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1 ORIGINAL ARTICLE

2 Urine levels of 5-aminoimidazole-4-carboxamide riboside (AICAR) 3 in patients with type 2 diabetes

4 Michael Mendler[1] · Stefan Kopf[1,2] · Jan B. Groener[1,2] · Christin Riedinger[1] · Thomas H. Fleming[1,2] ·
5 Peter P. Nawroth[1,2,3] · Jürgen G. Okun[4]

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8 **Abstract**
9 **Aims** 5-Aminoimidazole-4-carboxamide riboside (AICAR) is an endogenous activator of AMPK, a central regulator of
10 energy homeostasis. Loss and/or reduction of AMPK signaling plays an important role in the development of insulin resist-
11 ance in type 2 diabetes. The loss of AMPK in diabetes could be due to a loss of AICAR. The aim of this study was to char-
12 acterize urine levels of AICAR in diabetes and determine whether an association exists with respect to late complications,
13 e.g., retinopathy, nephropathy and neuropathy.
14 **Methods** Urine AICAR was measured by liquid chromatography tandem mass spectrometry in 223 patients consisting of
15 5 healthy controls, 63 patients with pre-diabetes, 29 patients with newly diagnosed type 2 diabetes and 126 patients with
16 long-standing type 2 diabetes. For statistical analyses, nonparametric Kruskal–Wallis test, one-way ANOVA and multivari-
17 ate regression analysis were performed to investigate the associations of urinary AICAR excretion within different groups
18 and different clinical parameters.
19 **Results** The mean urine AICAR for all 223 patients was 694.7 ± 641.1 ng/ml. There was no significant difference in urine
20 AICAR between the control and patients with diabetes (592.3 ± 345.1 vs. 697.1 ± 646.5 ng/ml). No association between any
21 of the biochemical and/or clinical parameters measured and urine AICAR was found, with the exception of age of patient
22 ($R = -0.34$; $p < 0.01$) and estimated glomerular filtration rate ($R = 0.19$; $p = 0.039$). These results were confirmed addition-
23 ally by linear regression analysis.
24 **Conclusions** Clinical diabetes is not associated with a change in endogenous AICAR levels. Loss of AICAR may therefore
25 not be a mechanism by which AMPK signaling is reduced in diabetes.

26 **Keywords** Diabetes · Late diabetic complications · Urine analysis · AICAR · AMPK

27

Abbreviations

ACR	Albumin to creatinine ratio	28
AICAR	5-Aminoimidazole-4-carboxamide riboside	29
AMPK	AMP-activated protein kinase	30
ASA	Acetylsalicylic acid	31
BMI	Body mass index	32
CBS	Cystathionine beta-synthase	33
CVD	Cardiovascular disease	34
HCT	Hydrochlorothiazide	35

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A2 Peter P. Nawroth and Jürgen G. Okun have contributed equally to
A3 this work.

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A6 supplementary material, which is available to authorized users.

A7 ✉ Michael Mendler
A8 michael.mendler@med.uni-heidelberg.de

A9 [1] Department of Medicine I and Clinical Chemistry,
A10 University Hospital of Heidelberg, INF 410, Heidelberg,
A11 Germany

A12 [2] German Center for Diabetes Research (DZD), Ingolstädter
A13 Landstraße 1, 85764 Neuherberg, Germany

[3] Institute for Diabetes and Cancer, IDC Helmholtz Center A14
Munich, Germany & Joint Heidelberg-IDC Translational A15
Diabetes Program, Neuherberg, Germany A16

[4] Dietmar-Hopp Metabolic Center, Center for Child A17
and Adolescent Medicine, University of Heidelberg, A18
Heidelberg, Germany A19

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36 NSS Neuropathy symptom score (NSS)
37 NDS Neuropathy disability score (NDS)
38 RAAS Renin–angiotensin–aldosterone system

