Lifetime macronutrient intake alters structural and functional risk factors for diabetic kidney disease.

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Introduction

Globally, the prevalence of chronic kidney disease (CKD) is estimated to range from 8-16%, up to 50% of which is attributable to diabetes (DKD) (1). CKD is characterised by changes in glomerular filtration rate (GFR) and structural abnormalities such as tubulointerstitial fibrosis (TIF) and glomerulosclerosis (GS). Many of the classical risk factors for CKD, including diabetes, being overweight or obese, hypertension and dyslipidaemia are modifiable by diet (1). The influence of consumption of specific macronutrient combinations on kidney function and structure, over the course of ageing has not been previously investigated. Therefore, this study uses the novel geometric framework (2) to explore the effects of different dietary macronutrient ratios on the structure and function of the kidney in ageing mice.

Aim: To determine the effects of dietary macronutrient intake on risk factors for diabetic kidney disease and kidney health in ageing mice.

Methods

The geometric framework study design	Assessed markers of kidney disease	Data analysis
<p>Randomised to 1 of 25 diets; Systematically varied in macronutrient composition, at 3 tiers of caloric intake</p> <p>Example diets</p> <p>High calorie Med calorie Low calorie</p> <p>Mice remained on their diet till 15 months of age Food consumption measured weekly</p> <p>Kidney analysis</p>	<p>Glomerular sclerosis (GS) GS, the expansion of mesangial matrix and formation of lesions in the glomeruli, was assessed histologically in 20 glomeruli per kidney. Glomeruli were scored based on the percentage of mesangial matrix expansion, as per the examples below.</p> <p>Score 0 0% Score 1, 1-25% Score 2, 26-50% Score 3, 51-75% Score 4, 76-100%</p> <p>Fibrosis % interstitial fibrosis of the kidney cortex was assessed histologically using Masson's trichrome stain. 10-20 fields of view per kidney were assessed.</p>	<p>Glomerular filtration rate (GFR) Serum cystatin C, a surrogate marker of GFR was measured by ELISA. Cystatin C was used as it is not influenced by age, gender, muscle mass or diet. Cystatin C is an inverse marker of filtration rate.</p> <p>Additional measures Gene expression of renal klotho was assessed using the Taqman platform, gene expression was normalised to housekeeping gene 18s. Blood pressure, blood lipids, HDL/LDL cholesterol, Body Composition had been assessed previously (published, (2))</p> <p>Generalised additive models (GAMs), a non parametric extension of generalised linear models, and thin plate splines were used to analyse and visualise the response variables (markers of kidney structural and functional changes) to protein, carbohydrate and fat intake. These were plotted using the mgcv package in R. Each mouse was plotted in a 3D nutrient space based on actual consumption of each macronutrient and the response variable superimposed on top of this. These are visualised as 3 heat map surfaces in 2D, sliced through the 3D model, each representing two of the macronutrient axes whilst holding the third macronutrient constant.</p> <p>Example of the 3D heat maps on 2D surfaces. Image adapted from (Solon-Biet, McMahon et al. 2014) (2).</p> <p>Example of how 2D surfaces fit within 3D nutrient space. Image adapted from (Solon-Biet, McMahon et al. 2014) (2).</p>

Results

Structural changes to the kidney

Dietary consumption of energy from protein increased kidney size

Glomerular sclerosis associated with fat consumption

Fibrosis increased with low protein + high or low carbohydrate

Functional markers of DKD

GFR is associated with protein intake

Surfaces showing the relationship of macronutrient to serum cystatin C, figure a), and serum cystatin C adjusted for lean mass, figure b). Cystatin C ranged from 126 to 1006 ng/ml ($N=153$). Increased protein consumption was associated with increased GFR (lower cystatin C), with mice consuming less calories from protein and more calories from carbohydrates having the lowest GFR. Both protein and carbohydrate as independent variables were highly significant ($P < .01$). When adjusted for lean mass, only protein remained significant as an independent variable ($P < .0001$), while protein and carbohydrate were found to be significant only as interacting variables ($P = .005$), $N=145$.

Renal klotho (relative gene expression)

Mice consuming a greater proportion of calories from protein showed the highest levels of renal klotho expression ($p = .05$). Neither fat nor carbohydrate were found to influence renal klotho expression, $N=151$.

Known risk factors for kidney disease do not explain the observed renal changes

Previously published figures adapted from Solon-Biet, McMahon *et al.* 2014, (2). Surfaces showing cardiometabolic risk factors for kidney disease; hypertension (BP), dyslipidaemia (triglycerides, HDLc, LDLc), glucose tolerance and adiposity. BP, glucose (area under the curve for i.p.GTT) and triglycerides did not show an association with the renal parameters examined. LDL and triglycerides showed an inverse relationship with GFR.

Conclusions

- The ratio of macronutrients in the diet appears to impact on structural and functional changes in the kidney, with macronutrients contributing both as independent and interacting factors.
- Lower dietary protein consumption was found to be associated with lower GFR, more fibrosis, lower renal klotho expression and smaller kidney mass. This was particularly evident in the context of compensatory high fat or high carbohydrate consumption to make up the caloric intake.