# Levels of retinol and retinoic acid in pancreatic cancer, type-2 diabetes and chronic pancreatitis

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**Aims.** Retinoids participate in multiple key processes in the human body e.g., vision, cell differentiation and embryonic development. There is growing evidence of the relationship between retinol, its active metabolite- all-trans retinoic acid (ATRA) – and several pancreatic disorders. Although low levels of ATRA in pancreatic ductal adenocarcinoma (PDAC) tissue have been reported, data on serum levels of ATRA in PDAC is still limited.
The aim of our work was to determine serum concentrations of retinol and ATRA in patients with PDAC, type-2 diabetes mellitus (T2DM), chronic pancreatitis (CHP) and healthy controls.
**Methods.** High performance liquid chromatography with UV detection (HPLC) was used to measure serum levels of retinol and ATRA in 246 patients with different stages of PDAC, T2DM, CHP and healthy controls.
**Results.** We found a significant decrease in the retinol concentration in PDAC ($0.44^{+/-0.18}$ mg/L) compared to T2DM ($0.65^{+/-0.19}$ mg/L, $P<0.001$), CHP ($0.60^{+/-0.18}$ mg/L, $P< 0.001$) and healthy controls ($0.61^{+/-0.15}$ mg/L, $P<0.001$), significant decrease of ATRA levels in PDAC ($1.14^{+/-0.49}$ ug/L) compared to T2DM ($1.37^{+/-0.56}$ ug/L, $P<0.001$) and healthy controls($1.43^{+/-0.55}$ ug/L, $P<0.001$). Differences between early stages (I+II) of PDAC and non-carcinoma groups were not significant. We describe correlations between retinol, prealbumin and transferrin, and correlation of ATRA and IGFBP-2.
**Conclusion.** Significant decrease in retinol and ATRA levels in PDAC compared to T2DM, healthy individuals and/or CHP supports existing evidence of the role of retinoids in PDAC. However, neither ATRA nor retinol are suitable for detection of early PDAC. Correlation of ATRA levels and IGFBP-2 provides new information about a possible IGF and retinol relationship.

**Key words:** all-trans-retinoic-acid, chronic pancreatitis, pancreatic ductal adenocarcinoma, type-2 diabetes mellitus, vitamin A

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## INTRODUCTION

Retinol and its derivatives (also known as retinoids) are obtained from the diet directly or as provitamin – carotenoids. Carotenoids form micelles with other hydrophobic molecules (e.g., bile salts, cholesterol...) and after absorption in the small intestine they are converted to retinol. Most of the retinol is transported by chylomicrons after esterification with long chain fatty acids. A minor percentage of unesterified retinol is transported directly to the liver in the blood. Up to 80% of whole-body retinol is stored in the form of retinyl-esters in hepatic stellate cells.

Retinol is a fat-soluble vitamin; thus, it can only be transported via the bloodstream in lipid droplets or bound to specific transporting proteins (e.g., retinol binding protein 4 (RBP4) in complex with prealbumin). Absorbed and internalized retinol may be either stored as retinyl esters in e.g., HSCs or PSCs or further metabolized by specific aldehyde dehydrogenase to all trans retinoic acid (ATRA) (ref.[1,2]).

Retinol has several important roles in the human organism. Well-known is its importance in vision, cell differentiation, cell proliferation and in the regulation of transcription. The last is involved primarily through its active metabolite ATRA via interaction with specific retinoic acid receptors[3].

Through the interaction with its receptors (retinoic acid receptors – RAR, retinoic X receptors – RXR) ATRA has impact on the expression of various (up to 500) genes involved in tissue differentiation, cell maturation, apoptosis and/or cell proliferation.

