High Fit	**SBP (mmHg)**	116 ± 2	120 ± 2	120 ± 2	120 ± 2	121 ± 2			
	DBP (mmHg)	69 ± 2	69 ± 2	69 ± 2	70 ± 2	70. ± 2			
	MAP (mmHg)	84 ± 2	86 ± 2	86 ± 2	86 ± 2	87 ± 2			
	HR ($b \cdot min^{-1}$)	51 ± 2	57 ± 2	56 ± 2	57 ± 2	58 ± 2			
	Femoral blood flow ($ml \cdot min^{-1} \cdot kg^{-1}$)	36.56 ± 2.91	33.28 ± 2.44	42.46 ± 5.05	41.80 ± 5.03	43.88 ± 4.81			
	Femoral vascular conductance ($ml \cdot min^{-1} \cdot kg^{-1} \cdot mmHg^{-1}$)	0.43 ± 0.03	0.39 ± 0.03	0.49 ± 0.06	0.48 ± 0.06	0.50 ± 0.05			

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; HR, heart rate. Values are means ± SE.

Table 2.3 Muscle sympathetic nerve activity (MSNA) responses to a mixed meal

		Time					ANOVA		
		Baseline	30	60	90	120	Group	Time	Interaction
Average Fit	**MSNA Burst Frequency (bursts•min^{-1})**	11 ± 2	18 ± 2	21 ± 2	20 ± 3	20 ± 2	0.199	<0.001	0.049
	MSNA Total Activity (AU•min^{-1})	293 ± 52	1096 ± 191	1261 ± 151	1138 ± 171	1124 ± 132	0.009	<0.001	<0.001
	Δ (AU•min^{-1})	0	804 ± 175	969 ± 149	845 ± 158	831 ± 139	0.004	<0.001	<0.001
	%Δ	0	335 ± 78	447 ± 95	353 ± 75	408 ± 100	0.393	<0.001	0.274
	MSNA Burst Strength (AU)	25 ± 2	62 ± 11	60 ± 0	54 ± 2	59 ± 4	0.014	<0.001	0.017
	Δ (AU)	0	37 ± 11	35 ± 3	29 ± 3	34 ± 4	0.044	<0.001	0.017
	%Δ	0	145 ± 40	139 ± 14	118 ± 13	132 ± 14	0.246	<0.001	0.049
High Fit	**MSNA Burst Frequency (bursts•min^{-1})**	12 ± 2	23 ± 1	24 ± 1	27 ± 2*	26 ± 3*			
	MSNA Total Activity (AU•min^{-1})	346 ± 49	1451 ± 144	1699 ± 155	2216 ± 250*	2238 ± 350*			
	Δ (AU•min^{-1})	0	1105 ± 143	1353 ± 128	1870 ± 220*	1892 ± 312*			
	%Δ	0	364 ± 68	426 ± 57	578 ± 94	556 ± 80			
	MSNA Burst Strength (AU)	29 ± 1	62 ± 4	70 ±3	81 ± 4*	83 ± 7*			
	Δ (AU)	0	33 ± 4	42 ± 3	53 ± 4*	55 ± 7*			
	%Δ	0	117 ± 15	147 ± 13	186 ± 19*	195 ± 29			

AU, arbitrary units. Values are means ± SE. *P<0.05 vs. average fit. MSNA total activity and burst strength are only included for completeness (see text for details).

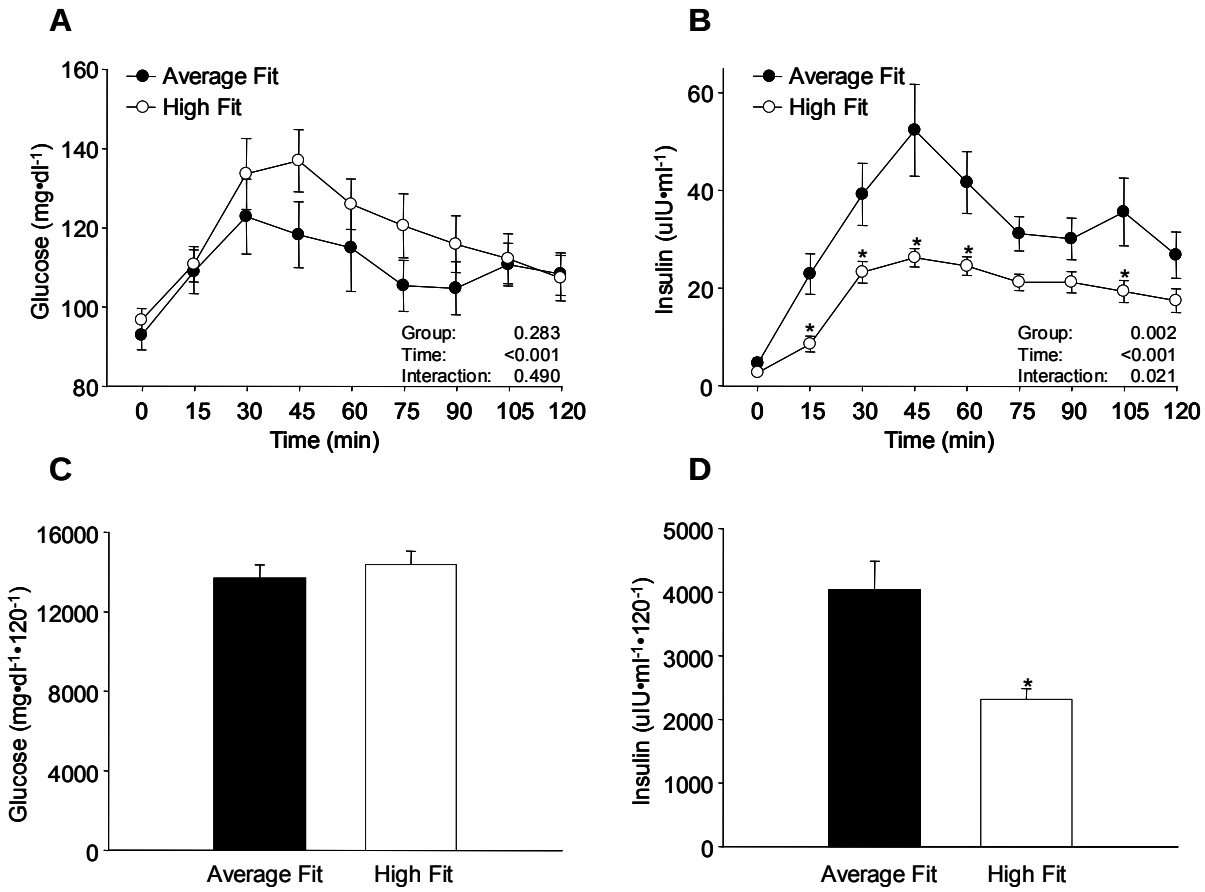

Figure 2.1 Mean plasma glucose (panel A) and insulin (panel B) at baseline (time 0) and for 120 minutes following consumption of the mixed meal in the average fit and high fit groups, as well as the area under the curve for glucose (panel C) and insulin (panel D). Values are mean ± SE. *P < 0.05 vs. average fit.

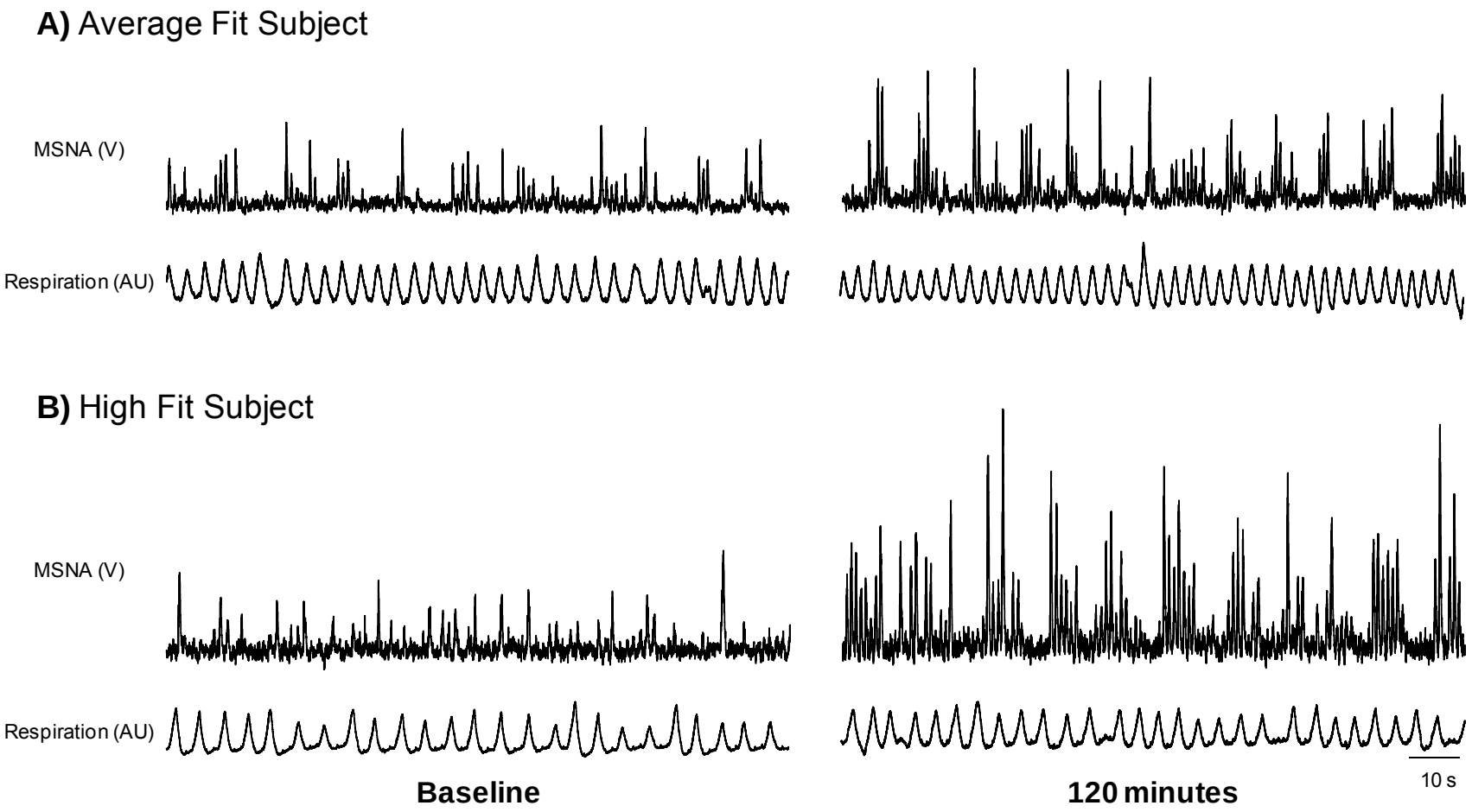

Figure 2.2 Original records from an average fit (panel A) and high fit (panel B) subject illustrating muscle sympathetic nerve activity (MSNA) and respiratory tracings at baseline and 120 minutes following consumption of the mixed meal. In response to the mixed meal, MSNA burst incidence increased 11 and 23 bursts/100 heart beats in the average fit and high fit subject, respectively. V, volts; AU, arbitrary units.

