2ª ed

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Estimation of protein intake and its impact in Esmoriz type 2 diabetics' renal function

Isabel Augusta Monteiro Tomás

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Acronims

ACEI – Angiotensin converting enzyme inhibitor

ACSS – Administração Central do Sistema de Saúde

BMI – Body Mass Index

DCCT – Diabetes Control and Complications Trial

CKD – Chronic kidney disease

DM2 – Diabetes mellitus tipo 2

GDH – Grupo de Diagnósticos Homogéneos

GFR – Glomerular Filtration Rate

HbA1c – Hemoglobina glicada A1c

IDF – International Diabetes Federation

INSA – Instituto Nacional de Saúde Dr. Ricardo Jorge

MDRD – Modification of Diet in Renal Disease

MEDS – Mestrado de Evidência e Decisão em Saúde

MGF – Medicina Geral e Familiar

USF – Unidade de Saúde Familiar

WHO – World Health Organization

Sumário

Introdução: A diabetes tipo 2 é uma patologia com uma prevalência de 11,7% na população portuguesa entre os 20 e os 75 anos. O seu controlo é um dos objectivos dos cuidados de saúde primários.

Objectivos: Calcular a média de estimativa de ingestão proteica em diabéticos tipo 2 da USF da Barrinha com e sem microalbuminúria. Calcular a diferença de médias da estimativa de ingestão proteica nos diabéticos tipo 2 com microalbuminuria e nos diabéticos tipo 2 sem microalbuminuria.

Material e métodos: Foi realizado um estudo *nested case-control* numa *coorte* de diabéticos tipo 2 da USF da Barrinha durante o ano de 2011. O protocolo deste trabalho foi aprovado pela comissão de ética do centro de saúde são João em maio de 2011. Durante a consulta de diabetes foi pedida a microalbuminuria e a ureia na urina de 24 h. A estimativa de ingestão proteica foi calculada com a aplicação de um calculador *online*. Foi calculada a diferença de médias da estimativa de ingestão proteica entre diabéticos tipo 2 com e sem microalbuminuria. Foi aplicado o teste t para significância estatística.

Resultados: A diferença de médias entre diabéticos tipo 2 com e sem microalbuminuria foi de -- 0,24 $\pm$ 0,76 g ($p>0,05$).

Discussão: A restrição da ingestão proteica em diabéticos com GFR<60 está recomendada pela IDF. Estudos recentes não mostraram preservação da função renal em utentes com restrição proteica.

Conclusão: Os diabéticos com microalbuminuria têm menor estimativa de ingestão proteica.

Abstract

Introduction: Type 2 diabetes has a 11,7% prevalence in portuguese population between 20 and 75 years. The monitoring of this pathology is one of the goals of primary care.

Objectives: To calculate average protein intake estimate in type 2 diabetics of USF da Barrinha with and without microalbuminuria. To calculate the mean difference between protein intake estimate in type 2 diabetics with and without microalbuminuria.

Material e methods: An nested case-control study was performed in a type 2 diabetic coorte from USF da Barrinha during 2011. This study was approved by Ethics Committee of Centro de Saúde São João in may 2011. During diabetes appointment it was asked microalbuminuria and urea in 24h urine. Protein intake estimate was assessed with an online calculator. The mean difference between protein estimate intake in type 2 diabetics with and without microalbuminuria was calculated. T test was applied to assess statistical significance.

Results: The mean difference between protein intake estimate in type 2 diabetics with and without microalbuminuria was -0,24 $\pm$ 0,76 g/day ($p>0,05$).

Discussion: IDF recommends protein intake restriction in diabetics with GFR < 60. Recent studies haven't shown renal function preservation with protein intake restriction

Conclusion: Diabetics with microalbuminuria have lower estimate for protein intake.

Rationale

Diabetes complications are an important consumer of time and resources in Health Care.

Between 2000 and 2008 diabetic hospitalizations in Portugal by genito-urinary complications increased from 5 to 7%. In this period hospitalizations caused by renal dysfunction increased from 6 to 8%. In 2006, according a cohort study of INSA, 0,2% type 2 diabetics over 25 years old were in hemodialysis. In 2008, according data of GDH of ACSS, 2,5% of the patients who were discharged from hospital care with diabetes diagnosis, left with renal dialysis. In the annual report of 2008 there were 25% of diabetics in the population who were in hemodialysis. Diabetes costs 0,7% of portuguese PIB and takes 7% of portuguese expense with health.

Protein intake is another risk factor associated to the development and progression of diabetic nephropathy, aside HbA1c, blood pressure, BMI and years since diabetes diagnose, usually monitored in diabetics. Studies aren't consensual concerning higher deterioration of renal function in diabetics with higher protein intake. However *Diabetes International Federation* still recommends protein restriction to preserve diabetic renal function.

Protein intake is difficult to quantify. Several studies have been published in this area using questionnaires with good predictive results.

In this work I´ve chosen to use an analytical value to assess an estimate of protein intake, using a mathematical formula. Thus, considering metabolization and excretion of proteins in human body, I found a formula based on Maroni's method (1985), validated in 2002, which allows us to estimate daily protein intake by measuring urinary urea in 24h urine. This formula is quite accurate in predicting protein intake since fecal nitrogen is minimal and its disregard in estimating protein intake is not considered incorrect.