There is growing evidence for a relationship between

retinol, ATRA and certain pancreas-related pathologies. Published data suggest the importance of retinoids for beta-islet maintenance and function[4]. Retinol contributes to the embryonic development of pancreatic tissue and to pancreatic cell differentiation. Some authors have described the relationship of retinol and its derivatives to pancreatic cancerogenesis and reduced blood levels of retinol in PDAC or lower levels of ATRA and/or its receptors in PDAC tissue have been reported[5,6]. It also appears that ATRA as a ligand of the peroxisome proliferator – activated receptor (PPAR) gamma, plays a role in regulating energy balance and increases insulin sensitivity[7]. ATRA signalling appears to be essential for maintaining beta-islet cell homeostasis[8,9]. Several studies have described the relationship between retinol and the insulin-like growth factor/IGF-binding protein (IGF/IGFBP) axis as a possible link to the relationship between retinol and cancerogenesis[10].

Another important link to pancreatic pathologies is the relationship between retinol, ATRA and pancreatic stellate cells (PSCs). PSCs are the only retinol storing cells in the pancreas. Retinol storing ability and typical fluorescence at 325 nm due to retinol containing droplets are the "signature" feature of these cells. which are thought to be key players in certain pancreatic pathologies e.g., PDAC, CHP and DM2).

These facts make retinol and its main metabolite ATRA (together with their signalling and metabolic pathways) promising diagnostic and therapeutic targets. The goal of this study was to measure serum concentrations of retinol and ATRA in patients with T2DM, CHP, distinct stages of PDAC, compared to healthy controls.

## MATERIALS AND METHODS

### Subjects

220 patients (M/F–124/96) in total were included in the study. Of these there were 59 patients with T2DM, 46 with CHP fulfilling the M-ANNHEIM criteria[11] 64 with PDAC (5 were stage I, 8 in stage II, 14 stage III, 37 stage IV according to AJCC (ref.[12])) and 51 matched healthy controls. Blood samples obtained from patients enrolled to our previous study[13] were analysed. Patients with stage IV PDAC were added to the set of probands. All were diagnosed at the General University Hospital in Prague. The DM2 patients were diagnosed more than 3 years prior to entering the study. None of the participants had taken retinol or beta-carotene supplementation.

Basic characteristics of the groups are summarised in Table 1. Laboratory data are presented in Table 2. The study was approved by the local Institutional Ethics Committee (1814/15 S-IV, 24.9.2015) and was conducted in accordance with the latest Declaration of Helsinki. All subjects had given informed consent to participation in the study.

### Laboratory analysis

Blood samples were collected at the General University Hospital in Prague. All samples were obtained through puncture of the cubital vein into anticoagulant-agent-free tubes (for obtaining serum). They were then centrifuged for 10 min at 1450 x g and serum aliquots were stored at −80°C until analysis was performed.

The laboratory analysis of retinol and ATRA in serum was performed by high performance liquid chromatography with UV detection (HP 1100 Series HPLC system, Agilent, Waldbronn, Germany) and acitretin as an internal standard. For obtaining the results, the samples were deproteinated with acetonitrile and extracted by ethyl acetate/hexane mixture. After evaporation and dissolution in methanol, ATRA was detected together with retinol after separation on a C18 column. For ATRA, the lower limit of quantification was 0.7 ug/L, for retinol 0.06 mg/L. A description of the method was published in detail in Zelenková et al.[14].

Measurement of CA19-9, nutritional and inflammatory parameters characterizing the studied groups and used for correlations with ATRA and retinol was described previously[13].

### Statistical analysis

The Kruskal Wallis and Mann-Whitney tests were used to test the differences in ATRA/retinol concentrations in the groups. Differences were considered significant when $P<0.05$.

