

(続紙 1)

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論文題目	Exploration of fibroblast growth factor 21-inducing rare sugars for the prevention of diet-induced obesity （線維芽細胞増殖因子21を誘導する希少糖による食餌性肥満予防の探索）		
（論文内容の要旨） Obesity is acknowledged as a global epidemic that has been rising over the years. Without comprehensive strategies, obesity adds a heavy burden to both healthcare system and socio-economic aspects of the affected communities. Overeating is a key factor for obesity progression. Specifically, the intake of dietary simple sugar as a rewarding food component promotes overeating and therefore obesity. Continuous sugar intake is detrimental to obesity development. Alternatively, low-calorie natural sweeteners such as rare sugars are used as the dietary simple sugar substitute for the purpose of replacing sweet taste. On the other hand, following sugar intake, a negative feedback mechanism exists to suppress further sugar intake. This mechanism consists of hepatic fibroblast growth factor 21 (FGF21) and oxytocin neuron in the paraventricular nucleus of the hypothalamus (PVH^{Oxt}). However, the ability to stimulate this negative feedback system had not been explored for the purpose of developing dietary simple sugar substitute. Rare sugars are group of monosaccharides that possess similar sweetness to the dietary simple sugar but unmetabolized in the body. Therefore, they are as rewarding as dietary simple sugar, but contribute little to no caloric value, which is safe for obesity progression. Inclusion of rare sugars into the diet has been reported to be beneficial for tackling obesity but the mechanism remains elusive. In this study, rare sugars are hypothesized to exert their anti-obesity property through the activation of FGF21-PVH^{Oxt} negative feedback mechanism, thereby suppressing sugar/sweet appetite. In chapter 1, FGF21-inducing rare sugars were searched among the selections of rare sugars using primary mouse hepatocyte. The identified sugars were then used to assess their sugar/sweet appetite-suppressing ability in C57BL/6 mice. In chapter 2, the mechanism underlying <i>Fgf21</i> expression by rare sugar induction was investigated using primary mouse hepatocyte. Finally, in chapter 3, the effect of chronic consumption of the identified rare sugars against diet-induced obesity (DIO) was evaluated in C57BL/6 mice that had been fed with high-fat high-sucrose diet (HFHSD). Some rare sugars are the epimers of dietary simple sugars such as glucose and fructose. Therefore, it is likely that rare sugars can induce FGF21 secretion. Forty-six rare sugars were screened in primary mouse hepatocytes. Among these, D-allulose, D-tagatose, and D-sorbitol were the FGF21-inducing rare sugars with higher capacity than D-glucose or D-fructose. Administration of single dose of these rare sugars to mice activated more PVH^{Oxt} and suppressed sweet appetite in C57BL/6 mice. The suppression was also observed when these sugars were mixed with sucrose solution. It was confirmed through lick microstructure analysis of the mice, that the inclusion of these rare sugars prolonged the interval of sucrose ingestion, while decreasing the frequency of sucrose ingestion, indicating the increasing replete state and suppression of sugar/sweet appetite in mice, respectively (chapter 1). D-Allulose, D-tagatose, and D-sorbitol were confirmed to induce FGF21 secretion, but the mechanism remained elusive. D-Glucose, D-fructose, and D-xylitol were previously reported to induce <i>FGF21</i> expression through the regulation of carbohydrate response element binding protein (ChREBP). With structure similarities among these sugars, it was assumed that the			

identified FGF21-inducing sugars shared the same *FGF21* expression regulation, as previously reported sugars. Analyses using mouse *Fgf21* promoter revealed that D-allulose, D-tagatose, and D-sorbitol required an intact *Fgf21* promoter region that does not contain carbohydrate response element (ChoRE). The mutation in this region significantly reduced *Fgf21* promoter activation by rare sugars. It was confirmed that activating transcription factor 4 (ATF4) can bind to this region, and knocking-down this transcription factor significantly reduced *Fgf21* expression by the rare sugars. Therefore, transcriptional regulation of *Fgf21* by rare sugars is different from that by dietary simple sugars (chapter 2).