Background

AMP-activated protein kinase (AMPK) is an evolutionary conserved serine/threonine kinase that functions as a master regulator of energy homeostasis [1]. Upon activation, AMPK signals through its downstream substrates to restore normal energy levels by stimulating metabolic processes that generate ATP, such as fatty acid oxidation, or by inhibiting those that use ATP, such as triglyceride and protein synthesis [2]. Regulation of AMPK is of great interest in the study of diabetes and metabolic syndrome as evidence would suggest that loss and/or reduction of AMPK signaling plays an important role in the development of insulin resistance. It has been reported in multiple animal models with a metabolic syndrome that there is decreased AMPK activity in the muscle [2] and that pharmacological activation can prevent and/or ameliorate the pathologies of diabetes [2]. Evidence also exists that AMPK activity is reduced in skeletal muscle and adipose tissues of humans with either type 2 diabetes or obesity [3, 4].

5-Aminoimidazole-4-carboxamide riboside (AICAR) is an endogenous activator of AMPK [5, 6]. It is an intermediate produced during de novo purine biosynthesis and an analog of adenosine monophosphate (AMP). It can be phosphorylated by adenosine kinase to become ZMP which can bind to the cystathionine beta-synthase (CBS) domains of AMPK's γ-subunit leading to an allosteric change [5]. This change makes AMPK a better substrate for its upstream kinases to phosphorylate it at Thr172 and inhibits dephosphorylation at this site by the protein phosphatases, PP2A and PP2C [7, 8]. This combined effect significantly increases the activity of AMPK ex vivo [9]. Treatment with AICAR has been shown to prevent and/or reverse metabolic syndrome in animal models. In ob/ob mice, fa/fa rats, as well as rats fed on a high-fat diet, AICAR treatment has been shown to improve glucose tolerance, whole-body glucose disposal, as well as reduce hepatic glucose output and plasma triglycerides and free fatty acid levels [10–13]. AICAR has also been shown to increase expression of genes involved in oxidative metabolism in muscle [14, 15]. Untrained mice that were treated with AICAR (500 mg $kg^{-1}day^{-1}$) over four weeks significantly improved their running endurance in a treadmill experiment by 44% [16]. Due to its performance-enhancing effects, AICAR has been prohibited for use in athletes by the World Anti-Doping Agency (WADA) since 2009 [17].

Renal clearance of endogenously produced AICAR has been described. In a cohort of nondoping athletes, the mean urinary concentration of AICAR, as determined by isotope dilution, liquid chromatography, tandem mass spectrometry, was 2186 ± 1655 ng/ml. The concentration was found to differ depending on gender, with females having significantly lower levels [18]. Based upon these measurements, it was concluded that AICAR concentrations > 20 µg/ml would be considered inconsistent with an endogenous production in healthy individuals. Elevated amounts of eliminated AICAR are known to associate with vitamin B12 and folic acid deficiencies, due to an impaired AICAR transformylase activity [19] as well as in leukemia patients and patients with hypoxanthine–guanine phosphoribosyl transferase deficiency [20–22]. To our knowledge, there is nothing known about the impact of other physiological parameters like nutritional purine intake or a seasonal or diurnal variation, although AMPK was shown to be involved in circadian regulation [23].

Urine was selected in this study over red blood cell measurement as it is not only well established [24] but also could be performed within our routine screening panel for inborn errors of purine and pyrimidine biosynthesis (Dietmar-Hopp-Metabolic Centre, quality assured for clinical use via the ERNDIM scheme [25]). Measurement in red blood cells is a better indicator of long-term levels [26], but this was not considered superior as a steady state was expected in the patients.

Within the context of diabetes, renal output of AICAR remains unknown. However, as the activity of AMPK has been reported to be decreased in diabetes and AICAR is a potent endogenous activator of AMPK, the loss of AICAR by an increased renal output may provide a noninvasive means for assessing those patients which are at risk of developing diabetes as well as at risk of developing late complications. The aim of this study was to characterize the urinary levels of AICAR in a cohort of healthy controls and patients with pre-diabetes, as well as patients with newly diagnosed and long-term type 2 diabetes, and determine whether an association exists with classical markers of diabetology, markers of metabolic stress and late diabetic complications.