Because of the small number of probands in early stages of PDAC, we combined PDAC stages I and II into one group as they share similar characteristics, mainly resectability (i.e., no distant metastases or involvement of the superior mesenteric artery and coeliac axis). We used Spearman's rank correlation ($r_s$) to evaluate the re-

**Table 1.** Basic characteristic of the studied groups.

| | n | Age (mean$^{+/- SD}$) | BMI (kg/m$^2$, mean$^{+/- SD}$) | Sex M/F |
|---|---|---|---|---|
| PDAC all | 64 | $63^{+/- 8.86}$ | $25^{+/- 6.41}$ | 54/23 |
| PDAC st I+II | 13 | $66^{+/-7.7}$ | $27^{+/- 2.8}$ | 6/7 |
| PDAC st III | 14 | $66^{+/-4.5}$ | $26^{+/-4}$ | 11/3 |
| PDAC st IV | 37 | $61^{+/-10}$ | $25^{+/-7.6}$ | 30/7 |
| DM2 | 59 | $63^{+/- 8.23}$ | $31^{+/-5.82}$ | 31/28 |
| ChP | 46 | $55^{+/- 12.28}$ | $24^{+/-4.27}$ | 31/15 |
| Healthy controls | 51 | $54^{+/-8.68}$ | $26^{+/-6.2}$ | 21/30 |

BMI, body mass index; PDAC, pancreatic ductal adenocarcinoma; DM2, type 2 diabetes mellitus; ChP, chronic pancreatitis; SD standard deviation.

lationship between ATRA, retinol and other variables. To estimate the left-censored (below quantification limit) values of ATRA and retinol, we used the method "Robust regression on order statistics" (ROS) (ref.[15]).

ROS is semi-parametric method based on a simple linear regression model using ordered detected values and distributional quantiles to estimate the concentration of the censored values. ROS is a regression procedure that uses the estimate parameters of a linear regression model of uncensored observed values vs their log normal quantile to impute the censored data.

ATRA and retinol levels were correlated with serum levels of CA19-9 and basic nutritional and inflammatory parameters. Statistical analysis was performed in the Python programming language using Spyder software (licensed under MIT, freeware, https://www.spyder-ide.org/) and Jupyter notebook (Jupyter Notebook: License. Open-source tool, version 5.1. Copyright (c) 2017).

## RESULTS

The detected concentration levels of retinol and ATRA are presented in Fig. 1. Levels of retinol ranged between 0.11–1.16 mg/L. Levels of ATRA ranged between 0.37–3.15 ug/L. The concentrations of retinol and ATRA were significantly different among the groups ($P$<0.001 and $P$<0.01 respectively for Kruskal Wallis test).

Retinol concentration was significantly lower (mean +/- standard deviation) in PDAC ($0.44^{+/-0.18}$ mg/L) compared to T2DM ($0.65^{+/-0.19}$ mg/L, $P$<0.001), CHP ($0.60^{+/-0.18}$ mg/L, $P$< 0.001) and healthy controls ($0.61^{+/-0.15}$ mg/L, $P$<0.001). The levels of retinol decreased stepwise with the highest levels observed in healthy controls and lowest in the PDAC group. (Healthy controls → DM2 → CHP → PDAC). However, differences between individual "non-PDAC" groups were not statistically significant.

Concentrations of ATRA were significantly lower in PDAC ($1.14^{+/-0.49}$ ug/L) compared to T2DM ($1.37^{+/-0.56}$ ug/L, $P$<0.001) and healthy controls ($1.43^{+/-0.55}$ ug/L, $P$<0.001). Interestingly, differences between ATRA levels in PDAC and CHP were not significant. After splitting the PDAC group to subgroups according to staging it was clear that both ATRA and retinol levels were significantly lower in stage III (ATRA: $1.08^{+/-0.47}$; retinol: $0.42^{+/-0.19}$) and IV (ATRA: $1.07^{+/-0.45,}$, retinol: $0.40^{+/-0.15}$) of PDAC compared to healthy controls (ATRA: $1.43^{+/-0.55}$, retinol: $0.61^{+/-0.15}$) and DM2 (ATRA: $1.37^{+/-0.56}$, retinol: $0.65^{+/-0.19}$). There was no significant difference between the stage I+II subgroup and non-PDAC groups.