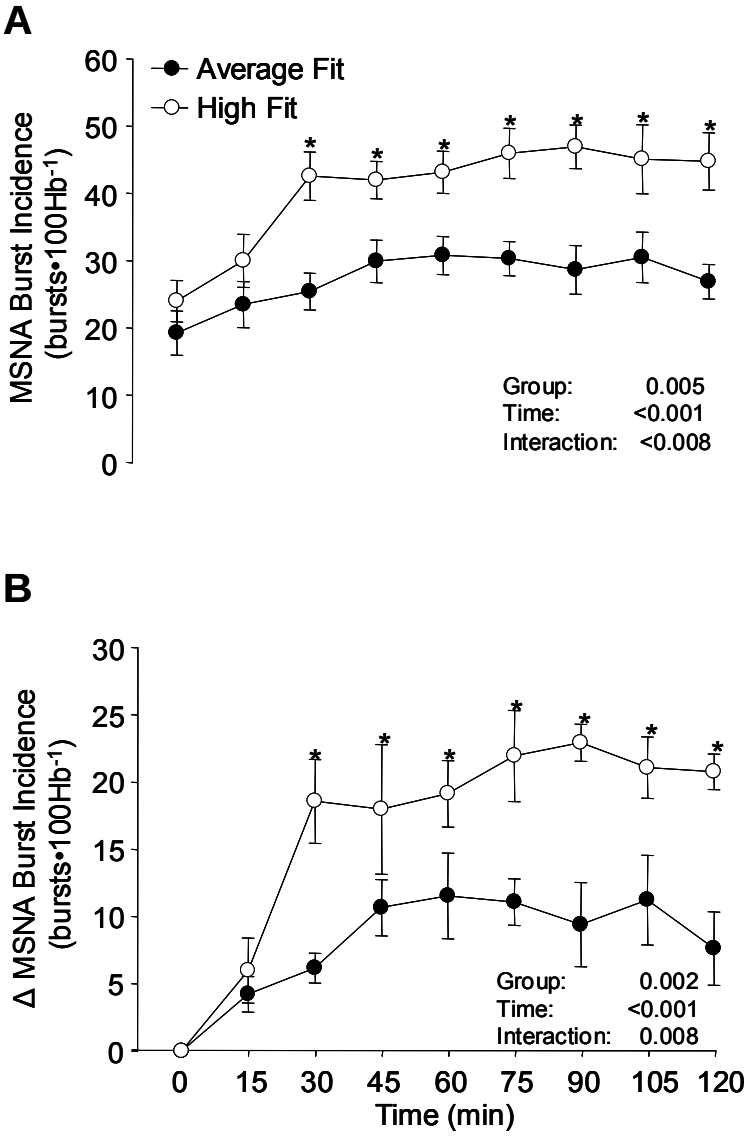

Figure 2.3 Mean muscle sympathetic nerve activity (MSNA) burst incidence responses (panel A) at baseline (time 0) and for 120 minutes following consumption of the mixed meal in the average fit and high fit groups. Panel B shows the changes in MSNA burst incidence from baseline in both groups. AU, arbitrary units; Hb, heart beats. Values are mean ± SE. *P < 0.05 vs. average fit.

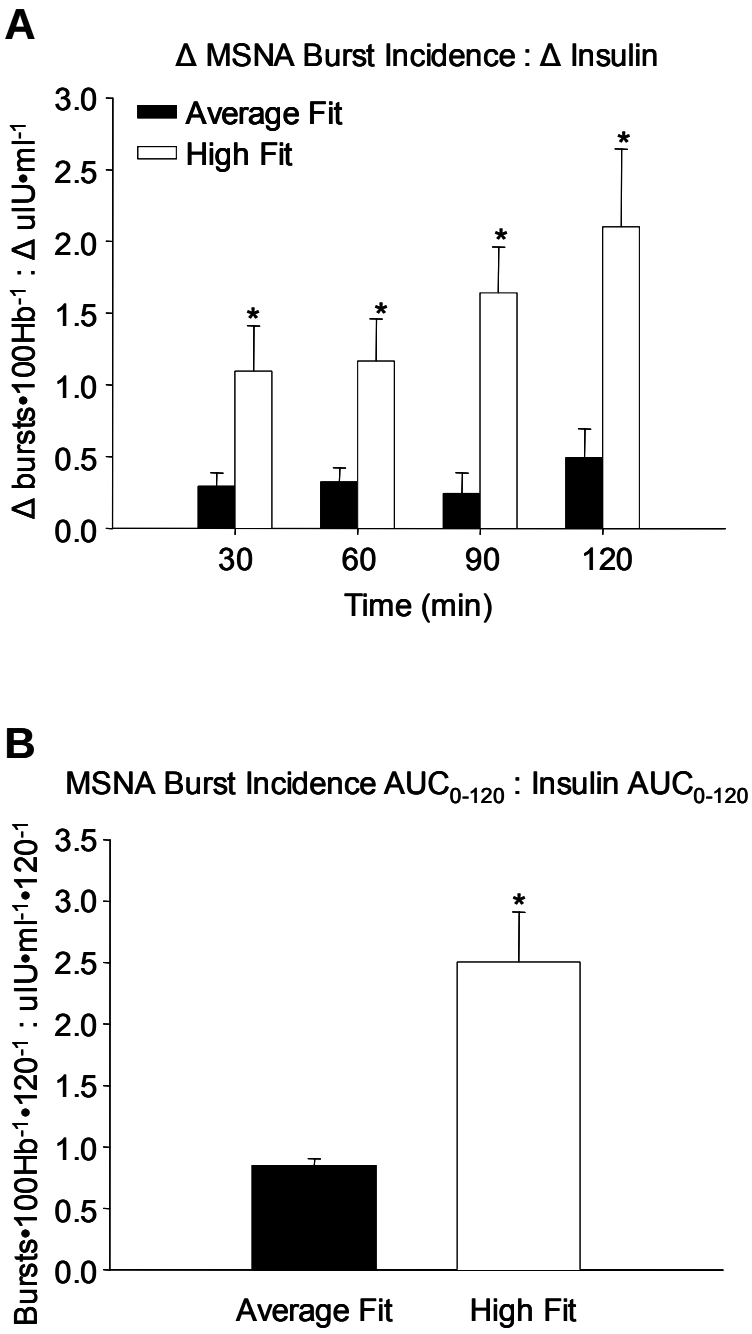

Figure 2.4 Group averages for the ratios relating changes from baseline in MSNA burst incidence (panel A) to changes in plasma insulin following mixed meal intake. Panel B shows the ratio of MSNA burst incidence area under the curve ($AUC_{0\text{-}120}$) to the insulin $AUC_{0\text{-}120}$. AU, arbitrary units; Hb, heart beats. Values are mean ± SE. *$P < 0.05$ vs. average fit.

Chapter 3.

Insulin enhances the gain of arterial baroreflex control of muscle sympathetic nerve activity in humans

Colin N. Young[1], Shekhar H. Deo[1], Kunal Chaudhary[2, 6], John P. Thyfault[3, 4, 6] & Paul J. Fadel[1, 5]

Departments of Medical Pharmacology & Physiology[1], Internal Medicine-Division of Nephrology[2], Nutrition and Exercise Physiology[3], Internal Medicine-Division of Gastroenterology and Hepatology[4], Dalton Cardiovascular Research Center[5], University of Missouri, Columbia, MO, and Harry S. Truman VA Medical Center[6], Columbia, MO.

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ABSTRACT

Recent animal studies indicate that insulin increases arterial baroreflex control of lumbar sympathetic nerve activity; however, the extent to which these findings can be extrapolated to humans is unknown. To begin to address this, muscle sympathetic nerve activity (MSNA) and arterial blood pressure (BP) were measured in 19 healthy subjects (27±1 yr) before and for 120 minutes following two common methodologies used to evoke sustained increases in plasma insulin, a mixed meal and a hyperinsulinemic euglycemic clamp. Weighted linear regression analysis between MSNA and diastolic blood pressure was used to determine the gain (i.e. sensitivity) of arterial baroreflex control of MSNA. Plasma insulin was significantly elevated within 30 minutes following meal intake (Δ34±6 uIU/ml; $P<0.05$) and remained above baseline for up to 120 minutes. Similarly, after meal intake, arterial baroreflex MSNA gain for burst incidence and total MSNA was increased and remained elevated for the duration of the protocol (e.g. burst incidence gain: -3.29±0.54 baseline vs. -5.64±0.67 120 minutes bursts/100 heart beats/mmHg; $P<0.05$). During the hyperinsulinemic euglycemic clamp, in which insulin was elevated to postprandial concentrations (Δ42±6 uIU/ml; $P<0.05$), while glucose was maintained constant, arterial baroreflex MSNA gain was similarly enhanced (e.g. burst incidence gain: -2.44±0.29 baseline vs. -4.74±0.71 120 minutes bursts/100 heart beats/mmHg; $P<0.05$). Importantly, during time control experiments, with sustained fasting insulin concentrations, the arterial baroreflex-MSNA gain remained unchanged. These findings demonstrate, for the first time in healthy humans, that increases in plasma insulin enhance the gain of arterial baroreflex control of MSNA.

INTRODUCTION

The arterial baroreflex modulates beat-to-beat oscillations in arterial blood pressure via the control of efferent sympathetic outflow to the vasculature. Due to the importance of the arterial baroreflex in the regulation of blood pressure a large body of research has been dedicated to understanding alterations in baroreflex sensitivity (i.e. gain) in physiological (e.g. exercise) as well as pathophysiological conditions[1-3]. In regards to the latter, numerous studies have indicated that hypertensive conditions are typically associated with reductions in arterial baroreflex sensitivity[4-7]. Interestingly, another common risk factor shown to be present in hypertensive patients is insulin resistance[8, 9]. In this regard, a number of recent studies have indicated that several conditions with known reductions in insulin sensitivity also demonstrate impairments in arterial baroreflex function and hypertension, including obesity[10] and the metabolic syndrome[11]. Although not causative, the concomitant occurrence of insulin resistance with reductions in arterial baroreflex function clearly suggests a link between the two that warrants investigation.

