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Fasting small vessels to prevent microvascular ageing? The experience of a microvascular research group working in the shadow of the leaning tower

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Microvascular dysfunction is currently regarded as the earliest organ damage in hypertension and a feature of metabolic diseases. The importance of microvascular function in preserving organ and tissue homoeostasis and in response to injury has become dramatically evident during the most recent COVID-19 pandemic, showing that the microvascular endothelium might represent a preferential site of SARS-CoV2 infection, promoting the viral spreading in the body, amplifying the tissue inflammatory response, compromising tissue oxygenation and accounting for some of the most important complications of the infection, such as the so-called COVID-19 coagulopathy.[1]

The microvascular research in Pisa

From the earliest '90s, our group has focused its attention on the mechanisms involved in the regulation of small vessel physiology and pathology in human, using a combination of *in vivo* (strain-gauge plethysmography) and *ex vivo* (pressurized micromyography) microvascular assessment methods. The initial interest of our research was the identification of factors involved in the regulation of endothelial function in physiological and diseased conditions. Using strain-gauge plethysmography, we found that ageing was associated with reduced endothelium-dependent vasodilation in small vessels of healthy subjects and in patients with arterial hypertension.[2] A few years later, we documented that the severity of microvascular endothelial dysfunction was correlated to the burden of microvascular remodelling, independently of cardiovascular risk factors.[3] Based on this evidence, we hypothesized that endothelial dysfunction might have an essential role in the age-related process of small vessel remodelling. Indeed, using the pressurized micromyography, we reported that ageing is associated with a progressive reduction in nitric oxide (NO) availability coupled with increased levels of oxidative stress within the microvascular wall. These alterations were accompanied by a greater vascular remodelling, increased vascular wall fibrosis and collagen deposition.[4] Intriguingly, similar alterations are the substrates of the increased arterial stiffness recorded in large vessels with ageing and considered a hallmark of 'early vascular ageing'.[5] Can we use the definition of 'early microvascular ageing' as a specular entity of the 'early vascular ageing' used in large arteries to identify the vascular remodelling resulting from the interaction between inherent and environmental factors?

Our experience supporting the concept of 'early microvascular ageing'

Ageing is a physiological and unavoidable consequence of organ and tissue function. In the attempt to preserve tissue and organ homeostasis, every change in the local environment induces adaptive responses to maintain physiological tissue functions. However, sustained modifications of the local environment require prolonged compensating efforts, which are paid with early exhaustion of the local tissue defenses, eventually resulting in an acceleration of the ageing process. How can these concepts be applied to small vessel ageing?

The critical function of microvascular structures is the regulation of tissue oxygenation and nutrition, transport of mediators, exchanges of gases, and metabolites to and from tissues. Thus, it is logical to suppose

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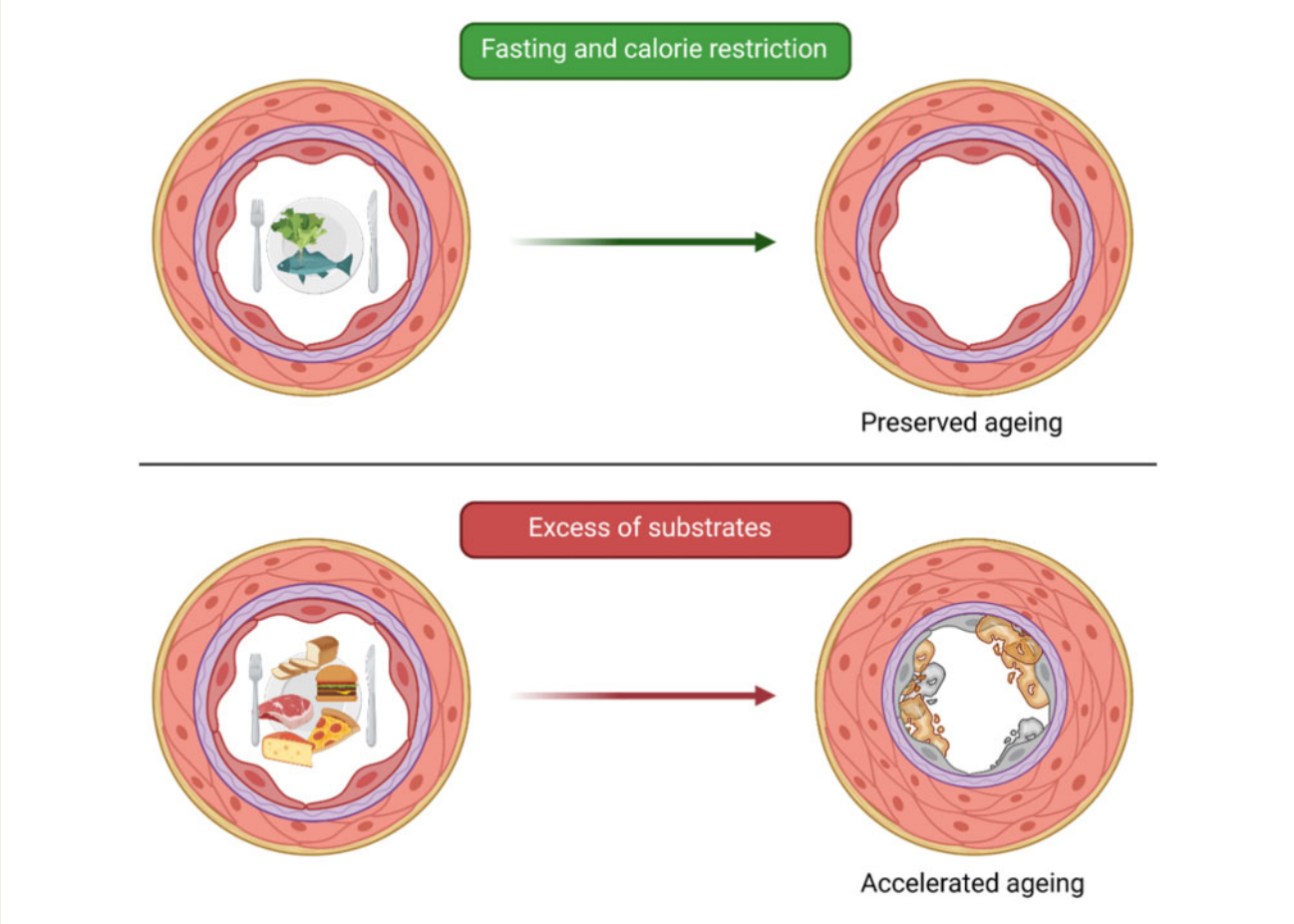