Methods

Patient cohorts

Patients with pre-diabetes and healthy controls were recruited from the Pre-diabetes Lifestyle Intervention Study (PLIS, multicenter study, local ethics number S-245/2013). Newly diagnosed patients with diabetes mellitus type 2 were recruited from the German Diabetes Study (DDS, multicenter study, local ethics number S-232-2013), and patients with long-standing type 2 diabetes mellitus were recruited from Heidelberg Study on Diabetes and Complications

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135 (HEIST-DiC, local ethics number S-383/2016). Informed
136 consent was obtained from all individual participants
137 included in the study, and blood and spot urine samples were
138 obtained under fasting conditions in all three studies. Fol-
139 lowing collection, urine was centrifuged (2000 rpm; 5 min;
140 4 °C) and ca.1 ml aliquots were frozen at − 80 °C. Preana-
141 lytical degradation was very unlikely to occur as a robust
142 stability of AICAR was described at room temperature even
143 in red blood cells for at least 5 days before [26]. All proce-
144 dures were approved by the local ethics committee of the
AQ1 University of Heidelberg. The characteristics of the different
146 patient cohorts studied are given in Table 1.

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147 Assessment of clinical parameters and late diabetic 148 complications

149 Standard laboratory parameters were assessed in the cen-
150 tral laboratory of the Heidelberg University Hospital. The
151 patients were starving 10 h before taking blood and urine
152 samples. The glomerular filtration rate was estimated by
153 using Chronic Kidney Disease Epidemiology Collabora-
154 tion (CKD-EPI) equation [27]. Blood pressure and heart
155 rate were measured on the left upper arm in a resting state
156 after 5 min, while patients were sitting, using OMRON
157 M8RC IntelliSense Dual Check System. Weight measured
158 using TANITA BWB-620A scale. Height was measured
159 using Längenmesstechnik GmbH Limbach-O System Dr.
160 Keller I. All devices are calibrated on a regular basis by
161 our clinical technicians. Retinopathy was assessed by
162 central 1-field fundus photography (NIDEK AFC-230,
163 NIDEK CO LTD, Padua, Italy; Canon EOS 5D Mark II,
164 Canon Deutschland, Krefeld, Germany). Study partici-
165 pants were screened for neuropathy by using neuropathy
166 symptom score (NSS) [28, 29] and neuropathy disability
167 score (NDS) [30]. Skin auto-fluorescence, which par-
168 tially reflects the accumulation of AGEs in the skin, was
169 determined by the use of noninvasive fluorescence-based
170 measurement (DiagnOptics AGE Reader SU, DiagnOptics

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Table 1 Clinical characteristics of the patient cohort