Emerging evidence from studies in experimental animal models has begun to establish a role for insulin in the central control of the arterial baroreflex. Recently, Pricher et al[12] demonstrated that increases in insulin within the brain, via lateral ventricular infusion, enhanced the gain of arterial baroreflex control of lumbar sympathetic nerve activity in rats. Other studies have indicated that neurons in central baroreflex regulatory regions (i.e. nucleus tractus solitarius) are responsive to insulin[13]. Collectively, these studies indicate that insulin can directly influence arterial baroreflex function within central neural cardiovascular control pathways. However, to date, no

work has been performed in humans evaluating the potential influence of insulin on arterial baroreflex control of sympathetic nerve activity, and therefore the extent to which these findings in experimental animals can be translated to humans remains unknown.

Therefore, the purpose of the current study was to examine a role for insulin in arterial baroreflex control of sympathetic nerve activity in humans. Although, insulin is not produced in significant amounts within the brain, increases in circulating insulin gain access to the central nervous system via transport mediated uptake across the blood brain barrier[14, 15]. Thus, we utilized two common methodologies to raise plasma insulin concentrations. First, a mixed meal was used as a physiological method to evoke increases in plasma insulin. In addition, to further isolate the influence of insulin on arterial baroreflex control we also performed hyperinsulinemic euglycemic clamps, in which insulin concentrations were elevated to a similar extent as postprandial conditions, without concomitant increases in plasma glucose. We hypothesized that increases in plasma insulin would enhance the gain of arterial baroreflex control of muscle sympathetic nerve activity in healthy humans.

METHODS

General Procedures

Nineteen healthy male subjects (age, 27 ± 1 yr; height, 180 ± 1 cm; weight, 80 ± 3 kg) volunteered for participation in these studies. No subject had a history or symptoms of cardiovascular, pulmonary, metabolic, or neurological disease and none were taking medications. Subjects were instructed to abstain from caffeinated beverages and food for 12 hours, alcohol for 24 hours, and physical activity for 48 hours prior to the experimental sessions. After receiving a detailed verbal and written explanation of the intended experimental protocol and measurements, each subject provided written informed consent. All experimental procedures and protocols conformed to the Declaration of Helsinki and were approved by the University of Missouri Health Sciences Institutional Review Board.

Experimental Measurements

Subjects were studied in the supine position at a constant ambient room temperature of 22-23 ºC. Heart rate (HR) was continuously monitored using a lead II electrocardiogram. Arterial blood pressure was measured on a beat-to-beat basis using servo-controlled finger photoplethysmography (Finometer, Finapres Medical Systems, Amsterdam, Netherlands). In addition, arterial blood pressure was measured with an automated sphygmomanometer (Welch Allyn, Skaneatles Falls, NY, USA) to confirm the finometer measurements of absolute blood pressure. Respiratory movements were monitored using a strain-gauge pneumograph placed in a stable position around the abdomen (Pneumotrace, UFI, Morro Bay, CA, USA).

Multiunit recordings of postganglionic muscle sympathetic nerve activity (MSNA) were obtained by inserting unipolar tungsten microelectrodes percutaneously through the intact, unanaesthetized skin and positioned into muscle nerve fascicles of the peroneal nerve near the fibular head. The nerve signal was processed by a pre-amplifier and an amplifier (Dept. of Bioengineering, University of Iowa, Iowa City, IA), band pass filtered (bandwidth 700 – 2000 Hz), rectified, and integrated (time constant, 0.1s) to obtain a mean voltage neurogram. MSNA recordings were identified by their characteristic pulse-synchronous burst pattern and increased neural activity in response to an end-expiratory apnea or Valsalva maneuver, without any response to arousal stimuli or stroking of the skin. MSNA was first identified by visual inspection and was then analyzed using custom designed software (MatLab, The Math Works, Natick, MA, USA), as previously described[16, 17]. The amplitude of the largest burst at baseline was assigned a value of 1000 (arbitrary units; AU) and all other bursts within a trial were normalized with respect to this value. All variables were sampled at 1000Hz and stored for off-line analysis (Chart v5.2 and Powerlab, ADInstruments, Bella Vista, NSW, Australia).

Plasma insulin and glucose were measured from venous blood samples drawn from an antecubital (Protocol 1) or a hand (Protocol 2 & 3) intravenous catheter. Insulin was determined using chemiluminescent enzyme immunoassay (Immulite 1000 Analyzer, Diagnostic Products Corp., Los Angeles, CA, USA) and glucose was determined using the glucose oxidase method (Thermo, Waltham, MA, USA or Beckman Instruments, Brea, CA, USA).

Experimental Protocols

Protocol 1: Arterial Baroreflex Control of MSNA Following a Mixed Meal

A mixed meal was utilized as a physiological method to evoke sustained increases in plasma insulin (n=12). MSNA, arterial blood pressure, heart rate and respiration were measured before and for 120 minutes following ingestion of a liquid mixed meal (Ensure Plus, Abbott Laboratories, Columbus, Ohio, USA; 57% CHO, 28% Fat, 15% Protein), corresponding to 20% of the subject's estimated energy expenditure calculated from body weight[18]. All neuro-cardiovascular variables were measured at baseline for 20 minutes and for a 5 minute period, every 30 minutes, following consumption of the mixed meal. Venous blood samples were similarly collected from an antecubital intravenous catheter and the resulting plasma was stored at -80° C for later analysis of plasma insulin and glucose.

Protocol 2: Arterial Baroreflex Control of MSNA During a Hyperinsulinemic Euglycemic Clamp

To further isolate the influence of insulin on arterial baroreflex control of MSNA, hyperinsulinemic euglycemic clamps were performed (n=8) in which insulin was elevated to postprandial concentrations while glucose was maintained constant[19]. Intravenous catheters were placed in a left antecubital vein and a right hand vein for the infusion of insulin/glucose and blood sampling, respectively. The right hand was placed in a heated box (50° C) for determination of arterialized venous blood samples[20]. Insulin (Humulin, Eli Lilly, Indianapolis, IN, USA) was diluted in 0.9% saline with 5 ml of the subject's blood and a 10 minute priming insulin infusion was followed by a constant

infusion at 30mU/m^2/minute, for a total of 120 minutes. Glucose was maintained at euglycemic concentrations throughout via a variable 20% dextrose infusion. Plasma glucose was determined every 5 minutes and plasma insulin was stored at -80° C for later analysis. MSNA, arterial blood pressure, heart rate and respiration were collected for 20 minutes at baseline and for a 5 minute period, every 30 minutes, during the hyperinsulinemic euglycemic clamp.

Protocol 3: Arterial Baroreflex Control of MSNA During Time Control Experiments

In a subset of subjects (n=4) time control experiments were also performed in which 0.9% saline was infused to match the volume administered during the hyperinsulinemic euglycemic clamp, while insulin was sustained at fasting concentrations.

Data Analysis

Baseline MSNA, arterial blood pressure and heart rate were calculated as mean values over a 6 minute period. Following consumption of the mixed meal and during the hyperinsulinemic euglycemic clamp or time controls, 3 minute averages were calculated from the 5 min data segments collected every 30 minutes. The same segments were used to evaluate arterial baroreflex control of MSNA by analyzing the relationship between spontaneously occurring fluctuations in diastolic blood pressure and MSNA, as previously described[21-24]. Briefly, the diastolic blood pressure for each cardiac cycle within a data collection period was grouped into 3 mmHg pressure bins. The burst

incidence within each pressure bin was calculated by determining the percentage of diastoles that were associated with a burst of MSNA and expressed as bursts/100 heartbeats. In addition, total MSNA was determined for each pressure bin by calculating the total area of all MSNA bursts, relative to the number of cardiac cycles, and expressed as arbitrary units (AU)/beat. The slope of the relationship between MSNA variables and diastolic blood pressure was identified using linear regression analysis (SPSS v17.0, SPSS Inc, Chicago, IL, USA), with a minimum r value of 0.5 used as a criteria for accepting slopes. The mean r value for all of the time points during each protocol was: Protocol 1 - 0.88 ± 0.01 (Range: 0.56-0.99), Protocol 2 - 0.90 ± 0.01 (Range: 0.78-0.99) and Protocol 3 - 0.86 ± 0.21 (Range: 0.74-0.98), indicating adequate fit of the linear regression analyses. All data were weighted to account for the number of cardiac cycles within each pressure bin; thus removing bias due to bins containing a small number of cardiac cycles. The diastolic blood pressure range used for the linear regression analyses was approximately 20 mmHg under resting conditions (mixed meal: 21 ± 1, hyperinsulinemic euglycemic clamp: 21 ± 2, time control: 19 ± 1) and importantly the range was the same at all time points examined. The slope of the relationship between spontaneous fluctuations in MSNA burst incidence and diastolic blood pressure was recently shown to be highly correlated with slopes derived using the more invasive modified Oxford approach for assessing arterial baroreflex-MSNA gain (i.e. bolus sodium nitroprusside and phenylephrine)[25]. In addition, van Schelven et al[26] have demonstrated that arterial baroreflex burst incidence gain was not altered from baseline during steady state nitroprusside infusions in which MSNA was robustly increased. Collectively, these data highlight the usefulness of the burst incidence-diastolic blood

pressure measures in assessing arterial baroreflex function; particularly in conditions in which multiple repeated measures are needed, such as in the current study following mixed meal intake and during a 2 hour hyperinsulinemic euglycemic clamp.

Statistical Analysis

Univariate repeated measures ANOVA was used and significant main effects were evaluated with Bonferroni post-hoc analyses when appropriate. Statistical significance was set at $P < 0.05$. Results are presented as mean ± standard error (SE).

RESULTS

Increased arterial baroreflex MSNA gain following a mixed meal

Mixed meal intake induced a significant rise in plasma insulin, as well as plasma glucose (Figure 3.1). The arterial baroreflex gain of MSNA burst incidence (Figure 3.2A) was significantly enhanced (i.e. more negative) within 30 minutes (Δ-1.91±0.53 bursts/100 heart beats/mmHg) and remained elevated for the duration of the study (e.g. 120 minutes: Δ-1.82±0.37 bursts/100 heart beats/mmHg). Similarly, arterial baroreflex control of total MSNA (Figure 3.2B) was increased at 30 minutes, and thereafter remained above baseline up to 120 minutes.