In each group, retinol and prealbumin levels correlated (PDAC $r_s$: 0.75, $P$<0.001; CHP: 0.69, $P$<0.001; T2DM: 0.60, $P$<0.001 and healthy controls $r_s$:0.83, $P$<0.001). In T2DM, ATRA levels negatively correlated to IGFBP-2 ($r_s$:−0.63, $P$<0.001). In CHP, retinol levels positively correlated with transferrin ($r_s$:0.60, $P$<0.01). We found no significant correlations between CRP, CA 19-9, serum lipids, total protein or albumin and retinol and/or ATRA. For significant correlations see Table 2.

The results show a significant decrease of retinol and

**Table 2.** Laboratory characteristics of the studied groups.

| | PDAC | | T2DM[%] | | CHP GROUP[%] | | Healthy controls[%] | KW | Signif. correlation (rs) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | mean$^{+/SD}$ | med | mean$^{+/SD}$ | med | mean$^{+/SD}$ | med | mean$^{+/SD}$ | med | | ATRA |
| CA19-9 (kU/L) | $2470.8^{+/3497}$ | 307 | $14.2^{+/10.4}$ | 12.4 | $22.8^{+/57.8}$ | 10.5 | $9.4^{+/6.1}$ | 8.4 | *** | |
| ALBUMIN (g/L) | $40.2^{+/4.6}$ | 41.1 | $45.1^{+/3.7}$ | 45.3 | $45.1^{+/4.6}$ | 45.7 | $45^{+/2.2}$ | 45.4 | *** | |
| PREALB (g/L) | $0.178^{+/0.1}$ | 0.175 | $0.3^{+/0.1}$ | 0.3 | $0.3^{+/0.1}$ | 0.3 | $0.3^{+/0.1}$ | 0.3 | *** | PDAC (rs: 0.75, $P$<0.001)<br>T2DM (rs: 0.6, $P$<0.001)<br>healthy controls (rs: 0.83, $P$<0.001)<br>ChP (rs: 0.69, $P$<0.001) |
| TP (g/L) | $67^{+/6.9}$ | 67.2 | $70.8^{+/5.1}$ | 70.5 | $71.4^{+/6.1}$ | 71.3 | $71.1^{+/4.8}$ | 70.6 | ** | |
| TRANSF. (g/L) | $2.36^{+/0.46}$ | 2.3 | $2.9^{+/0.6}$ | 2.8 | $2.7^{+/0.5}$ | 2.7 | $2.9^{+/0.5}$ | 2.8 | *** | CHP (rs: 0.60 $P$<0.01) |
| CHOL (mmol/L) | $5.04^{+/2.09}$ | 4.65 | $4.7^{+/1.1}$ | 4.4 | $4.8^{+/1.2}$ | 4.7 | $5.7^{+/1.1}$ | 5.7 | *** | |
| TAG (mmol/L) | $1.46^{+/0.84}$ | 1.25 | $1.8^{+/1}$ | 1.5 | $1.4^{+/1.1}$ | 1.2 | $1.4^{+/1}$ | 1.2 | NS | |
| HDL-CHOL (mmol/L) | $1.22^{+/0.49}$ | 1.22 | $1.3^{+/0.3}$ | 1.2 | $1.4^{+/0.6}$ | 1.3 | $1.5^{+/0.4}$ | 1.6 | *** | |
| CRP (mg/mL) | $30.22^{+/43.4}$ | 12.15 | $4.3^{+/5.2}$ | 2.7 | $9.4^{+/19.5}$ | 1.5 | $3^{+/4}$ | 1.7 | *** | |
| IGFBP-2 (ng/mL) | $756.75^{+/345}$ | 722.60 | $299.6^{+/198.9}$ | 255.7 | $575.6^{+/272.2}$ | 511.4 | $330.7^{+/143}$ | 315.3 | *** | T2DM (rs: −0.63, $P$<0.001) |

Significant correlations of analytes with ATRA and retinol are included. PDAC, pancreatic ductal adenocarcinoma; ATRA, all-trans retinoic acid; T2DM, type 2 diabetes mellitus; ChP, chronic pancreatitis; IGFBP, insulin-like growth factor binding protein; KW, Kruskal Wallis, NS, non-significant; Chol, cholesterol; Prealb., prealbumin; TAG, triacylglycerol; TP, total protein; Transf., transferrin. ***$P$<0.001, **: 0.001<$P$< 0.01 for Kruskal-Wallis test, [%] these groups are the same as in (13).

