

# New concepts in microvascular function and metabolic diseases: importance of cephalic phase of insulin response

## Abstract

During the process of human evolution, an enormous variety of mechanisms were selected in order to maintain metabolic homeostasis. Neurally-mediated anticipatory responses, also named as cephalic phase responses (CPR), and microcirculatory regulation are examples of these mechanisms. Beyond the already known insulin's metabolic effects, this hormone exerts clear hemodynamic effects in the entire vascular bed. One of the most studied aspects of the CPR is the one related to insulin's action. The cephalic phase of insulin response (CPIR) is involved in glucoregulation and seems to positively influence glucose homeostasis. While receptor and mainly post-receptor defects on insulin action are factors already well-known that regulate glucose homeostasis, actually pre-receptor defects directly related to microcirculatory dysfunction may also influence glycemia. With this view, microcirculatory dysfunction could be both cause and consequence of insulin resistance and glucose homeostasis dysregulation. In this review, we aimed to discuss an interaction hypothesis between CPIR and an early activation of microcirculation in order to maintain glucose homeostasis, as well as the possible impairment promoted by obesity and insulin resistance in this association.

**Keywords:** microcirculation; capillary recruitment; cephalic phase responses

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## Introduction

Homeostasis is defined as the property of a system in which variables are regulated to maintain internal conditions stable and relatively constant. It is a robust, dynamic, intergenerational, diachronic (across-time) mechanism for maintenance, perpetuation, and modification of physiological structure and function.<sup>1</sup> During the process of human evolution, an enormous variety of mechanisms were selected in order to keep physiological functions within tightly regulated and controlled limits. Neurally-mediated anticipatory responses, also named cephalic phase responses (CPR), and microcirculatory regulation are examples of these mechanisms. The hypothesis of an interaction between CPR and the microcirculation for maintenance of metabolic homeostasis and the search for consequences related to disruptions of these physiological mechanisms constitute an exciting field of research for understanding, not only human physiology but especially the pathophysiology of metabolic diseases.

## Microcirculation and its application in diagnosis and research of metabolic diseases

The microcirculation is the portion of vascular bed responsible for tissue nutrition. It is composed by arterioles (up to 100 µm of mean diameter) and their branches, including terminal arterioles (15 µm) and capillaries. The physiology, biochemistry and pharmacologic responses of the circulation vary accordingly to the size of the studied portion and its localization. Important endothelium-dependent responses to different stimuli are observed depending on the vascular segment analyzed.<sup>2,3</sup> Since smooth muscle cells tend to be reduced as arteriolar diameter decreases, remarkable changes are evidenced on the vascular wall. Capillaries are composed exclusively of a

single layer of endothelial cells overlying the basal membrane. These structural changes are also accompanied by changes in biochemical mechanisms, which regulate microvascular function. As the arteriolar diameter decreases, vasodilatation in microcirculation seems to be less dependent on nitric oxide (NO), being more related to prostacyclin and endothelium-derived hyperpolarizing factor.<sup>4</sup> Similarly, reactivity to specific agonists is also variable according to vessel diameter.<sup>5</sup>

The endothelium exerts many physiological actions, such as: regulation of vascular tone<sup>6</sup>; modulation of fibrinolysis, platelet aggregation, and coagulation<sup>7</sup>; participation on an inflammatory process through cytokines secretion<sup>7</sup>; and regulation of leukocyte activation and adhesion to vessel wall.<sup>8</sup> Endothelial damage caused by many cardiovascular risk factors such as hypertension, dyslipidemia, diabetes, and tobacco reduces the synthesis and secretion of protective factors activating the endothelium and promoting a pro-constrictor, pro-inflammatory, and pro-atherogenic state of blood vessels.<sup>9</sup> As a consequence of endothelium activation to a pro-atherogenic state, it is possible to observe disturbances in microvascular reactivity before the development of morphological changes in blood vessels.<sup>10</sup>

The use of skin microcirculation as a method to assess the microcirculatory function has been employed for many years, and skin microvascular dysfunction correlates both to coronary microvascular dysfunction, also known as microvascular angina or cardiac syndrome X,<sup>11–13</sup> as with increased coronary disease.<sup>14</sup> Patients with microvascular angina have a vasodilator deficit that affected both coronary and peripheral circulation,<sup>13</sup> forming an evidence that the study of skin microcirculation reflects findings in other vascular sites. Therefore, it seems that microvascular dysfunction is a systemic process, and it could be assessed using skin microcirculation tests.

