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ARTIFICIAL SWEETENERS IMPAIR ENDOTHELIAL VASCULAR REACTIVITY: PRELIMINARY RESULTS IN RODENTS

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Short Title: Effect of artificial sweeteners on vascular function.

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Abstract.

(1) **Background and Aims.** Prospective epidemiological studies highlighted recently the link between artificial sweeteners (AS) consumption and the risk of developing cardiometabolic diseases. However, underlying mechanisms remain unknown. Thus, the aim of this preliminary study was to characterize, in a healthy rat population, the effect of chronic AS consumption on body composition and vascular function, an early marker for cardiovascular disease.

(2) **Methods and Results.** Healthy Wistar rats followed a 10 weeks standard diet including the consumption of water sweetened or not with a sucralose/acesulfame potassium solution at different concentrations: for moderate consumption at 1 and 2 mg.kg⁻¹.day⁻¹ respectively or high intake at 15 and 15 mg.kg⁻¹.day⁻¹ for both molecules (Acceptable Daily Intake). Body fat composition has been evaluated and *ex vivo* aortic vasomotor function has been investigated with a pharmacological approach.

(3) **Conclusion.** Both groups of AS-treated rats showed a significant increase in subcutaneous and perirenal adipose tissue mass storage, without changes in total body mass. However, rats that have consumed AS at Acceptable Daily Intake concentration revealed a significative vascular endothelial dysfunction compared to the other groups. These results are interesting since they will help to better explain the observed increase in cardiometabolic risk.

Cardiovascular disease (CVD) is the single leading cause of death amongst those with obesity and type 2 diabetes (T2D).[1] Although these worsening trends are driven by a complex interaction between numerous lifestyle and socioeconomic factors, it is now well accepted that habitual excess sugar consumption is the dietary factor that is most consistently found to be associated with obesity or T2D.[2,3] Moreover, Malik et al. recently reported a positive, dose-dependent association between sugary drinks consumption and CVD mortality.[4]

As an alternative, artificial sweeteners (AS) have been increasingly used in food and beverages to replace high-energy, added sugars. By providing consumers with products that have a sweet taste and low-energy content, AS appear to be the magic bullet to enhance weight loss and reduce cardiometabolic diseases. Indeed, AS (e.g. sucralose, acesulfame potassium, saccharin, aspartame or stevia among others) are products that maintain perceived sweetness, without increasing caloric intake.[5] As a consequence, AS consumption does not acutely significantly change blood glucose concentration in healthy adults or in those with T2D.[6] These results encouraged health authorities to recommend AS consumption, as part of a weight loss strategy in overweight and obese individuals, and in patients with glucose intolerance or diabetes.[7] In addition, the European Food Safety Authority (EFSA) and the United States Food and Drug Administration (FDA), have approved several AS safe for human consumption.[8]

Despite the apparent neutral effects of AS on blood glucose, there is still a strong debate questioning their physiological inactivity and their long-term effect on human health.[9] Indeed, several recent epidemiological and descriptive studies have reported significant associations between the consumption of AS and weight gain, increased incidence of T2D, and CV events.[9–11] Recently, an increased risk in total

and CVD mortality has been identified in women consuming four or more AS beverages servings per day, but not in mixed-gender subjects drinking moderately.[4,12] However, the interpretation of these data is difficult since AS are typically consumed by individuals already suffering from obesity or cardiometabolic conditions. Very few prospective, randomized, controlled studies have assessed the effect of these molecules consumption on human health, highlighting the urgent need for more research into this question.[13]

Since the mechanisms through which artificial sweetened beverages (ASB) may be harmful on cardiovascular health remain unknown, we conducted a preliminary experimental study in rats focusing on the effects of chronic ASB consumption on vascular function. Indeed, vascular dysfunction is a well-recognized, early biomarker of atherosclerosis and a key contributor to the onset and progression of CVD. It could therefore explain health issues associated with AS consumption.

