ABSTRACT

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Title of dissertation thesis: Effects of isoflavonoids and their metabolites on vascular smooth muscles *in vitro* and *in vivo*

The dietary intake of flavonoids seems to be inversely related to cardiovascular mortality, in particular on coronary artery disease. The consumption of isoflavonoids, one class of flavonoids, has been increasing in the general population, especially due to the use of food supplements and a variety of isoflavonoid-rich foods. Although their bioavailability is low, they undergo extensive gastrointestinal metabolism by human bacteria, leading to smaller metabolites with a much higher degree of bioavailability. However, detailed studies on the impact of individual pure isoflavonoids on vascular system were mostly missing and much less was known for the effect of their colonic metabolites in this field. In the present study sixteen isoflavonoids, four metabolites and the racemic mixture of one of them were initially screened *ex vivo* for their vasorelaxant properties on rat aortas. The most potent of them, biochanin A, glycitein, O-desmethylangolensin (O-DMA), *S*-equol and *R,S*-equol were further tested for the mechanism of action on porcine coronary arteries. All abovementioned compounds induced an endothelium independent relaxation of the coronary vasculature *ex vivo,* with EC_{50} ranged from 5.5 to 17 μM. Biochanin A, *S*-equol and *R,S*-equol, but not

glycitein and O-DMA, were able to block the vasoconstriction caused by KCl, $CaCl_2$, serotonin and U46619 in a concentration-dependent manner. Another series of experiments suggested that the major mechanism of action of biochanin A was the inhibition of L-type calcium channels and this was further confirmed by experiments using human aortic and coronary smooth muscle cells, loaded with a calcium indicating fluorescent dye. Biochanin A in relatively small concentrations (2-4 μM) also interfered with the cGMP, but not cAMP, pathway in isolated coronary arteries. Moreover, O-DMA, *S*-equol and *R,S*-equol dilated smaller resistant mesenteric arteries *ex vivo*, while the more abundant human metabolite, O-DMA, decreased *in vivo* arterial blood pressure in spontaneously hypertensive rats, without impacting the heart function. Similarly to biochanin A, O-DMA blocked the calcium influx in human aortic smooth muscle cells, as well. These results indicate that several isoflavonoids, in particular biochanin A, and their metabolites are able to have vasodilatory effects in micromolar concentrations which is of potential clinical interest for the management of some cardiovascular pathologies.