Some aspects that relate microcirculation to metabolic disease are important to consider and are listed as follows:

- a) not only post-receptor or receptor defects on insulin signaling are involved in insulin resistance, but microvascular dysfunction also plays a role in the pathophysiology of insulin resistance<sup>15-19</sup> and glucose homeostasis,<sup>20</sup>
- b) impaired capillary recruitment seems to be involved in insulin resistance due to decreased tissue perfusion<sup>18,21</sup> and
- c) subjects with insulin resistance have microvascular dysfunction even in absence of hyperglycemia.<sup>20,22,23</sup>

From these points of view, it is supposed that both metabolic and hemodynamic actions of insulin could be related to the strong associations between vascular diseases and insulin resistant states, such as obesity, metabolic syndrome and/or type 2 diabetes.<sup>24</sup>

### The hemodynamic actions of insulin

In addition to the classical metabolic effects of insulin, which promote energy storage and glucose oxidation, this hormone also exerts important hemodynamic actions. Insulin promotes capillary recruitment, peripheral vasodilatation and increased regional blood flow,<sup>25-27</sup> and also possible positive effects on transendothelial transport of insulin's molecules.<sup>28,29</sup> To these hemodynamic effects of insulin, it adds up those classic metabolic effects of this hormone resulting in increments of glucose supply and exposure to the skeletal muscle, previously sub-perfused, to insulin action.<sup>30</sup> Up to 25% of insulin stimulating effect in glucose uptake in skeletal muscle is related to insulin's hemodynamic action.<sup>31</sup> The vascular effects of insulin are mainly exerted through effects derived from increased NO bioavailability, causing vasodilatation, increased capillary recruitment, improvement in muscle's permeability to insulin and its passage through a transcellular mechanism.

Although still a matter of intense debate, insulin's effects on muscle blood flow provoked some contradictory findings since vasodilatation *per se*, expressed by increased blood flow after insulin infusion, was not necessarily associated to increased glucose uptake in muscle tissue.<sup>32</sup> To lessen this possible contradiction, it is important to note that higher glucose uptake has been shown only when vasodilatation was associated with increased capillary recruitment.<sup>30</sup> Considering that at resting state, only 50% of muscle capillaries are perfused with whole blood, while the other 50% is filled only with plasma,<sup>33</sup> the metabolic action of insulin is increased by its hemodynamic action, which directly improves capillary recruitment. This improvement augments the micro flow and redirects blood from non-nutritive to nutritive capillaries, increasing exchange areas for tissue nutrition. Redirection of blood flow to insulin-sensitive tissues (basically fat and muscles) occurs in detriment of other less insulin-sensitive tissues, such as skin, bone, and tendons. Nevertheless, even with this physiological redirection of microflow, skin capillary recruitment secondary to exogenous insulin action has been shown by some authors.<sup>34,35</sup>

Data measuring muscle and skin microflow responses in humans during endogenous physiological insulin stimulus are still lacking. The role of insulin on capillary recruitment is exerted through the relaxation of terminal and resistance arterioles. In experimental models, insulin rapidly recruits skeletal muscle capillaries, a principle based on vascular smooth muscle relaxation on arterioles.

Experimentally, insulin rapidly recruits capillaries through a NO-dependent action<sup>27</sup> and such effect could be completely blocked by inhibition of NO-synthase.<sup>36</sup> In rats, capillary recruitment occurs without immediate change on total blood flow of the femoral artery; under physiological insulin levels, the recruitment precedes local blood flow increment.<sup>37</sup> These findings suggest that capillary recruitment is the first hemodynamic step to increase the perfused surface, and hypothetically to augment tissue substrate uptake. An experimental study evidenced that micro-occlusion of skeletal muscle microcirculation in rats was related to decreased insulin action,<sup>38</sup> since progressively larger microspheres, were infused, insulin availability to muscle and glucose uptake were, in parallel, progressively lower.