	Control n = 5	Pre-diabetes n = 63	Newly diagnosed T2D n = 29	Long standing T2D n = 126	*p* value
Age (years)	58.4 ± 14.1	55.8 ± 10.6	51.2 ± 10.7*	62.3 ± 8.7	< 0.001
Gender (f/m)	2/3	38/25	12/17	61/65	n.s.
Diab. duration (years)	–	–	< 1	12.5 ± 10.0*	< 0.001
BMI (kg/m^2)	25.7 ± 1.9	31.4 ± 5.4	31.3 ± 6.7	32.3 ± 6.4	n.s.
Hypertension [yes, *n* (%)]	2 (40)	31 (49)	8 (28)	102 (84)	< 0.001
CVD [yes, *n* (%)]	0 (–)	11 (18)	1 (3)	18 (14)	n.s.
Retinopathy [yes, *n* (%)]	–	–	3 (10)	25 (20)	n.s.
Polyneuropathy [yes, *n* (%)]	–	–	7 (24)	68 (54)	< 0.01
HbA1c (%)	5.8 ± 0.5	5.6 ± 0.3	6.4 ± 0.9*	7.1 ± 1.3*	< 0.001
HbA1c (mmol/mol)	40 ± 3	38 ± 3	46 ± 9	54 ± 14	< 0.001
Fasting glucose (mg/dl)	92.8 ± 5.0	105.9 ± 7.8	129.8 ± 33.3*	151.4 ± 48.6*	< 0.001
eGFR (ml/min*1.73 m^2)	88.4 ± 10.0	90.7 ± 15.3	100.8 ± 14.7	97.6 ± 27.3	n.s.
Urinary ACR (mg/g)#	6.4 (3.5/36.9)	6.1 (4.2/10.7)	6.0 (3.8/8.9)	12.1 (5.7/33.1)*	< 0.001
Urinary AICAR (ng/ml)	592.3 ± 345.1	801.3 ± 702.5	944.0 ± 711	588.1 ± 579.8	
Urinary AICAR (μM/mM crea)	0.156 ± 0.049	0.189 ± 0.109	0.390 ± 0.365	0.192 ± 0.136	
Oral antidiabetics [yes, *n* (%)]	–	–	14 (48)	86 (72)	< 0.05
Insulin therapy [yes, *n* (%)]	–	–	3 (10)	42 (35)	< 0.05
RAAS inhibitors [yes, *n* (%)]	0 (–)	28 (44)	8 (28)	77 (65)	< 0.001
Beta-blockers [yes, *n* (%)]	0 (–)	16 (25)	4 (14)	57 (48)	< 0.001
Ca antagonists [yes, *n* (%)]	0 (–)	10 (16)	2 (7)	27 (23)	n.s.
HCT [yes, *n* (%)]	0 (–)	14 (22)	2 (7)	31 (26)	< 0.05
Loop diuretics [yes, *n* (%)]	0 (–)	2 (3)	0 (–)	17 (14)	< 0.01
ASA [yes, *n* (%)]	0 (–)	11 (18)	2 (7)	41 (35)	< 0.01
Statins [yes, *n* (%)]	0 (–)	10 (16)	3 (10)	42 (35)	< 0.01

Data given in mean ± SD or n (%) or median (25./75. percentile) for #-values. Comparison within the groups was made via one-way ANOVA for metric variables and via Chi-square test for dichotomy variables

ACR albumin–creatinine ratio, *ASA* acetyl salicylic acid, *BMI* body mass index, *CVD* cardiovascular disease, *eGFR* estimated glomerular filtration rate, *HCT* hydrochlorothiazide, RAAS, renin–angiotensin–aldosterone system

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Technologies B.V., Groningen, Netherlands). Laboratory tubes as follows (Sarstedt, Nümbrecht, Germany) Monovette: urine Z 8.5 ml, EDTA KE/9 ml, EDTA K 2.7 ml, Li-Hep-Gel 7.5 ml, serum white 7.5 ml, glucose FE 2.7 ml. Standard blood tests including HbA1c were performed immediately after taking the samples in the central laboratory of Heidelberg University Hospital which is DIN EN ISO 15189 accredited. Urine samples for AICAR measurement were frozen at − 80 °C according to the German Federal Ministry of Education and Research (BMBF) guidelines.

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Measurement of urinary AICAR by liquid chromatography tandem mass spectrometry