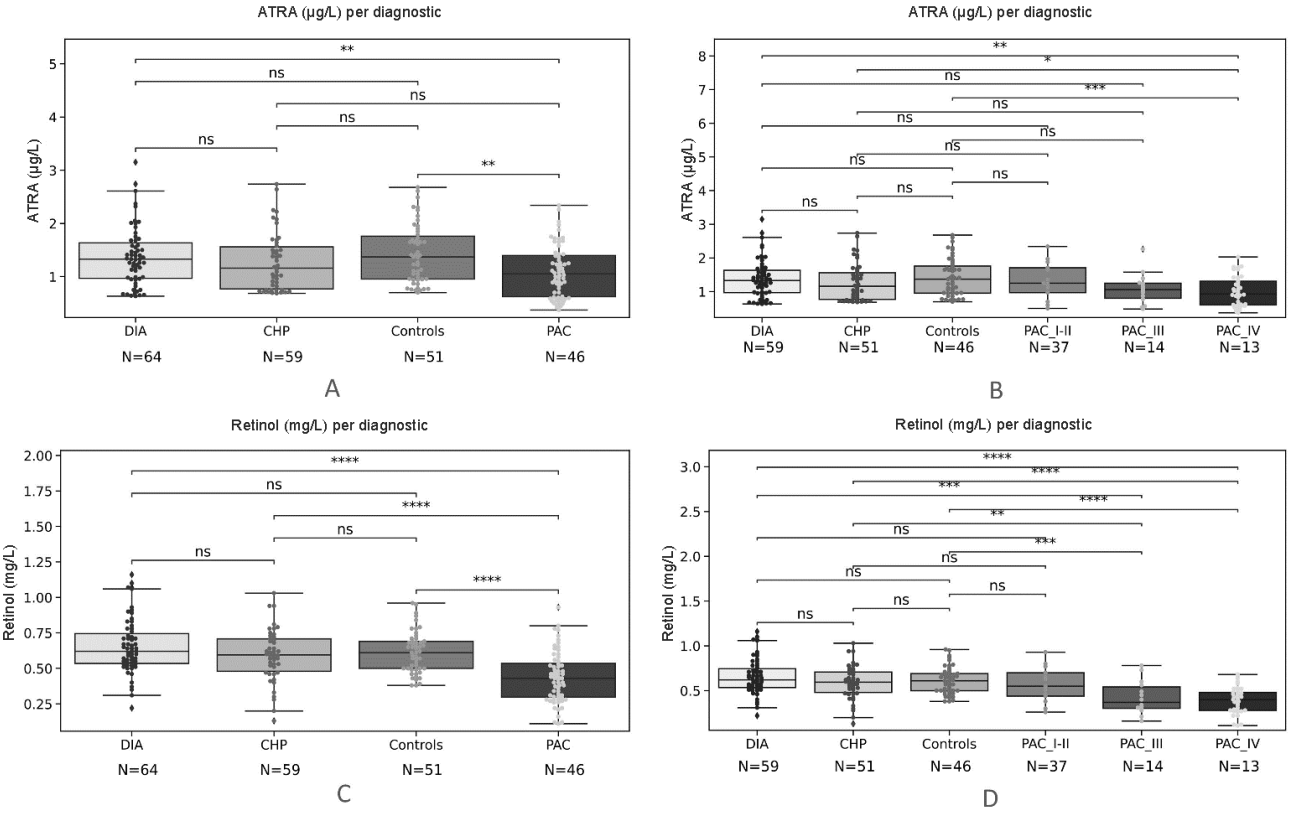


**Fig. 1.A–D.** Comparison of mean ATRA and retinol concentrations in studied groups.
A. ATRA levels in studied groups. B. ATRA levels in studied groups and PAC according to stages. C. retinol levels in studied groups. D. retinol levels in studied groups and PAC according to stages.