There is also data that contradict the possibility of the endothelial action of insulin in its global effects of sensitization to its own action in the muscle, since experiments of deletion of the insulin receptor in the endothelium of mice did not alter the insulin sensitivity tested by clamp technique.<sup>39</sup> However, subsequent data that specifically used animals with the insulin receptor-2 substrate (IRS-2) knockout in the endothelial cell demonstrated worsening of insulin sensitivity by clamp technique and also a reduced insulin transport through the endothelial barrier with subsequent lower insulin's levels in muscle interstitium.<sup>19</sup> This finding could suggest that in the insulin signaling chain in the endothelial cell, downstream of the insulin receptor, the specific knockout of IRS-2 promotes insulin resistance and possibly its function in the insulin's hemodynamic effects plays a crucial role. Hemodynamic effects of insulin promote increased transendothelial transport of insulin and capillary recruitment, with resultant blood flow increase, greater supply of insulin, and thus greater glucose uptake in skeletal muscle. It seems clear to suppose that these hemodynamic effects have an essential role in glucose homeostasis.

### CPR: the role of anticipatory stimulus on human homeostasis

CPR is innate and learned physiological responses to sensory signals that prepare the gastrointestinal tract for optimal processing of ingested foods.<sup>40</sup> They are typically initiated before or at the onset of ingestion,<sup>40</sup> during the pre-absorptive phase of digestion, and are triggered by meal expectations, followed by visual, olfactory, and gustatory stimuli, and finally by oropharyngeal stimuli including masticatory and swallowing sensations.<sup>41</sup> Rather than slowly generated, internal feedback signals, such as digested nutrients, allow rapidly measurable sensory information to control food selection, glucose supply for fight-or-flight responses or preparedness for digestion/absorption.<sup>42</sup> Cephalic phase secretions of the gastrointestinal tract are initiated by activated vagal motor neurons<sup>41</sup> and include both exocrine secretions, such as saliva, gastric acid, and pancreatic enzymes, and endocrine hormones, such as gastrin, insulin, pancreatic polypeptide, and glucagon, among others.<sup>40,43</sup> When a meal is directly administered into the stomach, trespassing sensorial receptors, the early phase of digestion does not occur, corroborating that the sense of sight and smell stimulation is physiologically crucial to release of aforementioned substances and primary organic responses to initiate digestion. Experimentally, the cephalic phase can be studied by "sham-feeding", classically involving an esophageal fistula preventing food from entering the stomach. In humans, this is mimicked by the "chew-and-spit" procedure, also known as "modified sham-feeding".<sup>41,43</sup>

The major purpose of CPR is to prepare the gastrointestinal tract for optimal digestion and absorption of nutrients, contributing to the maintenance of homeostasis and minimizing disturbances of

the internal *milieu* resulting from food intake.<sup>40</sup> Considering that many substances activated during CPR exert vascular effects, such as insulin<sup>25-27</sup> and pancreatic polypeptide,<sup>44</sup> it was suggested that CPR could also be related to microvascular activation, leading to a precocious role for microcirculation in the physiology of digestion and nutrient homeostasis.<sup>45</sup>

### Cephalic phase of insulin response and its relevance to metabolic effects

The cephalic phase of insulin response (CPIR) is defined as insulin release observed prior to nutrient absorption in response to sensory stimulation of the oral cavity by taste or food ingestion.<sup>43</sup> CPIR occurs during the pre-absorptive phase of digestion,<sup>46-51</sup> starting soon after the sensorial stimulation, peaking at 1-4 minutes and returning to baseline within 8-10 minutes.<sup>40,43,47,52,53</sup> Neural effector pathways related to CPIR begin in the ventromedial of the hypothalamus and in the dorsal motor nucleus of the vagus. Preganglionic fibers transverse the vagus and enter the pancreas,<sup>54</sup> terminating in intrapancreatic ganglia from which postganglionic fibers emerge to innervate the Langerhans islets.<sup>55</sup> Studies performed in animals and humans demonstrated that CPIR is independent of ATP-sensitive potassium channels<sup>56</sup> and can be abolished by vagotomy<sup>57</sup> or ganglionic blockade with muscarinic antagonists,<sup>58</sup> which reinforces that this stimulus is mediated by cholinergic neurons of the parasympathetic nervous system. However, in humans, there is evidence that CPIR is also mediated by noncholinergic mechanisms.<sup>59</sup>