Urine samples were diluted to 1 mmol/L creatinine with aqua dest. prior analysis. Creatinine determination in urine was performed on an Olympus AU 480 analyzer using the creatinine kit (Beckman Coulter, Krefeld, Germany). This test is based on the alkaline–creatinine–picrate method [31]. Then, 20 µl of 50 µM thymine-d4 internal standard (Cambridge Isotopes, Tewksbury, MA, USA) was added to a 180-µl aliquot of diluted urine and was filtrated with a centrifuge filter (Merck Millipore, Darmstadt, Germany) and a pore size of 0.1 µm. Analysis was performed on a HPLC system coupled to a Quattro Ultima triple quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with an electrospray ion source and a Micromass MassLynx data system according to Hartmann et al. [25] with minor modifications. In brief, optimized multiple reaction monitoring (MRM) experiment was performed on the most abundant ion transition (m/z 259–127), which was identified by the direct infusion of AICAR (Sigma-Aldrich, Darmstadt, Germany). Collision gas was argon with collision energy of 14 eV. The mass spectrometer was operated in positive ion mode with a needle voltage of 3.15 kV. The system was equipped with a Phenomenex Aqua C18 column (2.0 × 250 mm, 5 µm particle size, Aschaffenburg, Germany) preceded by a C18 2.0 × 4 mm pre-column cartridge (Phenomenex, Aschaffenburg, Germany). Chromatography was performed with a flux of 100 µl/min and a gradient profile between 0.05 M acetic acid (pH 2.8) [eluent A] and 0.05 M acetic acid (pH 2.8) and methanol (1:1, v/v) [eluent B]. The gradient started at 100% [A], held isocratic for 2.0 min, increased to 100% [B] in 8.0 min, switched to 100% [A] in 1.5 min and a re-equilibration step for 8.5 min at 100% [A]. The overall run time was 20 min, and the injection volume was 20 µl. Concentrations were calculated by signal AICAR toward signal internal standard ratio and a seven-point external calibration curve (0–5 µM). To make the data comparable to previous studies, we decided to calculate the AICAR concentration in ng/ml for the respective diagrams.

Statistical analyses

One-way ANOVA and nonparametric Kruskal–Wallis test were performed to compare urinary AICAR excretion levels within different patient groups. Furthermore, different groups of age were defined (≤ 39, 40–49, 50–59, 60–69 and ≥ 70 years) to analyze associations between age and urinary AICAR excretion. Correlation analyses between different metric variables were performed with Pearson's correlation coefficient (r). Linear regression analyses were performed forward and backward to analyze independent associations between urine AICAR as the dependent variable with the following predictors: age, gender, BMI, patient groups, history of CVD, arterial hypertension, fasting glucose, HbA1c, cholesterol, triglycerides, estimated GFR, urinary albumin excretion. Urinary ACR and urine AICAR were log-transformed for parametric tests (one-way ANOVA and multivariate analysis) to reduce skewness and achieve normal distribution. Nonparametric tests (i.e., Kruskal–Wallis test) were performed with the AICAR levels in original scales. Statistical p values < 0.05 were defined as significant. All analyses were performed with IBM SPSS 23.0 (International Business Machines Corp. Armonk, NY, USA). GraphPad Prism version 6.05 for Windows (GraphPad Software, San Diego CA, USA) was used to visualize experimental data.

Results

For the total study cohort of 223 patients (Table 1), regardless of patient classification, the mean urine AICAR was determined to be 694.7 ± 641.1 ng/ml (Fig. 1a). There was no significant difference in urine AICAR between the controls and patients with diabetes (592.3 ± 345.1 vs. 697.1 ± 646.5 ng/ml; nonparametric Kruskal–Wallis test, Fig. 1b). Subgroup analysis with respect to the patients with diabetes showed a trend with patients with a pre-diabetes and those with newly diagnosed type 2 diabetes having a 1.3-fold and 1.6-fold increase, respectively, in urine AICAR as compared to the control group; however, these differences were nonsignificant (nonparametric Kruskal–Wallis test, Fig. 1c). A significant difference was observed with respect to the patients with long-standing type 2 diabetes; urine AICAR was not different to the control group (592.3 ± 154.3 vs. 588.1 ± 579.8 ng/ml; $p > 0.05$ in the nonparametric Kruskal–Wallis test), but there was a significant decrease by approx. 38% between patients with long-standing type 2 diabetes compared to patients with newly diagnosed type 2 diabetes (828.0 ± 701.7 vs. 588.1 ± 579.8 ng/ml; $p = 0.0134$ in the nonparametric Kruskal–Wallis test) (Fig. 1c). The analysis in different age groups showed that the younger subjects < 39 years ($n = 11$) had significantly higher urinary