Results for Mann-Whitney test. Level of significance marked with asterisk: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; $^{****}P<0.0001$, ns: non-significant.
ATRA (ug/L) mean $^{+/-\,SD}$/median: PDAC: $1.14^{+/-0.49}$/ 1.05(PDAC I+II: $1.33^{+/-0.56}$ / 1.25; PDAC III: $1.08^{+/-0.47}$ / 1.06; PDAC IV: $1.07^{+/-0.45}$ / 0.93); DIA $1.37^{+/-0.56}$ / 1.37; ChP $1.28^{+/-0.55}$ / 1.28; Controls: $1.43^{+/-0.55}$ /1.43.
Retinol (mg/L) mean $^{+/-\,SD}$/median: PDAC: $0.44^{+/-0.18}$/ 1.05 (PDAC I+II: $0.57^{+/-0.2}$ / 0.55; PDAC III: $0.42^{+/-0.19}$ / 0.37, PDAC IV: $0.40^{+/-0.15}$/ 0.40); DIA: $0.65^{+/-0.19}$ / 0.65; ChP: $0.60^{+/-0.18}$ / 0.59; Controls: $0.61^{+/-0.15}$ / 0.61.
PAC, pancreatic ductal adenocarcinoma; ATRA, all-trans retinoic acid; DIA, type 2 diabetes mellitus; ChP, chronic pancreatitis; CON, healthy controls.

ATRA levels in PDAC compared to T2DM, healthy individuals and/or CHP as well as correlation of retinol with prealbumin and transferrin and a correlation of ATRA and IGFBP-2.

## DISCUSSION

### Retinol and ATRA in PDAC

The levels of retinol and ATRA (except for ATRA in PDAC vs CHP) in PDAC were significantly lower compared to other groups. Although the interpretation of retinol and ATRA levels in early stages of PDAC is difficult due to the small sample number, it is clear that PDAC is characterised by lower levels of retinol and ATRA. Lower levels of retinol (but not ATRA) are present even in non-metastatic stages of PDAC, which are often asymptomatic or present with mild symptoms. The low concentrations of retinol and ATRA in PDAC are even more evident in stage IV of the disease. Patients in stage I and II have comparable retinol levels to the rest of the probands. This suggests that ATRA and retinol are related to advanced PDAC or disease progression but fail to be useful markers of early (stage I+II) PDAC.

The question remains whether the low levels of retinol and ATRA in advanced stages of PDAC just reflect the overall poor nutritional status of these patients and metabolic changes driven by malignancy or whether they contribute to development and progression of the disease. The last point is discussed in some publications and possible mechanisms of retinoid influence on the development and progression of PDAC can be proposed:

a) One of the key points in PDAC development is acinar-to-ductal metaplasia (ADM). This is the process where acinar cells after injury (caused e.g., by an acute pancreatitis event) dedifferentiate to progenitor cells with expression of ductal markers[16]. This process is tightly connected to activation of *K-RAS* oncogene and inflammatory signalling. ATRA is a known actor in differentiating pancreatic acinar and ductal cells and ATRA signalling is

activated during pancreatic injury. This suggests a possible important role of ATRA in ADM (ref.[17,19]).