Increased arterial baroreflex MSNA gain during a hyperinsulinemic euglycemic clamp

During the hyperinsulinemic euglycemic clamp, plasma insulin was increased similarly to after the mixed meal; however, euglycemia was maintained throughout at fasting concentrations (Figure 3.1). Importantly, in line with the findings from the mixed meal, robust increases in the gain of arterial baroreflex control of MSNA burst incidence (e.g. 120 minutes: Δ-2.30±0.62 bursts/100 heart beats/mmHg) and total MSNA were observed with the concomitant increase in plasma insulin. In contrast, during the time control experiments, in which plasma insulin and glucose were not changed (data not shown), the gain of the arterial baroreflex was not different from baseline at any time point (Figure 3.3).

MSNA and cardiovascular parameters

As anticipated, following the mixed meal and during the hyperinsulinemic euglycemic clamp, MSNA burst frequency, burst incidence and total MSNA were increased within 30 minutes and remained above baseline for the remainder of the protocol. Systolic and diastolic blood pressures, as well as heart rate, were slightly, but significantly increased following consumption of the mixed meal. Arterial blood pressure and heart rate remained unchanged during the hyperinsulinemic euglycemic clamp and time control experiments (Table 3.1).

DISCUSSION

The primary novel finding of this investigation is that increases in plasma insulin enhanced the gain of arterial baroreflex control of MSNA. Indeed, physiological elevations in plasma insulin following the mixed meal were associated with an increased slope of the relationship between diastolic blood pressure and MSNA. Furthermore, during the hyperinsulinemic euglycemic clamp, in which insulin was increased to postprandial concentrations, while glucose was maintained at euglycemia, arterial baroreflex control of MSNA burst incidence was also augmented. Overall, these findings strongly support a role for insulin in the modulation of the sympathetic arterial baroreflex in healthy humans.

In addition to the well described sympathoexcitatory effect(s) of insulin[27, 28], recent findings obtained in animals illustrate a role for insulin in the central modulation of the arterial baroreflex[12]. During lateral ventricular infusion of insulin in rats, robust increases in the gain of arterial baroreflex control of lumbar sympathetic nerve activity have been demonstrated; illustrating that acute increases in insulin within the brain modulate arterial baroreflex control of sympathetic outflow[12]. The findings from the current study support and extend these findings by demonstrating for the first time in healthy humans that acute increases in insulin are associated with an enhanced arterial baroreflex MSNA gain. Importantly, the enhanced sympathetic arterial baroreflex control was noted during modest increases in plasma insulin under both postprandial (mixed meal) and experimental (insulin clamp) conditions; supporting a role for insulin within a normal physiological range in the modulation of the arterial baroreflex.

Insulin is not produced in large quantities in the central nervous system; however plasma insulin gains access to the brain via saturable transport-mediated uptake across the blood brain barrier[14, 15]. As such, circulating insulin can influence arterial baroreflex control via central neural pathways and indeed, insulin receptors are present in numerous, yet distinct cardiovascular regulatory regions, including hypothalamic and brainstem regions[29, 30]. In this context, Schwartz et al[31] have previously demonstrated in dogs that cerebrospinal fluid concentrations of insulin are increased within 30 minutes after systemic infusion of insulin; in line with our finding of an increase in arterial baroreflex MSNA gain within 30 minutes after the mixed meal or during the hyperinsulinemic euglycemic clamp. Moreover, the half life of insulin within cerebrospinal fluid has been reported to be approximately 140 minutes[32], which may explain the sustained enhancement in arterial baroreflex control of MSNA demonstrated in the current study.

Interestingly, the increase in arterial baroreflex MSNA gain was noted for the relationships between diastolic blood pressure and both, MSNA burst incidence as well as total MSNA. In this regard, previous investigations have suggested that the occurrence of a sympathetic burst (incidence) and the area of a burst may reflect distinct central sites involved in arterial baroreflex control of MSNA[21, 22]. The current data demonstrate that during elevations in insulin, for a given change in diastolic blood pressure there is a greater change in MSNA burst incidence and total MSNA (area/beat), relative to fasting conditions. As such, our findings suggest that insulin may influence central pathways involved in both the occurrence of a burst of MSNA as well as the size of a given burst. Due to the peripheral vasodilatory effect of insulin, the physiological significance of an enhanced arterial baroreflex control over the occurrence and size of a

burst of MSNA may provide optimal protection of arterial blood pressure during acute elevations in plasma insulin. Interestingly, it should be noted that arterial blood pressure was well maintained throughout the mixed meal and hyperinsulinemic euglycemic clamp in the present study. Furthermore, autonomic failure patients demonstrate robust falls in arterial blood pressure after meal consumption[33], illustrating the importance of the sympathetic nervous system in the maintenance of blood pressure during periods of increased circulating insulin.

Of note, although we found an increase in arterial baroreflex gain for the control of MSNA burst occurrence and area (i.e. total MSNA), methodological considerations of the use of MSNA burst incidence, in comparison to total MSNA, to derive spontaneous baroreflex measures is warranted. In this regard, previous findings have demonstrated that the gain of arterial baroreflex control of MSNA burst incidence was similar to baroreflex sensitivities obtained using pharmacological manipulations of arterial blood pressure[25, 26]; demonstrating the usefulness of these measurements. In contrast, spontaneous arterial baroreflex gains calculated from measurements using MSNA burst area (e.g. total MSNA) have been suggested to be influenced more by non baroreflex inputs[22, 25], which may limit the interpretation of such measures. Indeed, several studies have reported weak relationships between burst area and diastolic blood pressure, whereas burst incidence consistently demonstrates strong relationships with diastolic blood pressure[21-23, 25, 34]. Thus, although robust increases in arterial baroreflex control of total MSNA were observed following the mixed meal and during the hyperinsulinemic euglycemic clamp, we focused our interpretation on baroreflex measures of burst incidence.

From these human studies, although we cannot determine the precise region(s) of insulin action on arterial baroreflex control of MSNA, several areas are worthy of consideration. The aforementioned work in rats using lateral ventricular infusion of insulin suggests a hypothalamic region, such as the paraventricular nucleus, may play a major role[12]. In addition, neurons from brainstem regions involved in arterial baroreflex afferent processing (e.g. nucleus tractus solitarius) are responsive to insulin[13]. Furthermore, neural projections from the circumventricular organs, which lack a blood brain barrier, could also impact arterial baroreflex function during elevations in plasma insulin[15]. Indeed, the investigation of insulin effects on arterial baroreflex control of sympathetic outflow is in its infancy; however this area undoubtedly deserves further attention.

Perspectives

A number of conditions are characterized by reductions in arterial baroreflex gain, including hypertension[4-7], obesity[10], metabolic syndrome[11] and pregnancy[35, 36]. In addition, emerging evidence indicates that insulin resistant states are accompanied by reduced cerebrospinal fluid insulin concentrations, likely emanating from an attenuated transport of insulin into the central nervous system[35-40]. In line with this, Brooks and colleagues have advanced the hypothesis that a certain level of insulin within the brain is essential for normal arterial baroreflex function[12]. The present study lends support to this growing body of literature by demonstrating for the first time that insulin can modulate the sympathetic arterial baroreflex in healthy humans. However, whether chronic alterations in insulin transport and/or signaling in the central nervous system contribute to

a decreased sympathetic arterial baroreflex gain in insulin resistant conditions remains to be determined. Given the importance of the arterial baroreflex in the regulation of arterial blood pressure, clearly future studies, from experimental animals to humans, are warranted to delineate the precise neural pathways and mechanism(s) of insulin action on neurocardiovascular control in health and disease.

In summary, for the first time, we found that physiological increases in plasma insulin following a mixed meal and during a hyperinsulinemic euglycemic clamp enhanced the gain of arterial baroreflex control of MSNA in humans. Collectively, these findings extend recent studies in animals and strongly support a role for insulin in the modulation of the sympathetic arterial baroreflex.

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Table 3.1 Sympathetic nerve activity and cardiovascular responses to a mixed meal, hyperinsulinemic euglycemic clamp and time control experiments

		Time				
		Baseline	30	60	90	120
Mixed Meal	MSNA Burst Frequency (bursts/min)	12 ± 2	19 ± 2*	22 ± 2*	23 ± 2*	23 ± 2*
	MSNA Burst Incidence (bursts/100Hb)	21 ± 3	29 ± 3*	34 ± 3*	34 ± 4*	32 ± 3*
	Total MSNA (AU/beat)	32 ± 4	49 ± 5*	64 ± 6*	66 ± 11*	64 ± 10*
	Heart Rate (beats/min)	59 ± 2	67 ± 3*	66 ± 2*	68 ± 2*	70 ± 3*
	Systolic Blood Pressure (mmHg)	116 ± 2	119 ± 3	119 ± 3	118 ± 2	120 ± 3*
	Diastolic Blood Pressure (mmHg)	69 ± 2	70 ± 2	70 ± 3	71 ± 3	72 ± 2*
Hyperinsulinemic Euglycemic Clamp	MSNA Burst Frequency (bursts/min)	11 ± 2	17 ± 2*	19 ± 2*	20 ± 2*	23 ± 2*
	MSNA Burst Incidence (bursts/100Hb)	18 ± 3	26 ± 3*	31 ± 3*	32 ± 3*	36 ± 3*
	Total MSNA (AU/beat)	30 ± 5	45 ± 6*	55 ± 7*	54 ± 7*	74 ± 8*
	Heart Rate (beats/min)	61 ± 3	65 ± 3	62 ± 3	64 ± 3	65 ± 4
	Systolic Blood Pressure (mmHg)	114 ± 3	114 ± 3	115 ± 3	112 ± 3	113 ± 3
	Diastolic Blood Pressure (mmHg)	65 ± 2	64 ± 1	64 ± 1	62 ± 3	61 ± 2
Time Control	MSNA Burst Frequency (bursts/min)	12 ± 2	13 ± 1	11 ± 2	10 ± 2	12 ± 2
	MSNA Burst Incidence (bursts/100Hb)	21 ± 2	24 ± 3	19 ± 4	19 ± 6	20 ± 3
	Total MSNA (AU/beat)	34 ± 5	35 ± 5	30 ± 8	27 ± 9	31 ± 6
	Heart Rate (beats/min)	57 ± 4	56 ± 3	57 ± 5	57 ± 4	57 ± 3
	Systolic Blood Pressure (mmHg)	111 ± 2	110 ± 2	110 ± 2	112 ± 1	112 ± 1
	Diastolic Blood Pressure (mmHg)	63 ± 2	64 ± 5	63 ± 3	64 ± 4	62 ± 1

MSNA, muscle sympathetic nerve activity; Hb, heart beats; AU, arbitrary units. Values are means ± SE. *P<0.05 vs. baseline.