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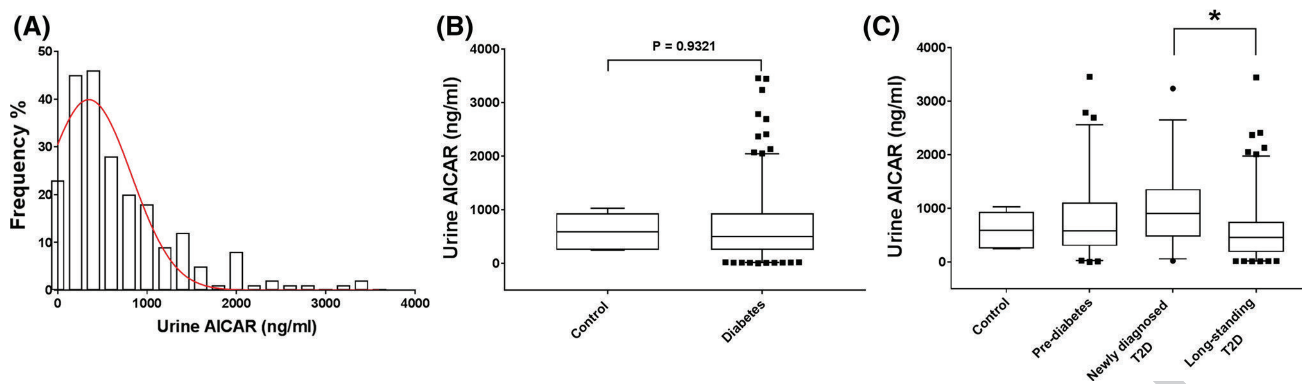

Fig. 1 Urine AICAR in individuals with and without type 2 diabetes. Histogram and density plot for skew-normal approximation of urine AICAR (ng/mL) in 223 urine samples from the study cohort (**a**). Urine AICAR in controls and patients with diabetes. Boxplots show 25–75 percentile, whiskers show 5–95 percentile, line indicates median (**b**). Urine AICAR in controls and subgroups of patients with diabetes. Boxplots show 25–75 percentile, whiskers show 5–95 percentile, line indicates median. *$p < 0.05$ (ANOVA) (**c**)

AICAR excretion compared to the rest of the participants ($F = 3.4$; $p < 0.01$). Similar results were shown with the Kruskal–Wallis test ($\chi^2 = 16.0$; $p < 0.01$). Normalization to the urine creatinine did not affect the relative differences observed between the different groups (Supplementary Figure 1).

Correlation analysis for the total study cohort showed no association between any of the biochemical and/or clinical parameters measured and urine AICAR. There was no association with skin advanced glycation end products (AGEs), a noninvasive marker for post-translational modifications (*data not shown*). There was also no association of urine AICAR with symptoms of retinopathy or neuropathy (*data not shown*). Multivariate regression analysis for the panel data with urine AICAR, as the dependent variable, confirmed this lack of association with exception for age and eGFR (Table 2). All joint effects of the parameters measured were found to be nonsignificant. The only exceptions were with respect to the age of the patients, which correlated negatively ($R = -0.34$; $p < 0.0001$; Fig. 2a), and glomerular

Table 2 Multivariate linear regression analysis for urine AICAR as dependent variable