Diabetics' diet is theoretically very strict (low sugar, fat and salt), but protein restriction is sometimes forgotten in the advices given to diabetics.

Considering that it is easier to intervene in the problem after recognizing it, it is essential to study the population to show benefits of simple preventive measures.

Intending to evaluate the influence of protein intake in diabetic renal function a mathematical formula based in 24h urinary urea was applied. I've proposed to determine if average protein intake in diabetics with microalbuminuria was higher than in diabetics without microalbuminuria.

There for the results of this study may influence the dietary advices to diabetics in order to prevent renal function deterioration.

1. Introduction / Motivation

The prevalence of microalbuminuria and the decrease in GFR increased between 1988 and 2004. The prevelence of chronic renal disease in stages 1 to 4 has been increasing from 10,0% in 1988-1994 to 13,1% in 1999-2004. The estimate of prevalence of stages of chronical renal disease in 1988-1994 and in 1999-2004, were respectively 1,7% and 1,8% to stage 1; 2.7% and 3.2% to stage 2; 5.4% and 7.7% to stage 3; and 0.21% and 0.35% to stage 4.

Diabetics with microalbuminuria eat higher amounts of proteins (20.5 $\pm$ 4.4 *vs.* 19.0 $\pm$ 3.5% of total energy intake; *p* 0.01). One model of multivariate logistic regression ajusted to age, gender, blood pressure, fasting glicemia, protein intake showed a positive association with microalbuminuria (OR 1.104; 95% CI 1.008 –1.208; *p* 0.032). The reduction of animal protein intake may reduce the risk of microalbuminuria.

On the other hand, a metanalysis of 2008 showed that a low protein intake is not associated with significant improvement of renal function in diabetics. Diabetics don´t comply with protein restriction. A study showed that protein restriction inicially proposed wasn't held by the end of the study, by the 12th month it was inexistant. This shows that protein restriction isn´t possible neither effective. A reduction of protein intake in ambulatory patients with nephropathy is difficult to accomplish, never the less even a small reduction has a high impact in reducing albuminuria. By the end of the 12th month of the study there was a reduction of 28% in albuminuria in the group with protein restriction compared with 18% reduction in the froup without protein restriction. Two or more meat portions, quantified by a questionnaire, were associated with microalbuminuria ((OR) 1.51, 95% CI 1.01-2.26).

A formula based on Maroni's method (1985) can be used to estimate protein intake from 24h urinary urea:

Estimated protein intake (g/d) = 6.25 × (Urine urea nitrogen (g/d) + 0,30 g/kg/d × Weight (kg).

This formula can be applied to estimate protein intake in adults with stable nitrogen balance, relentless renal function stage. Fecal nitrogen isn't related with protein intake according with 31 studies.

2. Objective

To calculate average protein intake in type 2 diabetics with microalbuminuria (30-300 mg/24h) and in type 2 diabetics without microalbuminuria in Esmoriz type 2 diabetics, ajusted to gender, BMI, HbA1c, blood pressure, years since diabetes diagnosis.

To calculate the media difference of protein intake estimate in both groups.

3. State of the art

IDF has a recomendation since 2005 in order to restrict protein intake in diabetics to preserve renal function. Recent studies showed that protein intake has no impact in renal function. To assess this apparent contradiction it was performed an electronic search on 5th October of 2010 in Pubmed and SCOPUS.

The search key used in Pubmed was the following: ("Diabetes Mellitus, Type 2"[Mesh] AND "Diabetic Nephropathies"[Mesh]) AND "Diet, Protein-Restricted"[Mesh]; com os limites Humans, English, French, Italian, Spanish, Portuguese, All Adult: 19+ years.

Eleven articles were retrieved, two were excluded by the title, four were excluded because the article wasn´t available, five of them were read and included in this review.

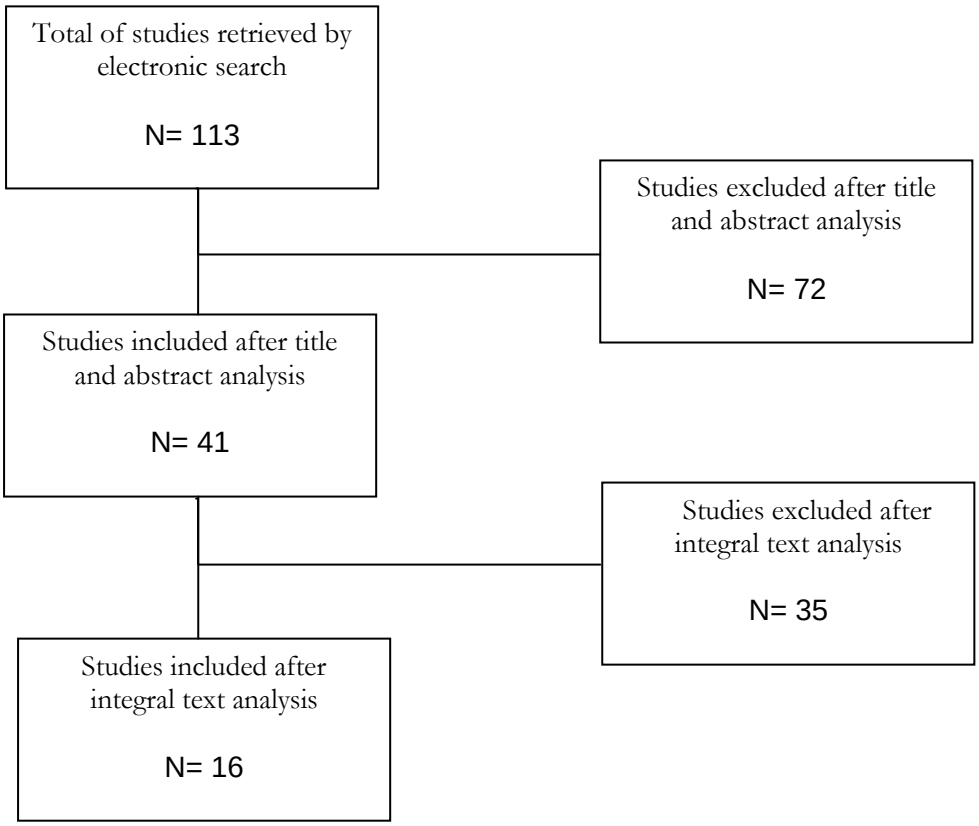
Another search key was created in order to obtain more articles: "diabetes" and "urinary urea" and "protein intake"; limited to Humans, English, French, Italian, Spanish, Portuguese, All Adult: 19+ years.

