An environmentally-friendly perlecan protein expression

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Abstract. The risk of high sugar level exposure can affect almost all parts of the body, organ systems, tissues, to the cellular level. The exposure to high sugar level caused disturbances in the joints in the form of changes in the microcellular environment. It will cause negative impact to microcellular component of articular cartilage, which increased the risk of joint degradation. Present study was to investigate excess carbohydrate diets on perlecan protein expression and Transforming Growth Factor alpha (TGF-ᾳ) in articular cartilage which still unclear, which perlecan function to chondrocytes articular cartilage was to support regeneration of articular cartilage by increasing the secretion of anabolic factors by chondrocytes, so the progressivity of articular cartilage damage decreased and the risk of osteoarthritis disease can be lowered too. Function of TGF-ᾳ expression was opposite to perlecan protein expression. Methods: six groups of eighteen adult’s male rats were divided into: control (K0), lower diet 1 (A), lower diet 2 (B), middle diet (C), over diet 1 (D), over diet 2 (E). Control group were not performed Anterior Cruciate Ligament Transection (ACLT), but others group were performed ACLT to develop osteoarthritis condition for two months’ maintenance. Perlecan protein expression, and expression of TGF-久しぶり were analyzed using Western Blotting method. Perlecan protein expression was fluctuate stable at group (a) < (b) compare to control group (K0), but decreasing at group (e) < (d) < (c) compare to control group, which opposite to increasing carbohydrate intake dose. TGF- العلي as catabolic factor was fluctuate increasing at group D > group C > E compare to control group, but slightly stable at group A and B. Interaction of perlecan and TGF- العلي as one of others factor which determine cartilage health under hyperglycemia condition. Results and discussion: This aim as early research as a based to next research for accomplish of pathogenesis of osteoarthritis caused by hyperglycemia. Present study concludes that hyperglycemia was influence to cartilage health due to changing of perlecan protein expression and TGF- العلي expression.
1. Introduction
Indonesians take carbohydrate diets as dominant component which is accompanied by other components covering, such as: vegetable-animal proteins, fats, minerals, vitamins, and waters. Excess carbohydrate diets increase of raising blood sugar levels. It will cause diabetes (diabetes mellitus). Today, the population of diabetes in the world, particularly in Indonesia, is increasing. Data of WHO in 2000 mentioned that there were 171,230,000 people with diabetes in the world. Indonesia was in the fourth rank with 8,426,000 diabetes sufferers. It continued to form osteoarthritis disease. In 2030, the number of people with diabetes in Indonesia is expected to increase to 21,257,000 people, so the risk of osteoarthritis disease increased, along with all the financial losses due to the morbidity of the disease and decreased work productivity caused by this disease. Excess carbohydrate diets will result in the exposure of high sugar level on the joint surface.

Chondrocytes as permanent resident of articular cartilage which produce many components of articular cartilage, such as major component: water (65-80%), collagen type II (10-20%), aggrecan (4-7%), glycosaminoglycans and hyaluronan, was weaved surrounding chondrocytes [1], minor component: Collagen type V, VI, IX, X, XI, XII, XIV, decorin, biglykan, fibromodulin, perlecan; non collagen protein: matrins, trombospondin-5/ COMP [2]. The chondrocytes production according to homeostasis of microenvironment of articular cartilage matrix, many influence was involved. External influence, such as: biomechanical factors, biochemical factors, biophysical factors. Internal influence, such as: chondrocytes mechanotransduction, anabolic factors, catabolic factors. Also involved of chondrocytes genetics factor. All above influence makes dynamical homeostasis to microenvironment of articular cartilage matrix. Homeostasis condition was one determined by Heparan Sulphate ProteoGlycan 2 (HSPG 2) also known as perlecan. Previous study of perlecan changing at endothelial aortic by hyperglycemia influence [3], which it shown that perlecan was reducing one of forth Heparan Sulphate (HS) chain. This perlecan changing by hyperglycemia influence not clear at articular cartilage matrix. Since perlecan was needed to chondrocytes as mechanoanducer [4], also to determine chondrocytes differentiation phase [5]. Perlecan was measure as anabolic factor of chondrocytes, also measure TGF-α as catabolic factor. Aim of present study as fulfil research gap of perlecan changing by hyperglycemia treatment at articular cartilage matrix, which can be used as basic to next research.