Independent variables	Parameter estimates (±SE)	Standardized ß-coefficient	T	p
Age (years)	−0.009 ± 0.003	−0.19	−2.7	< 0.01
Gender (f/m)	0.10 ± 0.06	0.08	1.2	n.s.
Diabetes (yes)	0.03 ± 0.07	0.01	0.1	n.s.
BMI (kg/m^2)	−0.01 ± 0.005	−0.11	−1.6	n.s.
Hypertension (yes)	0.02 ± 0.08	0.02	0.4	n.s.
CVD (yes)	0.10 ± 0.09	0.09	1.3	n.s.
HbA1c (%)	−0.21 ± 0.52	−0.01	−0.2	n.s.
Fasting glucose (mg/dl)	0.00 ± 0.001	0.01	0.2	n.s.
eGFR (ml/min * 1.73 m^2)	0.004 ± 0.001	0.18	2.6	< 0.05
Urinary ACR (mg/g)	0.08 ± 0.06	0.09	1.4	n.s.
RAAS inhibitors (yes)	−0.015 ± 0.08	0.01	0.1	n.s.
Beta-blockers (yes)	0.06 ± 0.08	0.06	0.9	n.s.
Ca antagonists (yes)	*0.16* ± 0.08	*0.11*	*1.7*	*0.09*
HCT (yes)	−0.08 ± 0.08	−0.08	−1.1	n.s.
Loop diuretics (yes)	−0.02 ± 0.12	0.02	0.3	n.s.
ASA (yes)	−0.03 ± 0.10	0.02	0.3	n.s.
Statins (yes)	0.10 ± 0.08	−0.02	−0.3	n.s.

Multivariate linear regression model for panel data was created by the stepwise inclusion of all explanatory parameters of urine AICAR, considering also joint effects of parameters. Results were expressed as parameter estimates (±SE) and standardized correlation coefficient (β); $R^2 = 0.12$. Values of $p < 0.05$ were considered statistically significant

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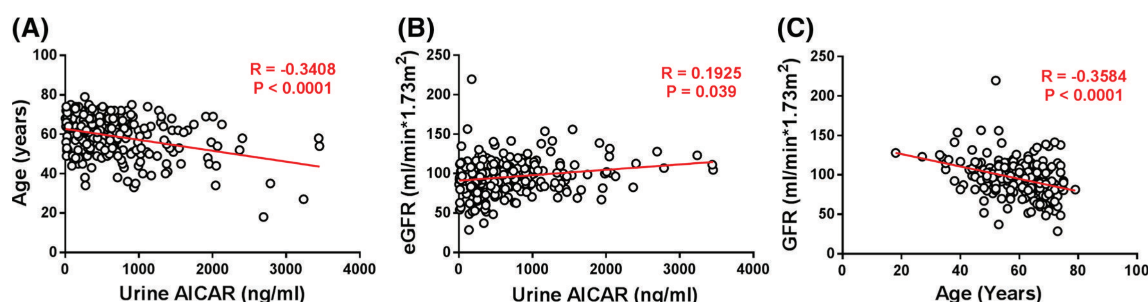

Fig. 2 Correlation of urine AICAR with age and glomerular filtration (eGFR). Urine AICAR correlated negatively with age (**a**) and positively with eGFR (**b**). eGFR correlated negatively with age (**c**). Univariate Spearman's rank correlation coefficient (*R*) was used for correlation analysis. Values of $p<0.05$ were considered statistically significant

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filtration, which correlated positively ($R=0.19$; $p=0.039$; Fig. 2b). Despite these significant correlations, analysis of either age or glomerular filtration with respect to the diabetes subgroups showed that the differences observed in urine AICAR could not be directly explained by either of these variables (*data not shown*). Normalization to the urine creatinine did not affect the correlation or multivariate analysis (*data not shown*).