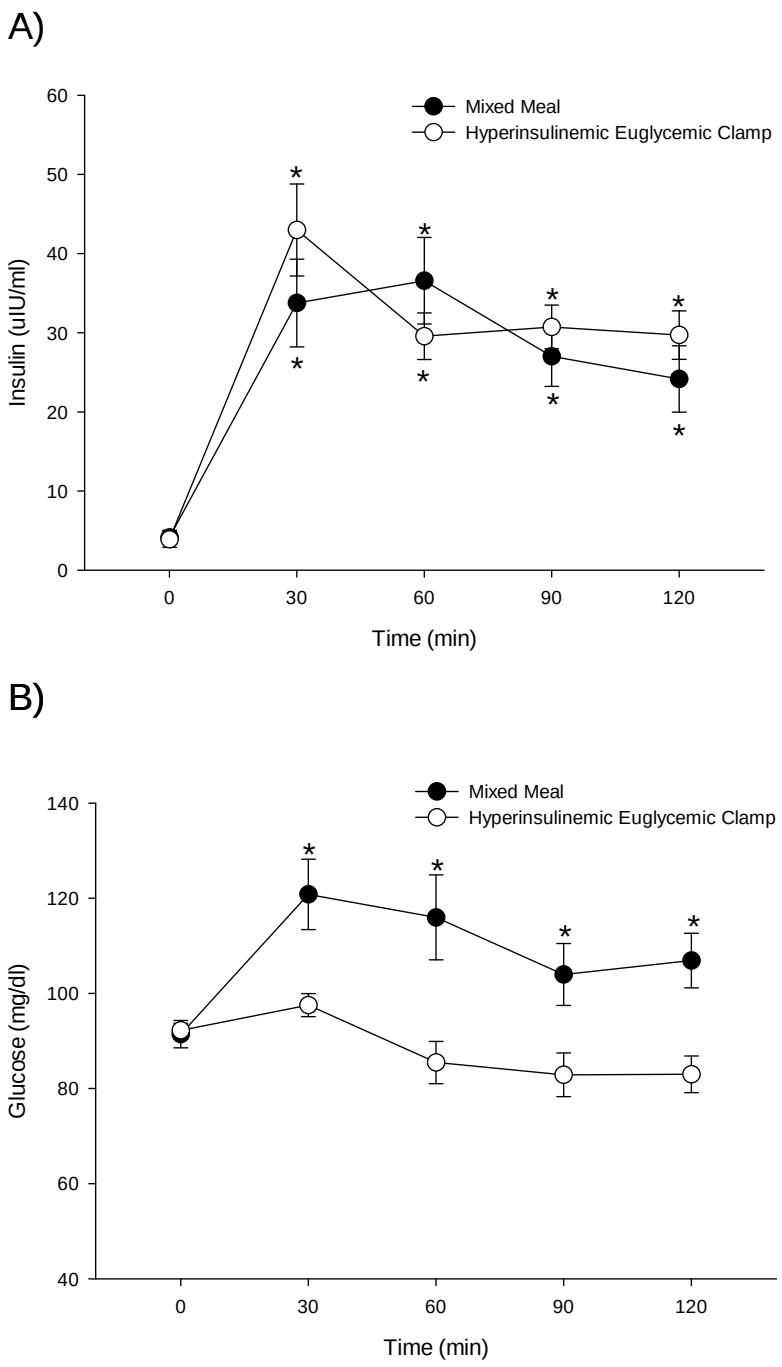

Figure 3.1 Mean plasma insulin (panel A) and glucose concentrations (panel B) at baseline (time 0) and for 120 minutes following consumption of the mixed meal and during the hyperinsulinemic euglycemic clamp.

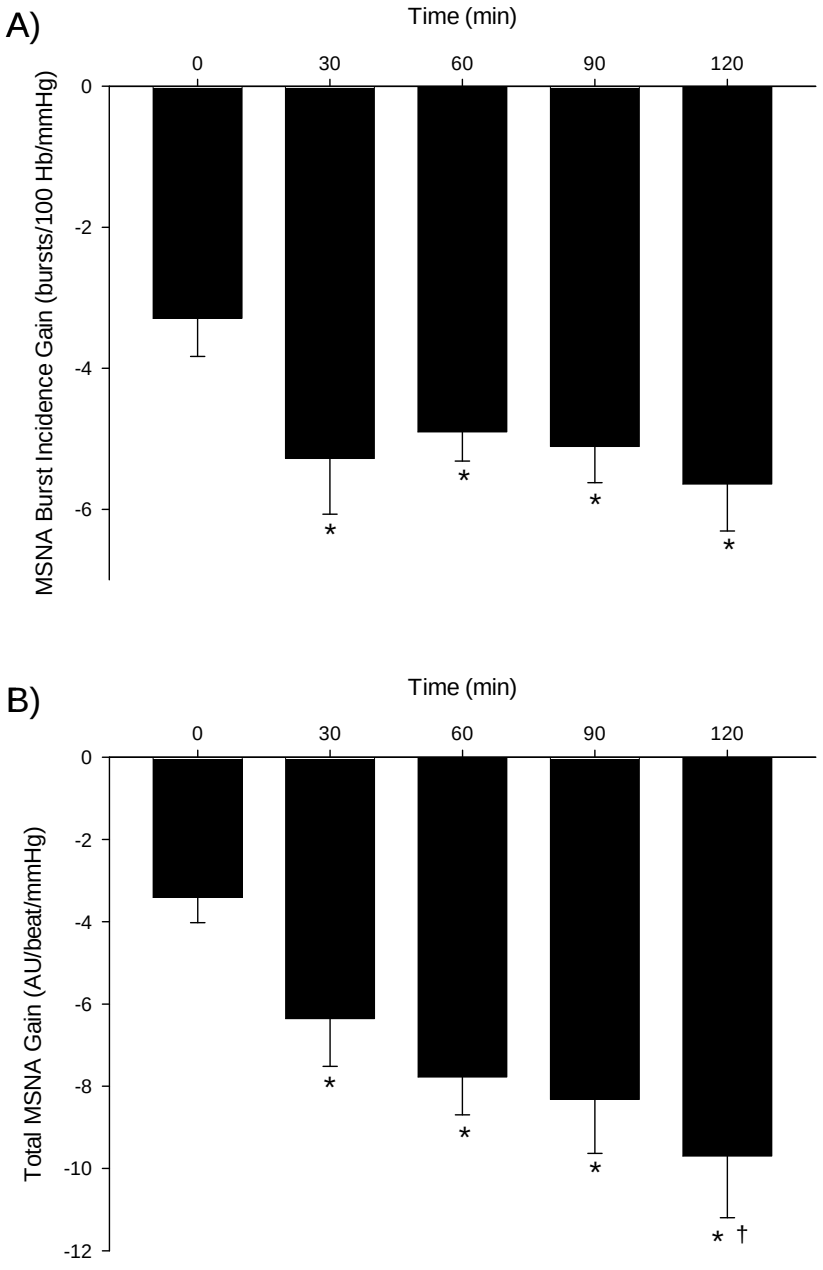

Figure 3.2 Summary data illustrating the gain of arterial baroreflex control of muscle sympathetic nerve activity (MSNA) burst incidence (panel A) and total MSNA (panel B) at baseline (time 0) and for 120 minutes following consumption of the mixed meal. Hb, heart beats; AU, arbitrary units. *P<0.05 vs. baseline. †P<0.05 vs. 30 minutes.

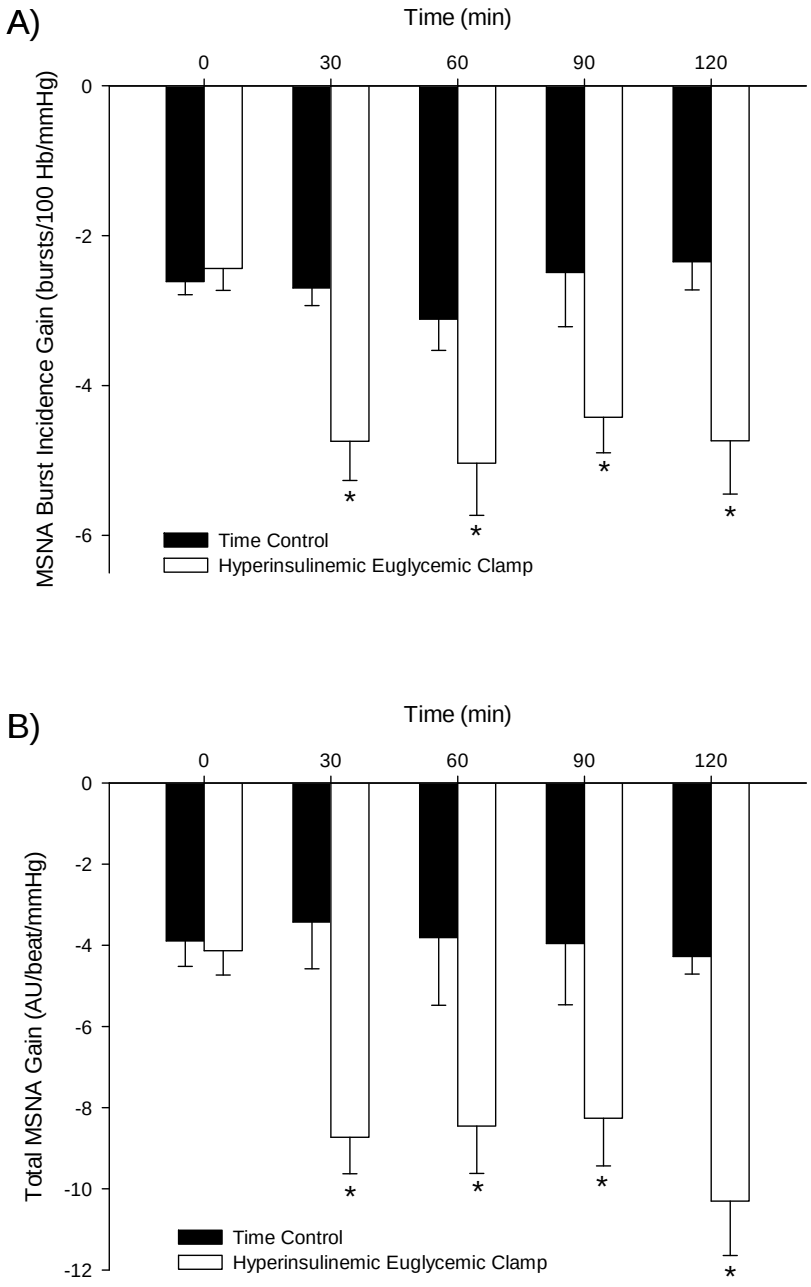

Figure 3.3 Summary data illustrating the gain of arterial baroreflex control of muscle sympathetic nerve activity (MSNA) burst incidence (panel A) and total MSNA (panel B) at baseline (time 0) and for 120 minutes during the hyperinsulinemic euglycemic clamp and time control experiments. Hb, heart beats; AU, arbitrary units. *P<0.05 vs. baseline.