b) Pancreatic stellate cells (PSCs) – the retinol storing mesenchymal-like cells – have been described as an important part of the PDAC stroma interplay. During activation, PSCs release retinol and their phenotype rapidly changes from a quiescent to a myofibroblast-like form. While activated, PSCs produce excessive amounts of extracellular matrix, which is an important compound of PDAC stroma. This process of transition can be reversed by ATRA (ref.[6,20]). Stability and quiescence of PSCs are key factors for maintaining the homeostasis of pancreatic tissue. Damage to ATRA signalling leads to impairment of the organisation of pancreatic acinar architecture[21]. PDAC-stroma interplay is essential for the migration of malignant cells. This could be indirectly hindered by ATRA via IL-6 downregulation of cancer-associated fibroblasts (CAF) (ref.[22]).

c) regulating certain signalling pathways. In our study we found inverse correlation between ATRA and IGFBP-2 in T2DM patients. This relationship confirms the fact, that IGF/IGFBP axis could be influenced by ATRA (ref.[23,25]). The relationship between *K-RAS* mutation, IGF mediated growth and ATRA is a possible factor influencing PDAC development.

Changes in concentration of retinol – an important micronutrient could be expected in malnourished patients with severe malignancy. Additionally, strong correlation between retinol and prealbumin concentrations suggests the impact of nutritional status on retinol levels. In the case of PDAC, retinol and prealbumin levels seem to reflect the stage of the disease. The simple mechanistic explanation for the decrease in retinol and its correlation with prealbumin levels is that prealbumin, also known as transthyretin, binds retinol in complex with retinol binding protein (RBP) and lower levels of prealbumin in PDAC necessarily lead to reduced levels of serum retinol. Surprisingly, other strong markers of nutritional status such as BMI, albumin or total protein did not correlate with retinol/ATRA levels. It is also known, that only about 33% of blood retinol levels are dependent on actual intake of retinol precursors. In healthy individuals, sufficient levels of retinol and its metabolites are regulated by release of retinol from the main storage organ – the liver[26]. This aside, metanalysis of eighteen studies failed to find any correlation between retinol intake and PDAC risk[27].

These facts show that other factors other than malnutrition possibly contribute to decrease of retinol in PDAC and make retinol and ATRA potential sensitive biomarkers of advanced PDAC.

Further research should be focused on the relationship between serum retinoid levels and prognosis of patients with PDAC as it has been reported that reduced levels of retinoids in PDAC tissue are associated with poor prognosis[5] and increased pancreatic cancer risk[9,28]. In contrast, upregulation of RAR receptors leads to a reduction in malignant phenotype of PDAC cells[27].

These facts and the reported influence of ATRA – the active metabolite of retinol on several malignancies (e.g., acute promyelocytic leukaemia) make ATRA, a promising therapeutic modality in PDAC (ref.[29]). Although the use of ATRA is limited by its hydrophobicity and instability, studies conducted with ATRA in nanoparticles show promising results in *in-vitro* studies[30,31].

Results from phase IIb of a clinical trial with ATRA in PDAC show that the decrease in plasma retinol levels during the treatment with ATRA + gemcitabine-nab-paclitaxel is associated with a poor CA19-9 response to the treatment[32].

**Retinol and ATRA in CHP**

The influence of ATRA on the pathogenesis of CHP has been reported[33]. Nevertheless, published studies on retinoid levels in CHP offer contradictory findings of retinol levels in CHP. Duggan et al. (2014) described a higher prevalence of retinol deficiency in CHP than in matched controls, but interestingly there were more CHP patients with excess of retinol than deficiency (19.4% vs 14.5%) and this result, according to the authors, could not be explained by excessive supplementation[34]. The authors further describe a relationship between BMI and retinol levels, but this observation was not confirmed in our study.

Sikkens et al. (2014) reported just 3% prevalence of retinol deficiency in CHP (ref.[35]). Finally, a metanalysis of Martínez-Moneo (2016) reports 15.9% prevalence of retinol deficiency in CHP (ref.[36]). In our work, the prevalence of retinol deficiency (levels below 0.15 mg/L) was 2.08%. Retinol also exhibits an anti-inflammatory effect in pharmacologically induced acute pancreatitis[37].