Discussion

In this study, it was found that urine AICAR was unchanged in patients with diabetes as compared to healthy controls, and while there was a tendency of patients with pre-diabetes and with newly diagnosed type 2 diabetes to having progressively higher urine AICAR, as compared to control patients, these differences were found to be nonsignificant. It remains speculative if further studies with larger patient cohorts might reveal a significant change, and if this then might be physiologically relevant. A limitation of our pilot study is that an a priori power calculation was not possible, as no comparable studies on diabetic patients have been published so far. Moreover, the small number of healthy controls is a major limitation of our study. Nevertheless, the values are consistent with the published data [18], and it is unlikely that an increase in the numbers would substantially change the outcome of this study. A significant decrease in urine AICAR was observed between patients with newly diagnosed type 2 diabetes and those with a long-standing type 2 diabetes. This patient group is well controlled with respect to their hyperglycemia and can therefore be considered as a representative of a "*healthy"* population with diabetes. The significant reduction in urine AICAR within this group could therefore be reflective of the good management of their condition. However, further analysis would be required to validate this and whether the observed trends are indeed a reflection of the diabetes status.

It was surprising to find that urine AICAR did not correlate with any of the standard biochemical and/or clinical parameters used in assessing diabetes and its associated complications, such as fasting glucose, HbA1c or BMI, suggesting that AICAR does not necessarily play a role in diabetes and the development of its complications. As the patient's diet was not assessed, we cannot evaluate its influence on the urine AICAR levels. Analysis of the entire study cohort showed that urine AICAR significantly correlated with the age of the patients and glomerular filtration. These positive and negative associations, respectively, could not, however, explain the observed differences in urine AICAR within the subgroups of patients with diabetes. It has previously been reported that age is associated with a decline in renal function [32], and within the current study cohort, a significant negative correlation between glomerular filtration and patient age, regardless of diabetes, was observed ($R=-0.3585$; $p<0.0001$; Fig. 1c). The decrease in urine AICAR with age may therefore only be a reflection of physiological reduction in glomerular filtration and not as a consequence of diabetes. Further patient cohorts are therefore required which include patients with diabetes with severely impaired renal function.

Overall, the mean urine AICAR level for the entire study cohort was found to be 694.7 ± 641.1 ng/ml, with control patients having a mean level of 592.3 ± 345.1 ng/ml. This amount of urine AICAR is approx. threefold lower than has been previously reported for healthy patients [18]. The differences observed could be explained by ages of the respective study cohorts. In the study by Thomas et al. [33], the urine AICAR was measured in elite athletes and while the age of the subjects is not stated, it can be assumed that the age demographic for the cohort ranged between 20 and 30 years. In the current study, the mean age of the entire study cohort was 58.91 ± 10.41 years. It can therefore be hypothesized that the lower levels of urine AICAR are due to age-dependent decrease in glomerular filtration. Future studies should therefore include patients of different ages,

363 particularly with respect to the control group, as to further
364 assess whether this variable affects urine AICAR.

Conclusions

The lack of any significant differences in urine AICAR would suggest that changes in AICAR do not underlie the loss in AMPK activity in diabetes. However, to substantiate this, a measure of AMPK activity, such as within the peripheral blood mononuclear cells, would be required. Although endogenous AICAR may not be changed in diabetes, exogenous supplementation with AICAR has been shown to be an effective means for inducting AMPK [10–13]. It is unlikely that AICAR will be used as treatment option in diabetes due to its poor bioavailability and a short half-life [34] and its nonspecific effects, such as the inhibition of fructose-1,6-bisphosphatase [35] and stimulation of muscle glycogen phosphorylase [36]. However, the use of AMP analogs as well as activators of AICAR/AITC metabolism may provide a more potent and specific means for inducing AMPK in the future [37].

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Authors' contributions MM and PPN designed the study, interpreted data and drafted the manuscript. JBG contributed substantially in the acquisition of data and revised the manuscript critically for important intellectual content. SK contributed substantially in the analysis and interpretation of data and preparing the tables and figures. CR revised the manuscript critically for important intellectual content. THF contributed substantially to the interpretation of data and revised the manuscript elaborately. JGO established the AICAR measurement, interpreted the data and revised the manuscript for important intellectual content. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical standards Urine samples were obtained from three studies that have been approved by the Ethics Committee Heidelberg (S-245/2013, S-232-2013, S-383/2016) that have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All patients gave their written consent prior to their inclusion in the studies.

Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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