Chapter 4.

Arterial baroreflex control of muscle sympathetic nerve activity in patients with type II diabetes

Colin N. Young[1,], Shekhar H. Deo[1], James P. Fisher[5], Joseph W. LeMaster[2], Abdullah Hanna-Moussa[3], Uzma Z. Khan[3] & Paul J. Fadel[1, 4]

Departments of Medical Pharmacology & Physiology[1], [2]Family and Community Medicine, [3]Internal Medicine, [4]Dalton Cardiovascular Research Center, University of Missouri, Columbia, MO; School of Sport and Exercise Sciences, [5]University of Birmingham, Birmingham, UK.

In Preparation

ABSTRACT

Type II diabetes is associated with acute and chronic alterations in the regulation of arterial blood pressure. However, whether impairments in arterial baroreflex control of sympathetic nerve activity contribute to alterations in blood pressure control in diabetic patients remains unclear. To begin to address this question, muscle sympathetic nerve activity (MSNA), and arterial blood pressure were continuously recorded in 8 type II diabetic patients and 6 healthy age, sex and body weight-matched control subjects during intravenous bolus injections of sodium nitroprusside followed 60s later by phenylephrine hydrochloride. The gain (i.e. sensitivity) of arterial baroreflex-MSNA control was identified from the linear relationships between total MSNA and diastolic blood pressure. The overall arterial-MSNA gain, which encompasses the MSNA responses to both a fall and rise in arterial blood pressure, was similar between the two groups (-6.96±1.17 diabetics vs. -6.54±1.17 controls, AU/beat/mmHg; P=0.45). Similarly, when the responses were examined separately for falls (-8.87±1.37 diabetics vs. -8.58±1.74 controls, AU/beat/mmHg; P=0.22) and rises (-6.57±1.24 diabetics vs. -6.49±1.13 controls, AU/beat/mmHg; P=0.44) in arterial blood pressure, no differences in arterial baroreflex control of MSNA were noted. These preliminary data suggest that patients with type II diabetes exhibit preserved arterial baroreflex control of sympathetic nerve activity.

INTRODUCTION

Arterial baroreceptors originating in the carotid artery and the aorta play a pivotal role in the rapid reflex adjustments that accompany acute cardiovascular stressors[1, 2]. Indeed, the ability to withstand acute falls and rises in blood pressure is dependent on a properly functioning arterial baroreflex[2]. In particular, arterial baroreflex control of sympathetic nerve activity to the peripheral vasculature is crucial for normal control of blood pressure[3-5]. Although previous findings have indicated that patients with type II diabetes mellitus exhibit postural hypotension[6-8], greater blood pressure reactivity to acute cardiovascular stressors[9-11], and an increased risk for the development of hypertension[12], the extent to which impairments in arterial baroreflex regulation contribute to these alterations in blood pressure control remains unclear.

Recent findings from experimental animal models of type II diabetes have provided insight into arterial baroreflex regulation of sympathetic nerve activity. In this regard, studies in the obese Zucker rat have demonstrated an impaired arterial baroreflex control of splanchnic sympathetic nerve activity[13, 14]. While adult obese Zucker rats do exhibit characteristics of type II diabetes, such as hyperinsulinemia and hyperglycemia, they are often hypertensive[15]. Therefore, the extent to which these findings in experimental animals can be translated to normotensive type II diabetic patients remains unknown. Investigations reporting a diminished increase in plasma norepinephrine, in response to standing, in diabetic patients suggests an impairment in the sympathetic arc of the baroreflex[16]. However, to date, no studies have directly examined arterial baroreflex control of sympathetic nerve activity in type II diabetes patients.

A further consideration when examining the arterial baroreflex, is the inherent asymmetry in the baroreflex-mediated responses to falls and rises in arterial blood pressure (i.e. hysteresis). In this context, differences in sympathetic baroreflex gain during increases versus decreases in blood pressure have been recently described in healthy adults[17]. As such, it appears necessary to account for the direction of pressure change when examining the sympathetic baroreflex in type II diabetic patients. Indeed, group differences in arterial baroreflex-mediated responses could potentially be masked by combining pressure falls and rises[17, 18].

With this background in mind, the aim of the current study was to characterize sympathetic and cardiovagal arterial baroreflex control in patients with type II diabetes. Pharmacological induced decreases and increases in arterial blood pressure were used as a robust stimulus while the resultant changes in muscle sympathetic nerve activity (MSNA) and cardiac interval were assessed. Furthermore, given that type II diabetic patients exhibit alterations in blood pressure control during both falls and rises in blood pressure, a secondary aim was to investigate baroreflex-mediated responses during directional changes in blood pressure separately. We hypothesized that type II diabetic patients would exhibit a decreased sympathetic and cardiovagal arterial baroreflex gain to both increases and decreases in arterial blood pressure, when compared to healthy age, sex and body weight matched control subjects.

METHODS

To date, 8 type II diabetic patients and 6 healthy age, sex and body weight matched control subjects have been studied. Diabetic subjects were all normotensive and were not being treated for hypertension, were free of signs of neuropathy, and had no history of cardiovascular disease. Patients were on a stable drug regimen for at least 3 months prior to participating in the study which included biguanides (n=7), sulfonylureas (n=5), insulin (n=4), incretin mimetic (n=1), statins (n=7), angiotensin converting enzyme inhibitor (n=1) and hydrochlorothiazide (n=2). All medications were withheld for a minimum of 12 hours prior to the experimental visit. All experimental procedures and protocols were approved by the University of Missouri Health Sciences Institutional Review Board and each subject provided written informed consent prior to participation.

Experimental Measurements

Studies were performed at an ambient room temperature of 22-24 ºC with external stimuli minimized. Heart rate was continuously monitored using a lead II electrocardiogram (Quinton Q710, Bothell, WA, USA). Beat-to-beat arterial blood pressure was obtained using servo-controlled finger photoplethysmography (Finometer, Finapres Medical Systems, Amsterdam, Netherlands). In addition, an automated sphygmomanometer (Welch Allyn, Skaneatles Falls, NY, USA) was used to measure arterial blood pressure by auscultation of the brachial artery and to confirm Finometer measurements. Respiratory movements were monitored using a strain-gauge pneumograph placed in a stable position around the abdomen (Pneumotrace, UFI, Morro Bay, CA, USA). Multiunit recordings of postganglionic MSNA were obtained as

described previously[19-21]. Briefly, a unipolar tungsten microelectrode was inserted percutaneously through the intact, unanaesthetized skin and positioned into muscle nerve fascicles of the peroneal nerve near the fibular head of the left leg. Postganglionic sympathetic action potentials were amplified, filtered (bandwidth 700 – 2000 Hz), rectified, and integrated (time constant, 0.1 s) to obtain a mean voltage neurogram (Dept. of Bioengineering, University of Iowa, Iowa City, IA). MSNA recordings were identified by their characteristic pulse-synchronous burst pattern and increased neural activity in response to an end-expiratory apnea or Valsalva maneuver, without any response to arousal stimuli or stroking of the skin[22-24]. Heart rate, arterial blood pressure, respiration and MSNA were sampled at 1000Hz and stored for off-line analysis (Chart v5.2 and Powerlab, ADInstruments, Bella Vista, NSW, Australia).

Experimental Protocol

Subjects were instructed to refrain from food and caffeinated beverages for 12 hours and strenuous physical activity and alcohol for 24 hours prior to reporting to the laboratory. Subjects were placed in the supine position on a medical examination table and an intravenous catheter was placed in an antecubital vein. Fasting blood samples were drawn for the measurement of triglycerides, cholesterol, lipoproteins, glucose and insulin. Following instrumentation for measures of heart rate, arterial blood pressure, respiration and MSNA, baseline variables were collected for a period of 20 minutes. Subsequently, sympathetic arterial baroreflex control was investigated using the modified Oxford technique[25]. MSNA, heart rate and blood pressure were continuously recorded while rapid pharmacologically-induced changes in blood pressure were elicited using

intravenous injections of sodium nitroprusside (100 µg) to lower blood pressure approximately 15 mmHg, followed 60 seconds later by phenylephrine hydrochloride (150-175 µg) to raise blood pressure above baseline levels. Two to three trials were performed for each subject with a minimum of 15 minutes between trials to allow for re-establishment of baseline cardiovascular and sympathetic variables.

Data Analysis

MSNA was first identified by visual inspection and was then analyzed using custom designed software (MatLab, The Math Works, Natick, MA, USA), as previously described[21, 26]. The amplitude of the largest burst at baseline was assigned a value of 1000 (arbitrary units; AU) and all other bursts within a trial were normalized with respect to this value. A 10 minute segment was used for the calculation of baseline variables.

Sympathetic Arterial Baroreflex Gain

Arterial baroreflex control of MSNA was determined by examining the relationship between MSNA and diastolic blood pressure (DBP) during the bolus drug infusions. DBP was chosen, as it has been shown to relate most closely with MSNA. Each modified Oxford trial was analyzed in 3 segments[17, 27]: First, overall arterial baroreflex gain was determined from the point at which blood pressure decreased following infusion of sodium nitroprusside until the blood pressure peak following phenylephrine hydrochloride infusion; thus encompassing the MSNA response to both a decrease and an increase in arterial blood pressure. The sympathetic responses to acute hypotension and hypertension were also separately examined using the segments from

the onset of the blood pressure drop after nitroprusside infusion until the nadir of the blood pressure change, and from the lowest blood pressure after phenylephrine administration until the blood pressure peak, respectively. The DBP within each segment was grouped into 3 mmHg pressure bins and total MSNA was determined within each pressure bin by calculating the total area of all MSNA bursts, relative to the number of cardiac cycles, and expressed as arbitrary units (AU) / beat. All data 3 mmHg above the greatest pressure associated with a burst of MSNA were excluded from analysis in order to ensure that the derived baroreflex sensitivity reflected primarily the linear portion of the arterial baroreflex curve. The slope of the relationship between MSNA and DBP was identified using linear regression analysis (SPSS v17.0, SPSS Inc, Chicago, IL, USA) and used as a calculation of sympathetic arterial baroreflex gain. A minimum r value of 0.5 was used as a criterion for accepting slopes. All data were weighted to account for the number of cardiac cycles within each pressure bin; thus removing bias due to bins containing a small number of cardiac cycles. Each modified Oxford trial was analyzed separately for each individual and then the gains from each trial were averaged together to provide a mean for each subject.