2. Method
Animal models divided six groups of eighteen adult’s male rats (Rattus norvegicus strain Wistar), 2-3 months old, 160-200 g body weight without physical anatomic defect or in healthy condition. The groups were: control (K0), lower diet 1 (A), lower diet 2 (B), middle diet (C), over diet 1 (D), over diet 2 (E). control group not performed Anterior Cruciate Ligament Transsection (ACLT), the fifth treatment groups were performed ACLT to develop osteoarthritis condition for two months maintenance, which given animal pelet (product of Comfeed®), once a day at evening for 30 g/ each animal model. After two months of ACLT, fifth groups of treatment were receiving group A received lower diet 1 (0.50 mg/kg of body weight); group B received lower diet 2 (0.75 mg/kg of body weight); group C received middle diet (1.00 mg/kg of body weight); group D received over diet 1 (1.50 mg/kg of body weight); group D received over diet 2 (2.00 mg/kg of body weight). All group of fifth was receive excess sugar by esophageal sonde three times a day at evening, for two months’ maintenance. After forth months of research period, perlecan protein expression, and expression of TGF-α were analyzed using Western Blotting method.

Ethics: This study had approved by research ethics committee of Brawijaya University, Malang, East Java, Indonesia.

3. Results and discussion
Treatment for five groups by increasing of sugar intake dose, as a representative of excess carbohydrate intake. This condition was maintaining for two months and the blood sugar was measure at the end of experiment time. The blood sugar level was listed at table.1 and it shown fluctuate data of blood sugar level. It caused by adaptive respond of sample individual was involved.
Table 1. Blood sugar level of control group and five control of treatment.

| Blood group (mg/dl) | Control group | A group | B group | C group | D group | E group |
|---------------------|---------------|---------|---------|---------|---------|---------|
| Sugar level         | 86.00         | 142.00  | 160.50  | 127.00  | 200.30  | 184.00  |

Result of treatment groups shown that excess of carbohydrate diets was influence to perlecan protein expression, also to TGF-α expression. Perlecan protein expression was decreasing was mention at figure 1.

Figure 1. Perlecan protein expression by Western Blot method, slightly decreased according to increasing of sugar intake.

Result data at figure 1 shown that perlecan protein expression was decreasing, but only at B group the expression was increasing, is caused by genetic variant factor of three samples at this group. Generally, protein perlecan expression was decline according to increasing of sugar intake. The TGF-α result data was mention at figure 2, it shown that TGF-α as catabolic factor was correlated to increase of sugar intake.

Figure 2. TGF-α expression by Western Blot method, slightly increased according to increasing of sugar intake.

Result of TGF-α was shown increasing fluctuate, at group B and E was not increased according to increase of sugar intake. Condition TGF-α expression at group B was similar to condition perlecan protein expression at group B, which not correlated to another group result. Generally, result group was: Perlecan protein expression was decreased, and TGF-α expression was increased according to increasing of sugar intake.

Group B has unique result data which significantly according to increasing of sugar intake, it can be caused by many chondrocytes apoptotic due to increasing of sugar intake dose, so production of TGF-α not so high.

Result data above, can be generated that hyperglycemia caused decrease of perlecan protein expression was not decreased and TGF-α was not increased too when the sugar intake dose was
increasing. The condition was caused by genetic variant factor of three samples. This sample has endurance to hold hyperglycemia, not to badly impact to perlecan protein expression and to TGF-α expression. So it can hold the increasing risk of articular cartilage matrix damage due to hyperglycemia, which can be lowering the risk of osteoarthritis disease occur. The result of TGF-α expression at group E was not increase protein expression and increase of TGF-α expression. Decreasing of perlecan protein expression badly impact to articular cartilage matrix, because perlecan act as chondrocytes mechanotransduction which stimulate chondrocytes to produce anabolic factor [6].

When the anabolic factor production was over the production of catabolic factor, the articular cartilage matrix growth and can be sustainable. Increasing of TGF-α expression was not good for cartilage health, because it stimulates chondrocytes at hypertrophy phase to produce more catabolic factors which can caused articular cartilage degradation by TGF-α [7]. If the hyperglycemia was continue furthermore, it can induce diabetic condition, which can have caused osteoarthritis disease occur [8]. Previous study had mention to that osteoarthritis disease at first was known as degenerative disease [9], then continued upgrading research of osteoarthritis known as inflammatory disease [10]. Now osteoarthritis is known as metabolic diseases [11]. Once osteoarthritis as metabolic disease, need to be preventive by avoid what kind of condition which act as trigger factor of this disease. Present study result as complement to pathogenesis of osteoarthritis disease.

4. Conclusion and conflicts of interest

Present study result was shown that hyperglycemia as trigger factor of metabolic disease which caused damage of articular cartilage matrix. Decreasing of perlecan protein expression and increasing of TGF-α expression, according to excess carbohydrate intake. Before diabetic occur, the negative impact was happen to homeostatic disturbances, which threaten the integrity of articular cartilage matrix. So early prevention of hyperglycemia condition was needed to prevent osteoarthritis, by avoid excess carbohydrate diets or reducing sugar intakes.

All authors declare no conflict of interests of present research related to the study presented in this manuscript to publish.

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