Figure 1 Small arteries overloaded with substrates (in particular, glucose and cholesterol) show a hypertrophic remodelling with impaired endothelial health and function. In turn, fasting and calorie restriction lower the glucose concentration and increase circulating ketone bodies that preserve endothelial function, resulting in healthy microvascular ageing.

that overburdening these capacities might promote an early and accelerated microvascular ageing. Following this conceptual framework, conditions characterized by an excessive availability of substrates compared with the metabolic requests could cause adaptive changes in the physiology of the microcirculation, ultimately resulting in 'early microvascular ageing'. Although these concepts might represent an oversimplification of the plethora of processes regulating microvascular ageing, some lines of evidence acquired during our experiments suggest that they could partially reflect reality. Indeed, we documented that conditions of excessive substrate availability, such as obesity, are characterized by a faster progression of the age-related microvascular remodelling than that observed in healthy subjects. Furthermore, vessels from obese patients had a more significant collagen deposition and early impairment of NO availability than healthy subjects (*Figure 1*). While a reduced production of NO by the endothelial NO synthase could partially account for these alterations, increased oxidative stress, and inflammation represented the primary driver of small vessel dysfunction in both obesity and ageing.[6] Further support to the hypothesis that the microvascular adaptation to an excess substrate availability might result from an early process of microvascular ageing is provided by the evidence that pathways involved in microvascular endothelial dysfunction and remodelling in metabolic diseases often overlap with those connecting cell metabolism and ageing. For example, we have shown that conditions of substrate excess promote microvascular inflammation and oxidative stress through the activation of the pro-ageing factors $p66^{Shc}$.[7] In turn, it has been shown that fasting and energy restriction can markedly improve microvascular endothelial function and that this is accompanied by activation of specific pathways involved in metabolic health. Fasting up-regulates the sirtuin axis and particularly the expression and activity of SIRT1, a potent epigenetic modulator of ageing that is involved in the down-regulation of inflammation, reactive oxygen species (ROS) production, and increases NO availability in small vessels of animals.[7] Beyond its influence on substrate availability, fasting might also prevent microvascular ageing through a switch on the type of metabolic substrates delivered to the microcirculation. Indeed, fasting can increase and maintain circulating ketone bodies, especially β-hydroxybutyrate (β-HB). An increased β-HB has been reported to prevent and alleviate the progression of various age-associated diseases, including cardiovascular diseases. Intriguingly, β-HB has been shown to prevent microvascular ageing through up-regulation of the Octamer-binding transcriptional factor 4 and Lamin B1 in both vascular smooth muscle and endothelial cells in

Figure 2 Our research group. From left to right: Alessandro Mengozzi, Agostino Virdis, Stefano Taddei, and Stefano Masi.

mice *in vivo*.[8] This evidence might help uncover the mechanisms underlying the significant cardioprotective effects of some new 'cardio-metabolic' drugs. One example is represented by the sodium-glucose cotransporter 2 (SGLT2) inhibitors, which induce a fasting-like state, thus increasing the production of ketones. This metabolic shift has been suggested to account, at least partly, for some of their remarkable cardio and renal protective actions, potentially through an improvement in microvascular health.[9]

Conclusions

In over 30 years of microvascular research, we reported several lines of evidence supporting the hypothesis of an early microvascular ageing process underpinning the small vessel remodelling in metabolic diseases. In our experience, this process seems to be tightly controlled by the microvascular substrate availability and potentially prevented by fasting. However, it remains to be defined whether the plastic adaptations of the pathways involved in 'early microvascular ageing' could be not only prevented but also reversed by fasting. In addition, it will be important to define the lower limit of microvascular substrates deprivation for which beneficial effects on the cardiovascular system can be expected. Indeed, we know that excessive fasting bordering on starvation may be detrimental for cardiovascular health, as sadly described in the autopsies conducted on men who had died of hunger in the Dachau and Warsaw ghettos during the Second World War.[10] Leveraging on the peripheral small vessels' capacity to regulate cardio-metabolic homeostasis, we will now focus on the delicate equilibrium of the energy balance and inflammation and how this might be meaningful in cardio-metabolic health and ageing. We (*Figure 2*) are enthusiasts to pursue this new challenge and have recently participated in the foundation of a new international working group on small arteries (https://www.eshonline.org/section-on-working-groups/).

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Figure 1 is created with biorender.com

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