Most of the studies evaluating CPIR focus in the role of insulin on glucose homeostasis, especially on postprandial glycemia.<sup>47,53,58-62</sup> Although several studies have shown that CPIR aids benefits in postprandial glucose,<sup>58,59,61,62</sup> other studies were unable to detect such improvements.<sup>47,53,60</sup> Teff et al.,<sup>58</sup> evaluated the effect of CPIR on postprandial glucose of lean and obese subjects infusing a tiny amount of insulin during the first 10 min of food ingestion, a procedure that would theoretically mimic CPIR. The total amount of insulin infused in this experiment was equivalent to the amount of insulin released peripherally during CPIR. While no effects on postprandial insulin or glucose levels were observed in lean subjects, insulin infusion lowered postprandial glucose area under the curve by approximately 27% in obese subjects, providing evidence that CPIR is able to optimize glucose homeostasis in obese subject's tested.<sup>58</sup> Similarly, prompt infusion of insulin during the first 15 min of food intake also increased glucose tolerance in subjects with type 2 diabetes.<sup>63</sup>

Moreover, when intragastric glucose administration that typically bypasses stimulation of CPIR is coupled with sham-fed, postprandial glucose levels are 30% lower compared to intragastric glucose administration without sham-fed.<sup>43,62</sup> All these evidences suggest that CPIR improves glucose homeostasis, being relevant to antihyperglycemic effects of insulin.

### Is CPIR relevant to hemodynamic actions of insulin?

According to Teff,<sup>43</sup> in light of the relatively small magnitude of CPIR (approximately 1-3% of normal postprandial insulin release or about 25% above baseline), it is unlikely that the observed effects of CPIR on postprandial glucose levels are due to muscle tissue uptake of glucose. Instead, the rapid increase in insulin, immediately prior to meal ingestion, would be most likely achieved by effects on hepatic glucose and fat metabolism, since insulin level in the portal vein

draining into the liver is 50% higher than measured peripherally.<sup>43</sup> However, considering that up to 25% of insulin stimulating effect in glucose uptake in skeletal muscle may be related to its hemodynamic action,<sup>31</sup> it is reasonable to consider that CPIR could activate the microvascular network, ensuring a microflow supply that primes tissues to glucose uptake and improves postprandial glucose homeostasis. If CPIR does not occur, postprandial glucose levels will be less well-regulated. This hypothesis draws attention to the importance of studying CPIR with special focus on its interaction with microvascular network and microflow supply.

The study of Buss et al.<sup>45</sup> was the first to demonstrate an association between CPR and functional changes in both non-nutritive and nutritive microcirculatory parameters, adding relevant knowledge to reinforce the above-mentioned hypothesis. Healthy male subjects were randomized into receiving cognitive-sensorial stimuli to elicit CPR or not. In the CPR group, neurally-mediated anticipatory responses of digestion elicited functional capillary recruitment, while no significant changes were evidenced in the control group.<sup>45</sup> Although CPIR could not be detected (insulin levels did not change from minutes 3 to 10 in both groups, which does not exclude the possibility of an early peak of insulin in CPR group or even an adrenergic inhibition of insulin release secondary to stress), CPR activation was demonstrated through a significant pancreatic polypeptide increase in the CPR group, which was positively associated to basal functional capillary density increase ( $\rho=0.527$ ,  $p=0.03$ ).<sup>45</sup> All these findings suggest a precocious role for microcirculation in the physiology of digestion and nutrient homeostasis in healthy men, related to pancreatic polypeptide,<sup>44</sup> and possibly to insulin, or other gastro-intestinal peptides.<sup>45</sup>

### Obesity and insulin resistance: how they could impact the relationship between CPR and microcirculation?

Learned anticipatory responses contribute to the maintenance of metabolic homeostasis in face of nutritional challenges. Increased absolute CPIR in obese subjects constitutes an evidence of an adaptive response, facilitating increased disposal of ingested food as quantity of food increases.<sup>43</sup> However, only obese individuals with insulin resistance exhibit CPIR of greater magnitude than lean ones,<sup>58</sup> initially suggesting that the magnitude of CPIR adapts to the metabolic state of the individual in an attempt to maintain its metabolic homeostasis. Unfortunately, it seems that there is an inadequate CPIR to compensate for impaired glucose homeostasis in obese humans since, when expressed as percentage of baseline insulin levels, the relative CPIR is attenuated in obese subjects.<sup>43</sup>

In an experimental model of obese rats, insulin-mediated capillary recruitment is decreased, as much as glucose uptake.<sup>64</sup> Similarly, in obese subjects, there was an inverse correlation between microvascular blood volume in muscle tissue, an index of capillary recruitment, and body mass index, suggesting that obesity per se abolishes insulin-mediated capillary recruitment.<sup>65</sup> Moreover, obese men had a delay at the beginning of insulin action when compared to lean subjects,<sup>66</sup> suggesting that there was a delayed transcappillary transport of insulin, possibly due to microvascular dysfunction.