Statistical Analysis

Group comparisons for baseline variables, as well as sympathetic arterial baroreflex gains derived from the bolus drug infusions for the overall, falls and rises in blood pressure were compared using unpaired Student t-tests. Statistical significance was set at $P<0.05$ and results are presented as means ± standard error (SE).

RESULTS

The type II diabetic patients were similar to the control subjects for age, height, weight and body mass index. As anticipated, plasma glucose and hemoglobin A_{1c} were higher in the diabetic patients. In addition, fasting insulin tended to be higher in the patient group (P=0.12) whereas cholesterol, lipoproteins and triglycerides were not different between the type II diabetic patients and healthy controls (Table 4.1).

Baseline heart rate, systolic, diastolic and mean arterial blood pressures were the same between groups. In addition, MSNA burst frequency and burst incidence indicated similar resting sympathetic nerve activity between the type II diabetic patients and healthy age, sex and body weight matched control subjects (Table 4.2).

Sympathetic Baroreflex Gain

Original relationships illustrating the derived arterial baroreflex-MSNA gains for one diabetic subject and a matched control subject are presented in Figure 4.1. The overall baroreflex gain during the bolus drug infusions, which encompasses the MSNA responses to both a fall and rise in arterial blood pressure, indicated similar sympathetic arterial baroreflex sensitivity in the type II diabetic subjects when compared to the control group. Furthermore, the sympathetic baroreflex gains during the acute decreases and increases in blood pressure were similar between groups (Figure 4.2).

DISCUSSION

The purpose of the present study was to examine arterial baroreflex control of sympathetic nerve activity in patients with type II diabetes. Contrary to our hypothesis, a comparable overall sympathetic arterial baroreflex gain was found between diabetic patients and healthy control subjects. These similarities in arterial baroreflex-MSNA gain were also evident when the responses were examined to falls and rises in arterial blood pressure separately. These preliminary findings indicate that arterial baroreflex control of MSNA is well preserved in type II diabetic subjects.

Type II diabetes is associated with acute and chronic alterations in the regulation of arterial blood pressure[6-12], although the underlying mechanisms remain incompletely defined. Indeed, during acute postural challenges, robust falls in blood pressure have been documented in diabetic patients[6-12]. In addition, type II diabetic patients exhibit an exaggerated blood pressure response to physiological stressors, such as exercise[9-11], and are highly prone to develop hypertension[12]. Given that the arterial baroreflex is crucial for beat-to-beat and potentially long term regulation of arterial blood pressure, we reasoned that arterial baroreflex control of MSNA would be impaired in the presence of type II diabetes. Interestingly, we found, that when the system was stressed across a wide range of pressures, overall arterial baroreflex-MSNA gain appeared normal in otherwise healthy, normotensive type II diabetic patients.

To date, limited information exists on arterial baroreflex control of sympathetic outflow in type II diabetic conditions. Findings in the obese Zucker rat, a commonly used model of type II diabetes, have indicated an impaired arterial baroreflex control of splanchnic sympathetic nerve activity[13, 14]. In these previous studies, the greatest

decrement in arterial baroreflex function was noted in adult rats, when overt diabetes, obesity and hypertension are present. Although, impairments in arterial baroreflex control were also noted in juvenile obese Zucker rats before the development of hypertension, comparisons of these animals were made to lean controls. Therefore, whether the reported decrease in arterial baroreflex control of sympathetic nerve activity was due to obesity or metabolic disturbances is unclear. To the best of our knowledge, this is the first study in type II diabetic humans utilizing direct recordings of efferent sympathetic outflow to examine the sympathetic arterial baroreflex. Our two subject groups were well matched, except for the occurrence of type II diabetes; strengthening the conclusion that type II diabetes alone does not alter arterial baroreflex control of MSNA. However, care should be taken when extending these findings to patients with a longer duration of diabetes, or conditions in which type II diabetes and other comorbidities (i.e. hypertension) concomitantly exist.

Although it was originally proposed that asymmetry did not exist in the sympathetic arm of the baroreflex[28], a recent reexamination by Taylor and colleagues[17] has demonstrated hysteresis in arterial baroreflex-MSNA gain during the modified oxford technique. Therefore, in the present study, we considered the baroreflex-mediated sympathetic responses to nitroprusside and phenylephrine separately. In support of the findings for overall gain, we found no difference in arterial baroreflex control of MSNA during acute falls and rises in pressure between the type II diabetic patients and healthy controls. This is an important consideration, given that differences in arterial baroreflex function could potentially be masked if the direction of pressure change is not taken into account[17, 18]. Indeed, Studinger et al[17] reported that age-related differences in arterial

baroreflex-MSNA gain were only revealed when the data were examined in response to hypo- and hypertension separately.

The measurement of the sympathetic baroreflex in the present study considers the entire arterial baroreflex arc; from the input into the system (arterial blood pressure) to the ensuing sympathetic response (MSNA). Thus, the derived gains comprehensively reflect arterial baroreflex afferent input, to central processing, to the resultant efferent output. Each of these points in the baroreflex arc work in concert to determine the integrated baroreflex response. In this regard, although we did not see differences between type II diabetic patients and controls in the "whole loop" baroreflex gains, it is still plausible that a diabetic state may alter a specific portion of the reflex. A reduction in baroreflex afferent signaling could be compensated for by an enhancement in the central neural processing, and vice versa, thus resulting in a preserved arterial baroreflex gain. Although speculative, in support of this idea, complex interactions between the afferent and efferent arcs for the cardiac baroreflex have recently been described during pharmacologically induced changes in blood pressure in healthy subjects[27].

Several previous findings have suggested that type II diabetes is associated with overactive sympathetic outflow to skeletal muscle[29, 30]. Interestingly, we found no difference in resting MSNA, expressed as burst frequency or burst incidence, between the diabetic patients and healthy controls. Although the reason for the discrepancy between the current findings and earlier work is unknown, it should be noted that in previous studies the type II diabetic patients were slightly older and had a higher resting arterial blood pressure (mean arterial pressure ~100mmHg) than the patient population in the present study. However, in line with our findings, plasma norepinephrine levels have

been reported to be similar or lower in type II diabetic patients, when compared to healthy control subjects[31, 32].

In summary, the results from the present study demonstrate that the overall gain for arterial baroreflex control of MSNA was similar between type II diabetic patients and healthy age, sex and body weight matched control subjects. The similarities in arterial baroreflex gain were also evident when examining the responses to acute hypo- and hypertension separately. These preliminary findings indicate a preserved arterial baroreflex control of MSNA in patients with type II diabetes.

Acknowledgments

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Table 4.1 Subject Characteristics

	Controls	Diabetics
Male / Female	3 / 3	2 / 6
Age (yr)	44 ± 3	47 ± 3
Height (cm)	167 ± 4	166 ± 3
Weight (kg)	86 ± 6	88 ± 5
Body Mass Index (kg/m^2)	31 ± 2	32 ± 1
Glucose (mg/dl)	93 ± 6	135 ± 14*
Hemoglobin A_{1C}	5.5 ± 0.1	7.5 ± 0.5*
Insulin (uIU/ml)	7.1 ± 1.0	12.2 ± 2.4
Cholesterol (mg/dl)	188 ± 12	183 ± 16
LDL (mg/dl)	118 ± 8	107 ± 16
HDL (mg/dl)	46 ± 8	40 ± 5
Triglycerides (mg/dl)	140 ± 13	181 ± 37

Values are means ± SE. *P<0.05 vs. controls.

Table 4.2 Resting Cardiovascular and Muscle Sympathetic Nerve Activity (MSNA)

	Controls	Diabetics
Heart Rate (beats/min)	64 ± 4	69 ± 4
Systolic Blood Pressure (mmHg)	117 ± 8	124 ± 4
Diastolic Blood Pressure (mmHg)	76 ± 4	76 ± 3
Mean Blood Pressure (mmHg)	90 ± 5	93 ± 3
MSNA Burst Frequency (bursts/min)	19 ± 3	22 ± 5
MSNA Burst Incidence (bursts/100Hb)	31 ± 4	31 ± 6

Hb, heart beats. Values are means ± SE.