Ten articles were obtained. Two were excluded by the title and the other five were excluded after reading the abstract. Two of these studies had already been selected in the previous search. One article was included.

The search key used in SCOPUS was the following: Diabetes Mellitus Type 2 AND (Diabetic Nephropathies or microalbuminuria or proteinuria or renal) AND (Protein-restricted diet or protein intake) and human and adult, limited to medicine area (LIMIT-TO(SUBJAREA, **"MEDI"**) OR LIMIT-TO(SUBJAREA, **"MULT"**) AND (LIMIT-TO(LANGUAGE, "English") OR LIMIT-TO(LANGUAGE, "French") OR LIMIT-TO(LANGUAGE, "Spanish")).

Ninety two articles were obtained. Fourty six were excluded by the title. Five were excluded because the article wasn´t available. Twenty eight were excluded after reading the abstract. Three were already included buy the previous search. Ten were read and included in this study.

Since there are studies available on other search engines that might have some interest to the present study, it was performed a search in *Google scholar* in 15th January of 2011 with the words “protein intake” “diabetes” “nephropathy”, which retrieved 23 articles which were included in this study.


Total of studies retrieved by electronic search
N= 113
Studies excluded after title and abstract analysis
N= 72
Studies included after title and abstract analysis
N= 41
Studies excluded after integral text analysis
N= 35
Studies included after integral text analysis
N= 16

Study (1st author, year)	Local	Study type	Participants	Intervention	Outcome	Results
Pijls, 1999	Netherlands	Observational	354 type 2 diabetics	Food frequency questionnaire	Urinary urea excretion	Food frequency questionnaire has good reproducibility, and to a very moderate extent reflected actual protein intake
Ascic, 2008	Bosnia	Observational	15 type 2 diabetics	-	Albuminuria	Glycaemia, blood pressure, cholesterol, ACEI, protein and salt restriction, smoking cessation can delay nephropathy progression
Higashiyama, 2009	Japan	Observational	7404 individuals	-	GFR	The higher protein intake was associated with higher GFR in both sexes and low prevalence of CKD in women.
Narita, 1999	Akita university hospital, Japan	Case-control	10 type 2 diabetics with microalb 10 type 2 diabetics with macroalb 7 diabetics with normoalb	Glicemic control therapy + low protein intake + ACEI	Urinary albimin excretion rate (15-200µg/min) in 24h urine samples	Lowering from 2,2 (0,81-44) to 0,57 (0,26-1,1) in microalbuminuric type 2 diabetics

Study (1st author, year)	Local	Study type	Participants	Intervention	Outcome	Results
Raal, 1994	South africa	RCT	22 type 2 diabetics	Moderate protein restriction	Urinay urea nitrogen	Protein restricted group had a decrease in proteinuria degree
Pijls, 2000	Netherlands	RCT	125 type 2 diabetics	Protein restriction	Urinary urea excretion and food frequency questionaires	After 12 months, the difference between protein restricted and normal intake was smaller
Pijls, 2000	Netherlands	RCT	335 type 2 diabetics	Protein restriction	Albuminuria	Protein restricted diet increases albuminuria in microalbuminuric type 2 diabetics and decreases albuminuria in normoalbuminurica
Hansen, 2002	Denmark	RCT	82 type 1 diabetics	Protein restriction	GFR	Moderate protein intake restriction improves prognosis in progressive nephropathy in addition to the beneficial effect od anti-hypertensive therapy.
Brinkworth, 2004	Australia	RCT	66 obese type 2 diabetics	Protein restriction		A high protein reduction weight may have a more benefic impact in cardiovascular disease than a low protein diet.
Pijls, 2007	Netherlands	RCT	160 type 2 diabetics	Protein restriction	Creatinine clearance	In long term, protein restriction is neither feasible nor efficacious
Giordano, 2008	Italy	RCT	6 type 2 diabetics 9 type 2 diabetics with nephropathy	Protein restriction	Albuminuria, fibrinogen pool, serum albumin	Low protein intake reduces inflammatory, proteinuiria