Short-term administration of FGF21-inducing D-allulose, D-tagatose, and D-sorbitol suppressed sugar/sweet appetite in C57BL/6 mice. To understand their chronic effect on DIO progression, rare sugar solution was given to C57BL/6 along with HFHSD for one month. The supplementation was effective to prevent diet-induced weight gain and reduced adiposity in the mice. It also improved obesity-associated phenotypes such as insulin resistance and glucose intolerance, with varying degrees. However, the supplementation was not effective in addressing an established DIO in mice, which had been fed with HFHSD for 4 months prior to the supplementation. It was found that continuous intake of HFHSD for 2 months could desensitize mice from FGF21-inducing rare sugar. Therefore, rare sugars are effective alternatives for DIO prevention, but not restoration.

In conclusion, rare sugar D-allulose, D-tagatose, and D-sorbitol are FGF21-inducing rare sugars. The intake of these sugars can significantly suppress sugar/sweet appetite, and they are effective for DIO prevention. Mechanistically, these sugars induce FGF21 secretion via the regulation of ATF4 and subsequently activate PVH^{Oxt} to suppress sugar intake. Since these sugars are generally recognized as safe (GRAS) and affordable, they are accessible to all communities.

注) 論文内容の要旨と論文審査の結果の要旨は1頁を38字×36行で作成し、合わせて、3,000字を標準とすること。

論文内容の要旨を英語で記入する場合は、400～1,100 wordsで作成し
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(論文審査の結果の要旨)

世界中で蔓延している肥満の原因の一つとして、シヨ糖の摂取過剰が挙げられる。糖分摂取のネガティブ・フィードバック制御を担う機構として、糖摂取時に肝臓から分泌されるFGF21が、視床下部オキシトシン神経を活性化し、糖分に対する食欲を抑制することが知られていた。しかし、同機序を刺激する食品成分、その分泌誘導機序、および、その抗肥満効果は未解明であった。本論文では、シヨ糖を代替し得る食品成分を細胞実験により同定し、マウスにおいて生体作用およびその抗肥満効果を、細胞を用いて作用機序を解析した。評価すべき主要な点は以下の3つに要約される。

1. FGF21分泌を誘導する希少糖として、D-アルロース、D-タガトース、D-ソルビトールの3種類を同定した。これらの糖をマウスに投与すると、FGF21の血中濃度が上昇し、FGF21の標的神経細胞である視床下部室傍核のオキシトシン神経を活性化できることを明らかにした。また、マウスにおいて、糖分に対する食欲を抑制し、糖溶液の摂取回数の減少、および、摂取間隔の延長効果を示すことを明らかにした。
2. 同定した3種類の希少糖によるマウス*Fgf21*遺伝子の発現誘導には共通したメカニズムがあり、転写因子ATF4が転写開始点の上流1,027 bpに存在するアミノ酸応答配列(AARE)に結合することによって起こることを明らかにした。
3. 同定した希少糖の抗肥満効果を比較し、アルロースが最も効果的であることを明らかにした。しかし、既に太ったマウスではFGF21抵抗性のために、肥満を是正する効果は認められなかった。すなわち、希少糖アルロースは、食餌性肥満に対して予防効果を示すが、治療効果は示さないことを明らかにした。

以上のように、本論文では、FGF21を誘導する希少糖によるFGF21発現の誘導メカニズム、その糖質嗜好性抑制効果、および、それに伴う抗肥満効果を明らかにした。これらの成果は糖分の摂り過ぎの是正および食事性肥満の予防法の新たな可能性を示すものであり、栄養化学、食品生理機能学、食品科学に寄与するところが大きい。

よって、本論文は博士(農学)の学位論文として価値あるものと認める。

なお、令和8年1月20日、論文並びにそれに関連した分野にわたり試問した結果、博士(農学)の学位を授与される学力が十分あるものと認めた。

また、本論文は、京都大学学位規程第14条第2項に該当するものと判断し、公表に際しては、当該論文の全文に代えてその内容を要約したものとすることを認める。

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