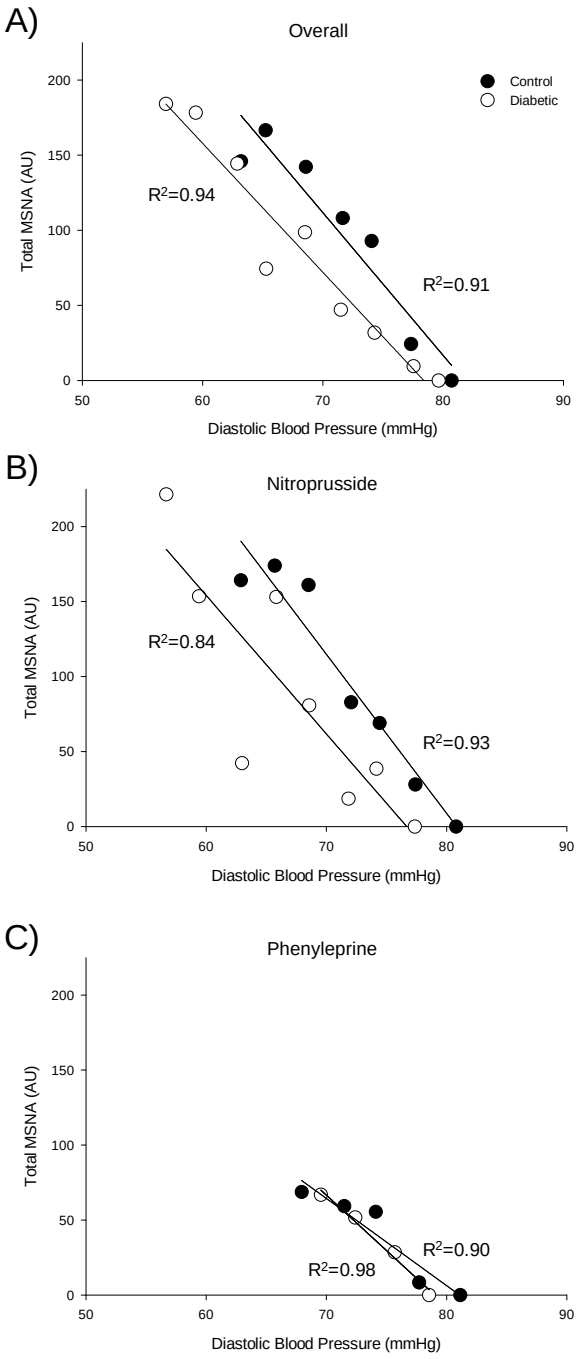
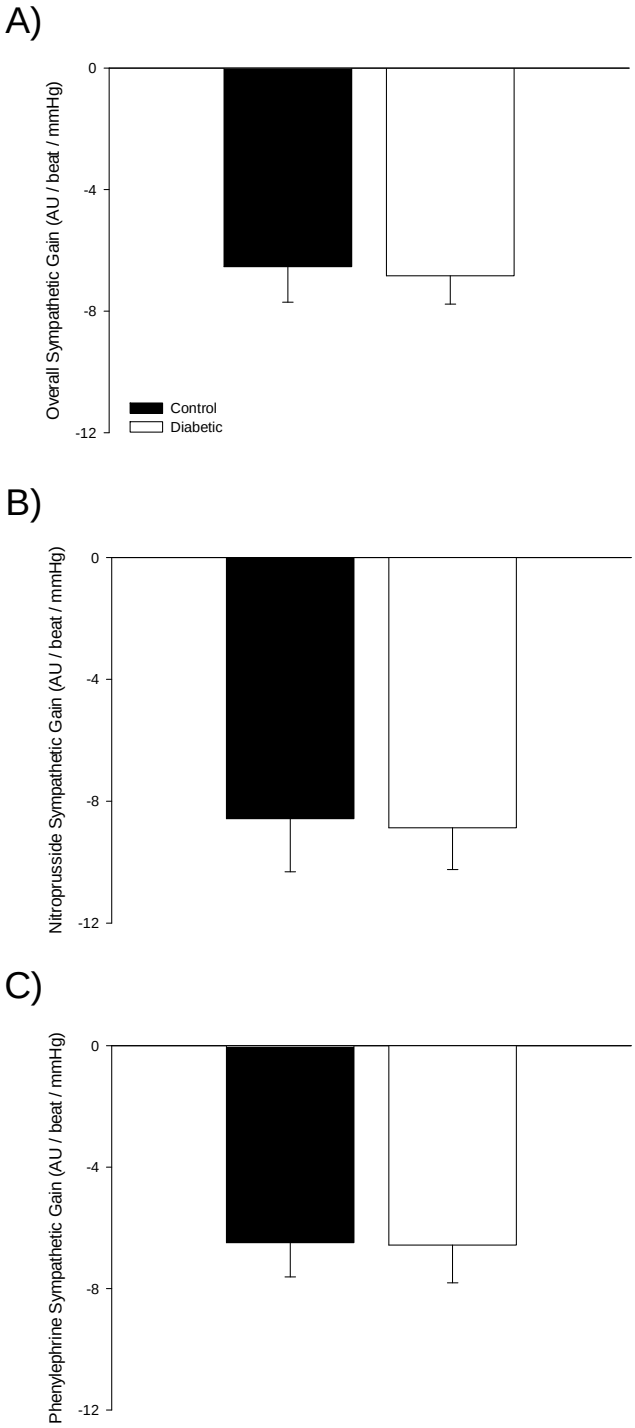


Figure 4.1 Examples of the derivation of arterial baroreflex control of muscle sympathetic nerve activity (MSNA) in one healthy control subject and one type II diabetic patient for overall baroreflex gain (Panel A), as well as the response to a fall (Panel B) and rise (Panel C) in arterial blood pressure. AU, arbitrary units; R^2, coefficient of determination.



F**igure 4.2** Group summary data for overall baroreflex gain (Panel A), as well as the response to a fall (Panel B) and rise (Panel C) in arterial blood pressure. AU, arbitrary units. Values are means ± SE.

Chapter 5. Discussion

Summary of Results

Although well recognized for metabolic regulation, a growing body of evidence has clearly established a role for insulin in the central control of cardiovascular control. Direct administration of insulin into the brain of experimental animals[1-3], or acute elevations in plasma insulin in healthy humans[4-12], result in robust increases in peripheral sympathetic nerve activity. In addition to evoking increases in sympathetic outflow, recent work in rats has reported a modulatory role for insulin in the regulation of arterial baroreflex control of sympathetic nerve activity[2]. The projects presented within this report support and extend this emerging area of research by: 1) Demonstrating an influence of insulin sensitivity on insulin-mediated sympathoexcitation. 2) Illustrating a role for insulin in the modulation of arterial baroreflex control of sympathetic nerve activity in healthy humans. 3) Translation of this area of research to an insulin resistant disease setting of type II diabetes mellitus.

In Aim 1, we sought to examine if an insulin sensitive state, such as chronic endurance training, enhances insulin-stimulated increases in central sympathetic outflow. Following consumption of a mixed meal, as a physiological stimulus to increases plasma insulin, we found that high fit subjects demonstrated a greater increase in muscle sympathetic nerve activity (MSNA) burst incidence compared to average fit subjects. Interestingly, when MSNA responses were expressed relative to plasma insulin, high fit subjects exhibited a greater change in MSNA for any given change in insulin. Collectively, these data suggest that, in addition to improved peripheral insulin

sensitivity, an insulin sensitive state (i.e. endurance training) may also enhance the central actions of insulin to increase MSNA.

In the second set of experiments we demonstrate, for the first time, a role for insulin in the modulation of the arterial baroreflex in humans. During increases in plasma insulin, under both physiological (mixed meal) and experimental (insulin clamp) conditions, an enhanced gain of arterial baroreflex control of MSNA was noted. These findings are in line with recent literature obtained in rats[2] and strongly support a role for insulin in the control of the sympathetic arterial baroreflex in healthy humans.

Given that cerebral spinal fluid levels of insulin are reduced in insulin resistant conditions[13-17] and that insulin enhances arterial baroreflex control of sympathetic outflow (Aim 2), we began to translate our research to a clinically relevant setting of insulin resistance in Aim 3. The gain of the sympathetic arterial baroreflex was investigated during pharmacologically induced changes in arterial blood pressure in type II diabetic patients and healthy age, sex and body weight matched control subjects. Interestingly, we found comparable overall sympathetic arterial baroreflex gains between the diabetic patients and healthy control subjects. These similarities in arterial baroreflex-MSNA gain were also evident when the responses were examined to falls and rises in arterial blood pressure separately. Overall, these findings suggest that arterial baroreflex control of sympathetic outflow is preserved in otherwise healthy, normotensive type II diabetic patients.

Future Directions

The investigation of insulin effects on neural-cardiovascular control is in its infancy and although the results from the present investigations support a role for insulin in the control of central sympathetic outflow in humans, this area of research undoubtedly deserves further attention. Potential implications of the findings from each Aim, as well as areas for future research are presented below.

The findings in Aim 1 clearly demonstrate that insulin sensitivity influences insulin-stimulated increases in central sympathetic outflow. These findings are in line with previous investigations which have suggested that the central stimulatory actions of insulin may be blunted in insulin resistant conditions[18-21]. To date, the exact mechanism(s) contributing to alterations in insulin stimulated changes in central sympathetic outflow to skeletal muscle remain unknown. Work illustrating that diet and pharmacologically induced insulin resistance results in decreased uptake of insulin into the brain, suggests that insulin delivery and/or rate of transport into the brain may be involved[13, 14]. However, the exact molecular mechanisms involved in the control of insulin transport into the central nervous system remain incompletely characterized. Indeed, the transporter and or receptor responsible remains unknown, although the insulin receptor itself has been suggested[17, 22, 23]. In addition, it is possible that central insulin-mediated signaling cascade pathways may be altered with changes in central insulin sensitivity. Findings from rodent models suggest that insulin stimulated increases in sympathetic outflow to skeletal muscle primarily occur through the PI3K pathway[3]. Given that this pathway have been shown to be modified in the periphery in insulin resistant states[24] and in response to endurance training[25], it is plausible that alterations in

insulin signaling pathways within the central nervous system occur with changes in insulin sensitivity. In line with this, the precise neural pathways and signaling mechanisms involved in insulin mediated increases in central sympathetic outflow deserve further attention. Interestingly, work in neuronal cell lines suggests that downstream molecules in the insulin signaling cascade, such as protein kinase C, modulate glutamatergic *N*-methyl-D-aspartic acid trafficking and gating[26]. Furthermore, a potential role for PI3K in the modulation of gamma amino butyric acid, the inhibitory neurotransmitter within the central nervous system, function has also been suggested[27]. These findings lend insight into insulin as a complex neuromodulator of excitatory and inhibitory neurotransmission; although future studies are clearly warranted.

Recent findings in rats have demonstrated a role for insulin in the modulation of arterial baroreflex control of sympathetic nerve activity[2] and in Aim 2 we extended these findings by providing evidence for the first time that insulin increases arterial baroreflex-MSNA gain in healthy humans. However, due to the inherent limitations of human investigation, we cannot determine the precise site of insulin action on arterial baroreflex control. The aforementioned work in rats clearly suggests that central brain regions are involved; although as mentioned above the precise cellular and molecular mechanism(s) of insulin action on central neural pathways remains to be elucidated. In addition, the potential for insulin to modulate afferent baroreflex input cannot be discounted, although no data exists to date investigating this particular pathway.

In addition to examining a role for acute increases in insulin to modulate the arterial baroreflex, we began to translate this area of research to a condition of chronic insulin resistance. Interestingly we found that arterial baroreflex control of sympathetic

nerve activity was preserved in patients with type II diabetes when compared to healthy control subjects. Although preliminary, the lack of a group difference in Aim 3, raises important questions about acute versus chronic influences of insulin on arterial baroreflex function. Furthermore, investigation of disease populations is complex and consideration for numerous other disease-related factors that may work in concert with and/or against insulin should be considered. Given the increasing incidence of type II diabetes, as well as numerous other insulin resistant conditions, future mechanistic studies from experimental animals to humans examining the interaction of the metabolic and neural-cardiovascular systems undoubtedly deserves further attention.

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VITA

Colin Neal Young, the second son of Robert and Denise Young, was born in Lancaster, Pennsylvania on February 23, 1981. He completed his primary education in Drumore, Pennsylvania and secondary education in Quarryville, Pennsylvania where he graduated from Solanco High School in June, 1999. He continued his education, studying Exercise Science at the University of Delaware, Newark, Delaware. After completion of his Bachelor of Science degree in May 2003, he began graduate studies at the University of Delaware in the Department of Health, Nutrition and Exercise Sciences under the supervision of William B. Farquhar. He received his Master of Science degree in May 2005 and joined the graduate program in the Department of Medical Pharmacology and Physiology at the University of Missouri, Columbia, Missouri in 2006. In 2010, under the direction of Paul J. Fadel, he received his Doctor of Philosophy in Physiology. Colin will subsequently pursue postdoctoral training with Robin L. Davisson at Cornell University, Ithaca, New York.