Study (1st author, year)	Local	Study type	Participants	Intervention	Outcome	Results
Koya, 2009	Japan	RCT	112 type 2 diabetics with overt nephropathy	Protein restriction during 5 years	Creatinine clearance, GFR	It is difficult to maintain long term protein restriction; protein restricted didn´t confer renoprotection.
Wheeler, 2002	Indiana	Randomized crossover trial	17 type 2 microalbuminuric diabetics	Plant based protein vs animal protein based protein	GFR	Protein source isn't relevant
de Mello, 2006	Brazil	Crossover controlled trial	17 type 2 diabetics with macroalbuminuria	Protein restriction, usual diet, chicken diet	Urinary urea exctretion	In macroalbuminuric patients, withdrawing of red meat reduces urinary urea excretion

Study (1st author, year)	Local	Study type	Participants	Intervention	Outcome	Results
Ryley, 1998	Australia	Cross-sectional-population based	178 insulin dependent type 2 diabetics	Food frequency questionnaire	microalbuminuria	There was no association between microalbuminuria and carbon or fat adjusted diet,
Nettleton, 2008	Minneapolis	Cross-sectional	5042 healthy subjects	Food frequency questionnaire	Albumin-to-creatinine ratio	Non diary animal protein intake was positively associated with albumin-to creatinine ratio.

Table 1. Included studies description.

3.1. Type 2 diabetes

3.1.1. Target-organ lesions and complications of Type 2 diabetes

Retinopathy, microvascular disease, neuropathy and nephropathy are complications of type 2 diabetes.

3.2. Diabetic nephropathy

3.2.1. Physiology of diabetic nephropathy

There are biochemical, hormonal and imunologic factors which contribute to diabetic nephropathy. They are: hyperglicemia, assessed by hemoglobina glycated (HbA1c), somatotrofine, hipopituitarism, imunocomplexes of insulin- anti-insulin anticorps which acumulate in the basal membrane, the diminuishing of flexibility of eritrocytes due to glicosilation and hipercoagulability due to fibrin. The increase in the glomerular basement membrane and the expansion of the mesangium due to increase of matriz are the main changes in the glomerulus in diabetic nephropathy. There is an glomerular hipertrophy in the early stage of diabetes. Transitory changes in the urinary albumin excretion depend of recente hiperglycemia, exercise, urinary tract infections, high blood pressure, cardiac insufficiency, fever.

Glomerulosclerosis is caracterized by increased glomerular basement membrane width , diffuse mesangial sclerosis, hyalinosis, microaneurysm, and hyaline artheriosclerosis. Micro and macroalbuminuric type 2 diabetics have more structural heterogeneity than patients with type 1 diabetics.

Electron microscopy reveals that the severity of glomerular lesions is related to GFR and UAE and to diabetes duration, degree of glycemic control, and genetic factors.

3.2.2. Evaluation of renal function

Proteinuria is the earliest and most sensitive predictor of diabetic nephropathy. Fisiologicaly the upper limit of normal proteinuria is 150 mg/24h. microalbuminuria must be present in at least 2 or 3 24h urine samples, colected during a several month period. The value of microalbuminuria can vary within an individual about of 40%.

Glomerular filtration rate

The National Kidney Foundation recommends for estimating GFR the MDRD (Modified Diet in Renal Disease) formula: GFR (ml/min/1.73 m2) x 186 serum creatinine (mg/dl)$^{-1.154}$ x age (years)$^{-0.203}$ x (0.742 if female) x (1.210 if African American)]. There is an online calculator available in www.kidney.org/klsprofessionals/gfr_calculator.cfm).

The Cockroft-Gault equation: creatinine clearance (ml/min) = [(140 - age (years)] x weight (kg)/[72 x serum creatinine (mg/dl) x (0.85 if female)] is less accurate.

The range of GFR values in young individuals is between 80 and 130 ml/min/1.73 m2, declining at ~10 ml/min/decade after 50 years of age.

The GFR is the best parameter to evaluate kidney function and should be estimated in micro or macroalbuminuric patients. In type 1 diabetics renal function declines 1.2mL/min/month without therapeutic intervention. In type 2 microalbuminuric patients renal function decline is variable. Patients with more rapid GFR decline usually have more advanced diabetic glomerulopathy and worse metabolic control. Levey in 2003 concluded that diabetics should be referred to a nephrologist when GFR reaches 30 mL/min/1,73 m2 because there is evidence of improvement in morbidity and mortality when patients start renal replacement therapy.

Stage	Description	GFR (mL/min/1,73m^2)
1	Kidney damage with normal or higher GFR	>90
2	Kidney damage with mild lower GFR	60-89
3	Moderate decrease in GFR	30-59
4	Severe decrease in GFR	15-29
5	Kidney failure with or without dialysis	<15

Table 2. Definition and classification of CKD stages.

Microalbuminuria definition

According with the Gento-Montecatini convention, microalbuminuria is present when the urinary albumin excretion rate in 24 hours or 12 hours urine collection is in the range of 30 – 300 mg/24h (20-200 μg/min). Urine collection should be done when the patient is at rest and his glicemic control is stable. No measures should be taken in patients with ketosis or poor control. If excretion is below 30 mg/24 hours, the patient is normoalbuminuric, if it is over 300 mg/24h the patient is macroalbuminuric. Microalbuminuria should be present in at least two of three urine samples collected over a period of several months. False positives may be present in the following situations: hyperglycemia, hypertension, urinary tract infection, fever, acute intercorrent disease, heavy exercise, cardiovascular decompensation, contamination with menstrual or seminal fluid.