Kraemer-Aguiar et al.<sup>67</sup> showed that when normoglycaemic subjects with metabolic syndrome were taking metformin, a drug which is known for its insulin sensitization effect, they had an important improvement in all microcirculatory variables assessed through nailfold videocapillaroscopy. In addition, when a technique

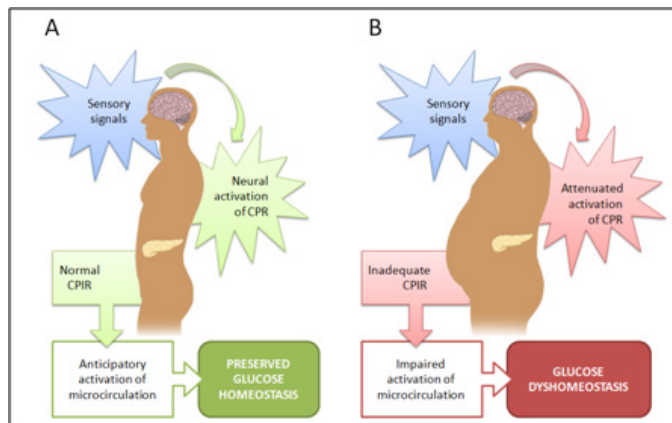


that assesses the endothelial function of resistance arterioles was employed, the same subjects with obesity also had an improvement on endothelium-derived vasodilatation.<sup>68</sup>

Considering that:

- I. obesity and insulin resistance promote impairments in both CPIR<sup>43</sup> and microcirculatory function;<sup>64,65</sup>
- II. CPIR aids benefit in postprandial glucose homeostasis;<sup>43,58,59,61–63</sup>
- III. a procedure that mimics CPIR is able to optimize postprandial glucose in obese subjects, without additional effects in lean individuals;<sup>58</sup>
- IV. an intervention that promotes benefits in both insulin resistance and glucose metabolism also improves microcirculatory function;<sup>67,68</sup> and
- V. there is an association between CPR and functional changes in microcirculatory parameters;<sup>45</sup>

it is reasonable to hypothesize that attenuated CPIR in individuals with obesity and/or insulin resistance leads to impairment in the precocious phasis of microvascular reactivity activation, which in turn compromises the precocious role of microcirculation in the physiology of digestion and nutrient homeostasis. If this hypothesis is tested and proved, possible implications of capillary recruitment impairment at the pre-ingestive phase of digestion would be associated, or even predict, an altered glucose homeostasis, adding new findings for the knowledge of insulin resistance states due to pre-receptor defects in the signaling pathway. Figure 1 provides a schematic illustration of above-mentioned hypothetical associations between CPIR, microcirculation, obesity, and insulin resistance.



**Figure 1** Hypothesized effect of CPIR on early activation of microcirculation in order to maintain glucose homeostasis:

(A) Under normal physiological condition

(B) During obesity and insulin resistance.

CPR: cephalic phase responses

CPIR: cephalic phase of insulin response

## Conclusion

CPR is innate and learned physiological responses that contribute to the maintenance of metabolic homeostasis in face of nutritional challenges. It involves many peptides derived from the gastrointestinal tract, such as insulin and pancreatic polypeptide, and CPIR seems to

improve postprandial glucose. Given the fact that insulin has not only metabolic but also hemodynamic actions, it may be involved in the association between CPR and early activation of microcirculation. This effect is probably impaired in subjects with obesity and insulin resistance, maybe resulting in additional impairments of glucose homeostasis. Future studies on this focus could add knowledge to this hypothesis, bringing a new potential for therapeutic interventions in metabolic diseases such as diabetes.

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## Conflict of interest

Author declares there is no conflict of interest.

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