All animal experiments were performed according to the European Parliament Directive and approved by the local research ethics committee. Adult Wistar rats (Janvier Labs, France) underwent 10 weeks of a normal chow diet (A04, SAFE, France), supplemented by ad libitum water or ASB. Sucralose (Su) and acesulfame potassium (AceK), both high intensity sweeteners were used in this protocol since they are the most used sweeteners in overall foods and beverages (accounting for 62 % of the global artificial sweetener market). To assess the health effect of a daily consumption of artificial sweeteners, we provided Su and AceK at low (LD-ASB) concentrations, closely following Young et al. study that reported ASB metabolic impact in human and corresponding to moderate user consumption (1 and 2 mg.kg⁻¹.day⁻¹, respectively).[14] ASB effect at a high (HD-ASB) dose was also studied, reaching the maximal limit of Acceptable Daily Intake (ADI), (15 mg.kg⁻¹.day⁻¹ for both

Su and AceK) respectively.[15] At the end of the diet period, rats were sacrificed to collect adipose tissues and aorta (Figure 1, A). Aortic rings (2mm) were placed into an isometric force transducer. After a one-hour incubation in either normoglycemic (11 mM) or hyperglycemic condition (100 mM), rings were constricted with phenylephrine and endothelium-dependent vasodilatation was tested using increasing doses of Acetylcholine (ACh). For comparison of multiple experimental conditions, analysis of variance (ANOVA) or repeated measures ANOVA followed by the Tuckey test were used. A value of $p < 0.05$ was considered statistically significant. Statistical analysis was done using GraphPad Prism version 8 (GraphPad Software Inc.).

No difference was observed regarding vasodilatory function in normoglycemic condition. However, after one-hour hyperglycemic stress, endothelial function in both ASB groups was impaired compared to control group, although statistical significance was reached only with HD-ASB (Figure 1, B). When expressed as a difference between NG and HG conditions, we observed a two-fold higher decrease in maximal relaxation in HD-ASB than in water group (Figure 1, C). Interestingly, we reported significant changes in adipose tissue deposition of both ASB groups, with increased sub-cutaneous and visceral perirenal fat mass (Figure 1, E), while total body weight remained comparable between the three groups (Figure 1, D).

As part of a weight loss strategy in overweight and obese individuals and in patients with glucose intolerance or type 2 diabetes, several health authorities or medical practitioners recommend the use of ASB consumption.[16] Nevertheless, there is no consensus regarding the safety of these molecules and further studies are needed to understand their metabolic impact on healthy subjects or patients with cardiometabolic disease. Our experiments conducted in rats showed, for the first

time, that commonly used AS in food or drinks (sucralose and acesulfame potassium) induced a decrease in endothelium-dependent vascular function in a hyperglycemic condition. This is of high interest, since we recently demonstrated that acute hyperglycemic stress induced by a commercial sugary drink impaired vascular endothelial function reactivity, in healthy young adults, thus confirming the high sensitivity of vascular function to disruption of glucose metabolism.[17] Therefore, the present study suggest that AS consumption could increase the vascular dysfunction in response to a glucose load. Moreover, this vascular dysfunction was also associated with a significant modification in adipose tissue repartition in the body, supporting the hypothesis that AS are able to affect body homeostasis, potentially explaining the increase in cardiometabolic risk observed in epidemiological studies.[9–11] However, the literature is not clear about AS effects on adipose tissue, describing deleterious or neutral effects depending on the study.[18,19] In human overweight and obese population, recent work described positive effects of AS, by limiting weight gain.[20] Whether these changes are found in humans by direct or indirect effect of AS on vascular function, and whether age and sex may affect the clinical findings[4,12], remain to be determined. The discrepancies observed between human and animal studies suggests that more studies are needed to understand AS cardiovascular effects in healthy population but also in patients with metabolic disease.