Since the duration of diabetes type 2 is often unkown microalbuminuria may be present even before the diagnosis.

Microalbuminuria in terms of predictive power is the best marker of diabetic nephropathy. There are other markers of nephropathy.

Transition from normoalbuminuria to microalbuminuria

Longitudinal studies show that patients who become microalbuminuric have previous albumin excretion in the upper limit. It has been demonstrated as well that microalbuminuric patients have HbA1c in the range 7,5% - 8%. Some authors have described an elevation of arterial pressure after microalbuminuria appears. This might sugest that microalbuminuria might be a predisposing factor to hypertension than a consequence of elevated blood pressure.

Management of microalbuminuria in diabetes

In patients with microalbuminuria the prescription of ACEI is essential. The risk factors for microalbuminuria are high lipid levels, smoking, poor glucose level control, alcohol excess, overweight, family history of hypertension and renal disease, lack of exercise.

Blood glucose levels controlled, with HbA1c below 7% are associated with decreased risk for clinical and structural manifestations of diabetic nephropathy in type 1 and 2 diabetics. In the Diabetes Control and Complications Trial (DCCT), intensive treatment of diabetes reduced the incidence of microalbuminuria by 39%. In this study patients allocated to receive strict glicemic control had a long lasting reduction of ~40% in the risk of development of microalbuminuria and hypertension in the 8 years after the end of the study. UKPDS in 1998 showed a 30% risk reduction for the development of microalbuminuria in the intensively glycemic controlled group. Kumamoto Study also showed a reduction in the rate of development of microalbuminuria. Intensive treatment of glycemia towards HbA1c < 7% should started as soon as possible to prevent microalbuminuria development. The effect of strict glycemic control on the progression from micro to macroalbuminuria patients is still controversial. In the DCCT study intensified glycemic control did not decrease the rate of progression to macroalbuminuria in type 1 diabetic with microalbuminuria at the beginning of the study. The Microalbuminuria Collaborative Study Group reported similar findings.

Treatment of hypertension reduces the risk of cardiovascular and microvascular events in diabetics. About 40% of type 1 diabetics and 70% of type 2 diabetics with normoalbuminuria have blood pressure levels > 140/90 mmHg. In UKPDS 1998 a reduction of 154 mmHg to 144 mmHg decreased the development of microalbuminuria by 29%. Blood pressure targets for patients with diabetes are lower (130/80 mmHg) than those for patients without diabetes. In the HOT (Hypertension Optimal Treatment) study a reduction of diastolic blood pressure from 85 to 81 mmHg resulted in a 50% reduction in the risk of

cardiovascular events in diabetic but not nondiabetic patients. In patients with type 2 diabetes ACE inhibitors and ARB's both diminish the risk for diabetic nephropathy and recurrence of cardiovascular events. MICRO-HOPE 2000 (Heart Outcomes Prevention Evaluation) study showed that ramipril 10 mg a day decreased the risk of nephropathy by 24% and the risk of cardiovascular death in patients with type 2 diabetes who were over 55 years old with one additional cardiovascular risk factor by 37%. Moreover, ramipril reduced UAE at 1 year and at the end of the study. These results reinforce the importance of ACE inhibitors in cardio and renal protection of type 2 diabetics. In microalbuminuric type 1 and type 2 diabetics numerous studies have demonstrated that treatment of hypertension, irrespective of the agent used, produced a beneficial effect on albuminuria.

Rennin-angiotensin system blockade with ACE inhibitors or ARBs confers an additional benefit on renal function, independently from blood pressure reduction and may be related to decreased intraglomerular pressure and passage of proteins into the proximal tubule. These drugs decrease UAE and the rate of progression from microalbuminuria to more advanced stages of diabetic nephropathy. According to the MDRD (Modification of Diet in Renal Disease) trial, the lower the blood pressure, the greater the preservation of renal function in nondiabetic patients. In Peterson JC 1995 patients with proteinuria > 1 g/day and renal insufficiency had slower decline in renal function when blood pressure < 125/75 mmHg. Although this study included mainly nondiabetic patients, this goal also has been recomended for proteinuric diabetic patients.

	Goal	
Intervention	Microalbuminuric	Macroalbuminuric
ACE inhibitor and/or ARB and low protein diet (0,6 to 0,8 g/Kg/day) *	Normoalbuminuria or reduction of microalbuminuria	Proteinuria < 500 mg/day or as low as possible
	GFR stabilization	GFR decline < 2 mL/min/year
Anti-hypertensive agents	Blood pressure < 130/80 mmHg or < 125/75 mmHg †	
Strict glycemic control	HbA1c<7%	
Statins	LDL cholesterol ≤ 100 mg/dL ‡	
Acetyl salicylic acid	Thrombosis prevention	
Smoking cessation	Prevention of atherosclerosis progression	

* low-protein diet: efficacy not proven in long term studies in microalbuminuric patients. † Goal: 125/75 mmHg with increased serum creatinine and proteinuria > 1,0 g/24h. ‡ LDL cholesterol < 70 mg/dL in cardiovascular disease.

Table 3. Interventions and goals for reno and cardioprotection in patients with diabetic nephropathy (Gross JL 2005).