Lower levels of retinol in CHP may be due to several mechanisms. The most common aetiology of CHP is excessive use of alcohol which is associated with impaired intake of nutrients including retinol or carotenoids. In chronic pancreatitis, the exocrine portion of the gland is damaged, and this leads to decreased secretion of enzymes important for fat and fat-soluble vitamins (A, D, E, K) absorption. Besides that, chronic pain associated with food intake in CHP can lead to reduced food intake and malnutrition. Despite these facts, we found no significant reduction of serum retinol or ATRA in CHP compared to T2DM or controls. Thus, lower retinol levels in CHP could be one of the possible 'red flags' leading to more detailed examination to exclude PDAC in such patients.

**Retinol and ATRA in T2DM**

In T2DM, the results of published studies are controversial, with both increased and decreased levels of retinol reported[38,39]. In a study on Korean population, the levels of retinoic acid and retinol appear to be a valid marker of T2DM development in patients with impaired fasting glucose and the fact, that sufficient levels of retinol and ATRA are likely essential for maintaining glucose homeostasis mainly by involving the insulin secretion and beta cell maintenance, we found no significant difference between retinol and ATRA levels in T2DM group compared to healthy controls or CHP (ref.[4,40]).

## CONCLUSION

Our knowledge of the storage and release of retinol via PSCs as well as its role in certain pancreatic pathologies, is still limited. Thus, our data provide fresh insight into retinoids in PDAC, T2DM and CHP. Whether the low levels of ATRA and retinol in PDAC and CHP patients reflect increased activity of PSCs, as both PDAC and CHP are characterised by excessive production of ECM by these cells, needs to be proven by further research. In this study we demonstrate that advanced, but not early, stages of PDAC are accompanied by significant decrease in retinol and ATRA levels compared to other non-carcinoma groups. Thus, serum ATRA and retinol unfortunately do not seem to be useful for detection of early PDAC either in normal populations or in patients with increased risk of PDAC development. The correlation between retinol and prealbumin levels with no significant correlation to total protein, BMI or albumin suggests that retinol levels reflect more the metabolic than nutritional status of the patient. A question worthy of further research is whether the lower plasma levels of retinol and ATRA are results of PDAC or an independent risk factor.

## ABBREVIATIONS

ADM, acinar-to-ductal metaplasia; AJCC, American Joint Committee on Cancer; ATRA, all-trans retinoic acid; BMI, body mass index;, CA19-9, carbohydrate antigen 19-9; CHP, chronic pancreatitis; HPLC, high performance liquid chromatography; HSC, hepatic stellate cell; IGF, insulin like growth factor; IGFBP, insulin like growth factor binding protein; PDAC, pancreatic ductal adenocarcinoma; PPAR, peroxisome proliferator-activated receptor; PSC, pancreatic stellate cell; RAR, retinoic acid receptor; RBP4, retinol binding protein 4; RXR, retinoic X receptor, T2DM, type 2 diabetes mellitus.

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**Author contributions:** PH: participated in study design, co-analysed the data, was involved in data interpretation and wrote the manuscript; MZ: performed laboratory analyses, contributed to manuscript writing; MK: designed the study, was involved in laboratory analyses, contributed to interpretation of the results and manuscript writing; JS, MV: were involved in the clinical part of the study, collected samples and data; TK: contributed to the study design, collected samples and data, contributed to the interpretation of the results; TH: supervised the clinical part of the study; LP, SS, AZ: were involved in the clinical part of the study, supervised samples' and data collection; TZ: supervised laboratory analyses, and interpretation of the results. All authors discussed the results, contributed to the manuscript and read and approved the final version of the manuscript.

**Conflict of interest statement:** The authors state that there are no conflicts of interest regarding the publication of this article.

**Ethic approval and consent to participate:** This study was performed in adherence to the principles of the Helsinki Declaration and approved by the Ethics Committee of The General University Hospital in Prague. All subjects gave their informed consent with participation in the study.

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