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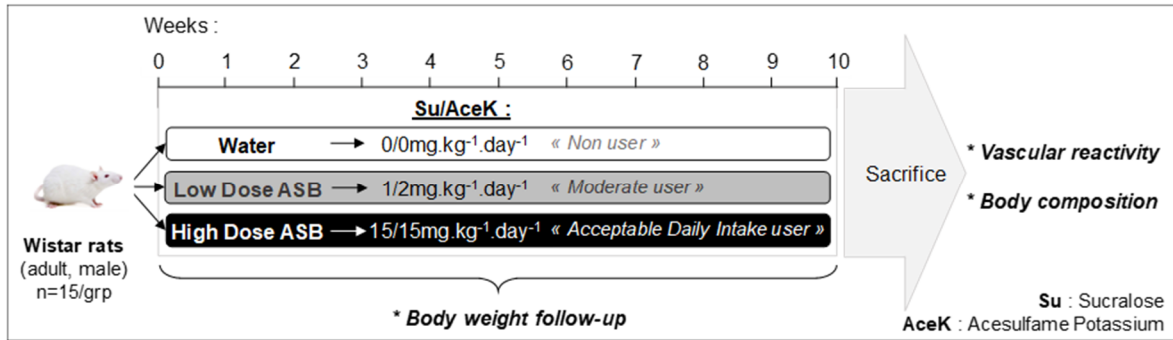
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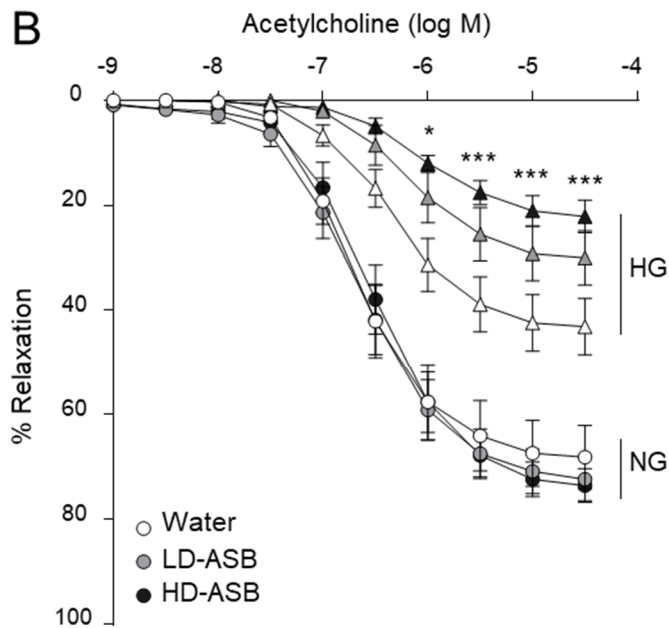
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Figure 1. Impact of chronic artificial sweeteners consumption, at moderate and Acceptable Daily Intake, on endothelial dysfunction during acute hyperglycemia. A, Diet protocol for rat fed with normal chow and consumed water supplemented or not (Water) with artificial sweeteners at low (LD-ASB) or high (HD-ASB) dose, respectively mimicking moderate and Acceptable Daily Intake consumption. After ten weeks of diet and body weight follow-up, animals are sacrificed for vascular reactivity and body composition assessment. B, Endothelium-dependent relaxation induced by acetylcholine (ACh) in rat aorta following one hour of normoglycemic condition (11 mM glucose) or hyperglycemic stress (100 mM glucose). C, Maximal relaxation delta between NG and HG condition. D, Body weight following ten weeks diets. E, Body weight percentage of visceral (perirenal, epididymal and inguinal), subcutaneous and brown adipose tissue. Data are represented as Mean \pm SEM, * $p < 0,05$, ** $p < 0.01$, *** $p < 0.001$.

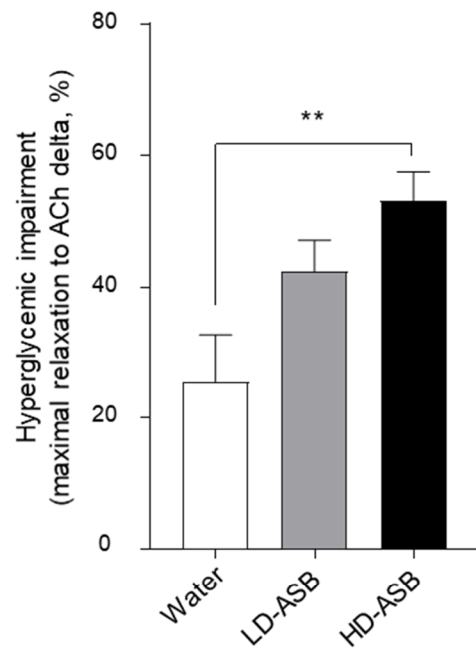
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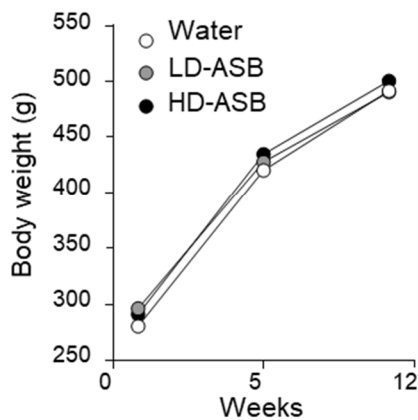
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C



D



E