Summary of the importance of microalbuminuria

Microalbuminuria is an important predictor of renal and cardiovascular disease, mainly in type 2 diabetics. New technologies have failed to replace microalbuminuria in the prediction of disease. One of the risk factors for progression to diabetic nephropathy, renal failure and early cardiovascular morbidity and mortality is microalbuminuria.

3.2.3. Risk factors to diabetic nephropathy

Nephropathy develops in aproximately 40% of diabetics. Epidemiological and family studies demonstrated that there might be genetic susceptibility to the development of diabetic nephropathy. Hyperglycemia and hypertension are the main risk factors to progression of diabetic nephropathy. Other risk factors are glomerular hyperfiltration, smoking, dyslipidemia, proteinuria levels, diet, particularly considering protein and fat content.

3.3. Protein intake estimate

3.3.1. Maroni's method

In 1985 Maroni et al developed a method to estimate protein intake. In 2002 a formula based on this method was validated.

3.3.2. Other methods to estimate protein intake

Protein intake can also be measured by Frequency food questionnaires with accuracy.

3.4. Eating behaviour

Lifestyle modification such as a change in diet is even more difficult to achieve than adherence to drug treatment. This is also true concerning preventive measures which have less compliance than treatment regimens.

Pijls et al, 2000 performed a study in order to evaluate compliance with protein restricted diet in type 2 diabetics. This study showed that compliance was kept only in the first 6 months of the study. This study found some predictors of diet compliance with limited predictive power. Knowing these predictors may help health care professionals addressing patients with appropriate strategies in order to obtain the compliance desired. Concerning drug treatment it has been demonstrated that even the most effective interventions achieve little improvement.

There are studies such as Pijls et al, 1999, which showed that self-reported data tended to overestimate compliance with proposed diet. This finding suggests that diabetics understand the concept of protein restricted diet but rarely put it into practice. In Pijls et al, 2000 it is suggested that confronting non-compliant patients with estimated protein intake determined by 24-h urinary urea may increase diet

adherence. One of the main reasons to low adherence to protein-restricted diet is probably the low perception of importance of compliance to the regimen to prevent renal failure in asymptomatic diabetics. MDRD study demonstrated that the higher the protein-restriction required the higher the time needed to be spent explaining the regimen. Pijls et al 2000 suggest that more frequent consultation with dietitians can improve compliance to the regimen.

Nicholson, 1999 implemented a plant-based diet under the assumption that this designation might be better understood by type 2 diabetics. Considering pharmacological therapy, it has been estimated that one third of patients adhere to physicians' drug prescription, one third adhere partially and one third do not adhere at all. The percentage of compliance with diets is even lower. A regimen will only be followed by those who choose to follow it, generally. This decision is the turning point needed to adhere to a new diet. In Pijls et al 2000 patients with habitual high protein intake were the ones with the lowest decrease in protein intake. These patients where the ones for whom protein restriction was the most needed. Patients who were satisfied with their previous diet showed good compliance. The MDRD study showed that diet satisfaction was a predictor of adherence during the follow up. Pijls et al 2000 showed that people who lived alone had better diet adherence. This suggests that taking into account partner's preferences outweighed the support to adhere. Kiley et al, 1993; Van Horn et al, 1997; Pijls et al 2000 found no assotiation between educational level and compliance with diet. Reid et al, 1984 found an association between education level and compliance. Pijls et al 2000 concluded that protein restricted diet addressed to type 2 diabetics without nephropathy is difficult to implement. Adherence was lower in patients with higher baseline protein intake, in those who were the least satisfied with previous diet, in those not living alone, and possibly in those who were overweight. All these factors predicted adherence only to a moderate extent. To health care professionals these predictors provide some guidance in order to choose the best approach to obtain diet compliance in a particular patient.

Wheeler ML et al 2002 concluded that there is no clear advantage to recommend diets containing plant protein rather than animal protein for type 2 diabetics with microalbuminuria.

4. Material and methods

This is a *nested case-control study* in a type 2 diabetics cohort from Esmoriz population.

The participants of this study were type 2 diabetics monitored in USF da Barrinha with renal function in stage 1 or 2.

I've considered as cases the type 2 diabetics with microalbuminuria (between 30-300 mg/24h) and as controls type 2 diabetics without microalbuminuria (below 30mg/24h).

Sample size calculation was performed assuming na expected media difference of 10g between median protein intake in type 2 diabetics with microalbuminuria and type 2 diabetics without microalbuminuria. Considering an amplitude of 20g, in order to obtain a significance of 5% and a power of 80% there would be necessary to study 61 cases and 61 controls.

The data was gathered consulting clinical data in Programa de Diabetes do SAM of USF da Barrinha. It was created a data base in PASW 18.0 acording with the table in the anexes.

Variable	**Numerical/nominal**	**Continuous/categorica**	**Unit**
Age	Numerical	Continuous	Years
Gender	**Nominal**	**Dicotomic**	**-**
BMI	Numerical	Continuous	Kg/m2, centesimals
HbA1c	Numerical	Continuous	%, centesimals
Total cholesterol	Numerical	Continuous	mg/dL
HDL	Numerical	Continuous	mg/dL
Triglicerids	Numerical	Continuous	mg/dL
Creatinine	Numerical	Continuous	mg/dL
Systolic blood pressure	Numerical	Continuous	mmHg
Diastolic blood pressure	Numerical	Continuous	mmHg
24h Microalbuminuria	**Numerical**	**Dicotomic**	**mg/24h**
24h Urinary urea	Numerical	Continuous	mg/24h
Protein intake estimate (Maroni et al 1985)	Numerical	Continuous	g/day/kg weight

Table 4. Variable description.

The estimate of protein intake was made by Maroni's method (1985), using the online calculator available in the link

http://nephron.org/nephsites/nic/protein_intake.

The inclusion criteria were type 2 diabetics under 75 years old, with renal stage 1 or 2, monitored in USF da Barrinha. Exclusion criteria were absence of 24h urinary urea measurement in 2011.

Statistical analysis was assessed with PASW 18.0, licensed by Oporto University's Faculty of Medicine.

Statistical significance was accessed by independent samples T-test and by ANOVA.

The population was described according with their basic characteristics

The mean difference between average protein intake of type 2 diabetics with microalbuminuria and type 2 diabetics without microalbuminuria was calculated, ajusted for age, gender, BMI, year of evolution of type 2 diabetes, HbA1c, arterial pressure.

The formula that was used to access the mean difference of protein intake between type 2 diabetics with or without microalbuminuria was the following:

Mean Difference=$\overline{X1}$-$\overline{X2}$

$\overline{X1}$ - protein intake in the group with microalbuminuria
$\overline{X2}$ - protein intake in the group without microalbuminuria

Standard error of Mean Difference = $\sqrt{\frac{s1^2(n1-1)+s2^2(n2-1)}{n1+n2-2}}$

5. Results

Were excluded type 2 diabetics who performed analysis including microalbuminuria dosing in hospital care, because it was not possible to measure urinary urea in 24 hours urine.

	Type 2 diabetics without microalbuminuria (< 30 mg/24h) N=44	Type 2 diabetics with microalbuminuria (≥ 30 mg/24h) N=29
Gender		
Men (N=38; 52,1%)	20	18
Women (N=35; 47,9%)	24	11
Age (years)	60,02 ± 9,06	58,55 ± 9,35
Years of diagnosis (years)	5,44 ± 5,49	8,10 ± 6,33
BMI (Kg/m2)	29,36 ± 3,66	29,52 ± 3,38
Waist (cm)		
Men	99,47 ± 8,42	99,53 ± 9,84
Women	98,78 ± 10,30	98,36 ± 6,83
Systolic blood pressure (mmHg)	126 ± 15	137 ± 16
Diastolic blood pressure (mmHg)	79 ± 8	85 ± 10
HbA1c (%)	6,74 ± 0,96	7,82 ± 1,50
Total cholesterol (mg/dL)	187 ± 31	201 ± 46
LDL (mg/dL)	113 ± 32	118 ± 31
Triglicerídes (mg/dL)	117 ± 46	178 ± 107
Creatinine (mg/dL)	0,93 ± 0,12	0,93 ± 0,18
GFR[1] (mL/min/1,73m2)		
Men	88,61 ± 26,86	96,19 ± 26,50
Women	82,48 ± 15,58	83,90 ± 14,07
Microalbuminuria (mg/24h)	11,27 ± 7,29	58,97 ± 35,79
Protein intake estimate[2] (g/Kg of weight/24h)	2,42 ± 0,76	2,18 ± 0,77

Table 5. Type 2 diabetics included in the study description.

[1] GFR men =((140 – age (years)) * weight (Kg)) / (72 * cretinine (mg/dL))); GFR women= ((140 – age (years)) * weight (Kg)) / (72 * creatinine (mg/dL))) * 0.85.

[2] Protein intake estimate (g/day) = 6,25 * (24 h urinary urea (g/day) + 0,31 * weight (Kg)).

Adjusting these variables to gender:

	Type 2 diabetics without microalbuminuria (< 30 mg/24h) (N= 44)		Type 2 diabetics with microalbuminuria (≥ 30 mg/24h) (N=29)	
Gender	Men (N= 20)	Women (N=24)	Men (N=18)	Women (N=11)
Age (years)	60,75 + 9,27	59,42 + 9,04	57,44 + 10,13	60,36 + 8,04
Years of diagnosis (years)	3,06 + 3,11	7,30 + 6,26	7,89 + 7,09	8,45 + 5,16
BMI (Kg/m2)	28,09 + 2,82	30,41 + 3,97	29,79 +3,90	29,09 + 2,42
Systolic blood pressure (mmHg)	126,65 + 18,32	127,00 + 11,83	134,78 + 16,24	140,55 + 16,25
Diastolic blood pressure (mmHg)	78,75 +7,66	78,5 + 9,00	83,83 + 9,48	85,91 + 10,78
HbA1c (%)	6,32 + 0,79	7,06 + 0,97	7,59 + 1,62	8,16 + 1,27
Total cholesterol (mg/dL)	183,55 +34,74	189,63 + 27,76	199,89 + 49,57	203,36 + 40,67
LDL (mg/dL)	115,60 + 37,63	110,28 + 26,62	117,9 + 33,57	118,56 + 29,81
Triglicerídes (mg/dL)	126,30 + 54,10	108,67 + 37,62	178,50 + 125,65	177,18 + 77,29
Creatinine (mg/dL)	1,00 + 0,11	0,86 +0,10	1,01 + 0,17	0,79 + 0,09
Microalbuminuria (mg/24h)	9,37 + 7,26	12,86 + 7,07	51,21 + 30, 62	71,69 + 42,29
Protein intake estimate[3] (g/Kg of weight/24h)	2,72 + 0,95	2,25 + 0,59	2,17 + 0,79	2,22 + 0,77

Table 6. Description of the 73 diabetics, adjusted by gender. (p values for without microalbuminuria and with microalbuminuria, respectively – Age p=0,63, p=0,43; Years of diagnosis p=0,08; p=0,82; BMI p=0,03, p=0,59; Systolic blood pressure p=0,94, p=0,36; Diastolic blood pressure p= 0,92, p=0, 59; HbA2c p=0,01, p=0,33; Total cholesterol p=0,52, p=0,85; LDL p=0,60,p=0,96; Triglicerides p=0,21, p=0,98; Creatinine p=0,00, p=0,00; Microalbuminuria p=0,11, p=0,14 ; Protein intake estimate p=0,12; p=0,89)

[3] Protein intake estimate (g/day) = 6,25 * (24h urinary urea (g/day) + 0,31 * weight (Kg)).

After the adjustment for gender, comes apparent that there is association between microalbuminuria and BMI, HbA1c and creatinine.
In order to access other possible confounders, another adjustment was performed considering age, years since diagnostic, BMI, blood pressure and HbA1c. (Table 7)

	Type 2 diabetics without microalbuminuria (< 30 mg/24h) (N= 44)	Type 2 diabetics with microalbuminuria (≥ 30 mg/24h) (N=29)
Gender		
Men (N=38)	2,72 + 0,95	2,22 + 0,77
Women (N=35)	2,24 + 0,59	2,17 + 0,79
Age (years)		
<40 (N=1)	2,56*	-
41 – 45 (N=0)	-	-
46 – 50 (N= 6)	2,71 + 0,27	1,48 + 0,37
51 – 55 (N= 7)	2,27 + 0,46	2,12 + 0,40
56 – 60 (N = 8)	2,08 + 0,26	2,43 + 0,53
61 – 65 (N= 8)	2,97 + 1,19	2,71 + 1,04
66 – 70 (N= 10)	2,07 + 0,77	2,43 + 0,57
71 – 75 (N= 9)	2,74 + 0,21	2,52 + 1,16
> 75 (N=2)	-	1,50 + 0,54
Years of diagnosis (years)		
0 – 2 years (N=8)	2,30 + 0,48	2,72 + 0,97
2 – 5 years (N=15)	2,52 + 1,11	1,99 + 0,51
5 – 10 years (N=8)	2,21 + 0,79	2,07 + 0,92
>10 years (N=14)	2,68 + 0,34	2,09 + 0,81
BMI (Kg/m2)		
< 18 (N=0)	-	-
18 – 25 (N=5)	2,79 + 2,73	2,13*
25 – 30 (N=21)	2,64 + 2,50	2,24 + 0,80
30 – 35 (N=17)	2,21 + 2,29	2,28 + 0,82
35– 40 (N=5)	1,93 + 1,65	1,55 + 0,62
> 40 (N=0)	-	-
Blood pressure (mmHg)		
< 130/80 mmHg (N=36)	2,49 + 0,83	2,23 + 0,76
> 130/80 mm Hg (N=12)	2,32 + 0,64	1,77 + 0,95
HbA1c (%)		
< 6,5 % (N=16)	2,58 + 1,06	2,01 + 1,16
> 6,5 % (N=32)	2,32 + 0,47	2,24 + 0,64

Table 7. Protein intake estimate adjusted to gender, age, years since diagnosis, BMI, Blood pressure, HbA1c. (Gender p=0,12, p=0,89; Age p= 0,41, p=0,27; Years of diagnosis p=0,82, 0,70; BMI p=0,30, p=0,71: Blood pressure p=0,58, p=0,44; HbA1c p=0,39, p=0,58)

The mean difference between type 2 diabetics with and without microalbuminuria was of -0,24 $\pm$ 0,76 g protein/Kg/day.

6. Discussion

This was an observational, transversal study. In order to relate protein intake
estimate and microalbuminuria it was assumed that average eating patern of people doesn't change. Sample size obtained is very small considering the size predicted by sample size calculation.
It was collected one 24 h urine sample for each type 2 diabetic, which limits the interpretation of the results since a considerable variation in daily protein intake can't be excluded.
This study showed that diabetics with microalbuminuria have lower mean estimate of protein intake than diabetics without microalbuminuria.

7. Conclusions and recomendations

According 2005 IDF recommendations, protein restriction should be recommended to type 2 diabetics to preserve renal function. Recent studies show that it seems to be no relation between protein intake and progression of renal failure. Type 2 diabetics with renal disease are at higher risk of under nourishing by protein depletion. This shows that protein restriction should be recommended according international recommendations, but without inducing under nourishing in type 2 diabetics. This study shows that diabetics with microalbuminuria have lower mean estimate of protein intake. Attending at limitations of this observational, transversal study, it is not possible to infer about the risk of higher protein intake and progression of renal disease. Monitoring estimate of protein intake during a longer period of time in type 2 diabetics would more accurately assess the eating pattern of type 2 diabetics.

8. Future work

A longitudinal study would better assess the relation between protein intake and renal function. In this case it could be measured urinary urea and microalbuminuria in 24 h urine and compared the mean of the estimate of protein intake with the difference of microalbuminuria between the beginning and the end of the study; or in alternative, with the difference between GFR in the beginning and the end of the study.

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Appendix

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Table to register data in PASW 18.0