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IDO activity forecasts obesity in males and premenopausal females in a 10-year follow-up study:The Cardiovascular Risk in Young Finns Study

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ABSTRACT

Background and aims: Indoleamine 2,3-dioxygenase (IDO) is an intracellular enzyme associated with artery wall inflammation. Previous studies have verified correlation between IDO activity and early signs of atherosclerosis especially in females. We aimed to elucidate the relationship between an estimate of IDO activity and atherosclerotic risk factors related to non-alchohol-fatty liver (NAFLD) in a 6- and 10-year follow-up.

Methods: Estimates of IDO activity along with complete risk factor data were measured from females (n = 506; age 24–39) and males (n = 421; age 24–39) in 2001. Risk factor measurements were conducted again in 2007 and 2011. Statistical examinations were carried out by Pearson correlation and risk ratio analysis.

Results: In females, age-adjusted IDO correlated with body mass index (BMI) ($p = 0.0008$), waist ($p = 0.0009$), C-reactive protein (CRP) ($p = 0.0014$) and logarithmically modified triglycerides ($p = 0.0488$) in 2007. Correlation remained significant with BMI ($p = 0.0007$) and waist ($p = 0.0063$) in 2011. In males, age-adjusted IDO correlated with waist ($p = 0.0367$) and high-density lipoprotein cholesterol (HDL-C) ($p = 0.0489$) in 2007. Correlation remained significant with HDL-C ($p = 0.0348$) in 2011. In risk ratio analysis, relationship between IDO and obesity was confirmed in females after 10 years (RR = 1.026, $p = 0.0147$, 95% CI) and in males after 6 and 10 years (RR = 1.019, $p = 0.0091$, 95% CI and RR = 1.015, $p = 0.0404$, 95% CI, respectively) when the data was adjusted for age and BMI.

Conclusions: IDO activity correlated with obesity and factors related to NAFLD, namely obesity of visceral type, hypertriglyceridemia and CRP (in females), well-characterized risk factors for diabetes and atherosclerosis in 6- and 10-year follow-up in males and premenopausal females.

1. Introduction

Association of indoleamine 2,3-dioxygenase (IDO) enzyme with various medical conditions has been widely established in recent years. Among these conditions, IDO plays an important role in regulating progression of atherosclerosis characterized by chronic inflammation. Earlier studies have shown that the main function of IDO, degradation of an essential amino acid tryptophan (trp) to kynurenine (kyn), which promotes suppression of T-cell activity by starvation [1,2], leads to reduced artery wall inflammation. Even though influence of IDO in atherosclerosis has clearly been established, interplay between IDO and risk factors promoting atherosclerotic events, such as inflammation, metabolic syndrome (MetS), visceral obesity, and non-alcoholic fatty liver disease (NAFLD), especially in a longitudinal, long-term follow-up is still largely unknown.

A connection between IDO, inflammation and obesity was already

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speculated in 2007 [3]. Since then many cross-sectional studies have described statistically significant correlations between IDO activity, obesity and MetS. For instance, research performed by Mangge and co-workers recognized an interplay between systemic inflammation, IDO activity and MetS in an obese study population. In obese juvenile males, neopterin levels were lower and MetS did not impact kyn/trp ratio compared to obese adults, suggesting an age-related activity of IDO. Indeed, authors speculated that Th2-driven activation may protect juveniles while a later shift to production of Th1-type cytokines may predispose adults to atherosclerotic events. Authors also suggested that the key source for increase of kyn/trp ratio in adults was abdominal fat content [4]. Similarly, Mallmann and co-workers reported a connection between inflammation, IDO activity and MetS in young adults. In addition, they noticed that kyn/trp ratio was significantly increased in hyperuricemia and positively correlated with uric acid, indicating a connection between IDO activity, MetS, chronic kidney disease and a risk for cardiovascular disease (CVD) [5].

NAFLD is the most common liver disease in the world (25% in the western world). NAFLD is a hepatic manifestation of MetS that includes central abdominal obesity along with other components such as hypertriglyceridemia and components of insulin resistance [6]. Up to 80% of patients with NAFLD are obese, defined as a body mass index (BMI) > 30 kg/m^2. However, the distribution of fat tissue plays a greater role in insulin resistance than BMI. Excess free fatty acids and chronic low-grade inflammation (increase in CRP) from visceral adipose tissue are considered to be two of the most important factors contributing to liver injury progression in NAFLD.

Previous studies have also showed differences in IDO activity between sexes. For instance, in a report from the Cardiovascular Risk in Young Finns Study, Pertovaara and co-workers found that IDO activity correlated cross-sectionally with risk factors and early markers of atherosclerosis, especially in female participants [7]. These results may be related to increased autoimmune disease risk in young females [8]. Other studies from female participants have also emphasized the influence of IDO activity in promoting obesity [9] and BMI [10,11]. In their research, Groer and co-workers found that IDO activity advanced BMI in pregnancy by contributing tryptophan 2,3-dioxygenase (TDO) activity along with correlation of plasma neopterin with kyn/trp ratio [11]. Promotion of BMI could also occur in adipose tissue by kynurenine 3-monooxygenase activation, which could lead to the production of 3-hydroxykynurenine and xanthurenic acid in kynurenine pathway [10].

These examples underline the complexity of IDO activity and intensity by which it influences atherosclerotic risk factors in different study populations. Causes of variations seem to depend on various factors, such as age, sex, BMI and pregnancy status, or a mixture of these, thus forming a complex network of possible impact avenues. It is clear, though, that IDO activity is increased in obesity, but the link between obesity and IDO as well as its role in MetS is still not well-understood [5,9]. For instance, in a previous mouse-model study, inhibition of IDO lead to substantial inflammatory response and atherosclerotic plaque development [12], whereas recently Laurans and co-workers reported that in mice *IDO* deletion (*Idol1*$^{-/-}$) or inhibition decreased chronic inflammation along with other beneficial effects [13].

Since visceral obesity is one of the main features influencing MetS, we felt the need to further examine the role of IDO in this interplay. We accomplished this by investigating the relationship between an estimate of IDO activity determined from IDO measurements in 2001 and several atherosclerotic risk factors including obesity in 2007 and 2011 (6- and 10-year follow-up). First, we determined correlations between IDO and several atherosclerosis risk factors. Then, we performed a risk ratio analysis to determine whether IDO activity increases risk for obesity.

2. Materials and methods

2.1. Participants

In this study, we used data from the Cardiovascular Risk in Young Finns Study. This multi-centre cohort study was originally initiated in 1980 and has been conducted in five university hospital cities (Turku, Tampere, Helsinki, Kuopio, and Oulu) and their rural surroundings in Finland. In 1980, altogether 3596 participants, aged from 3 to 18 years, participated in a cross-sectional survey. Tryptophan and kynurenine concentrations along with complete risk factor data were determined only in 2001 from 927 participants, females (n = 506; age 24–39; weight 40.5–134.4 kg) and males (n = 421; age 24–39; weight 55.2–157 kg). Follow-up risk factor surveys were conducted in 2007 for 2204 participants [14] and again in 2011 for 2063 participants. All participants gave written informed consent. In addition, the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and the protocol was approved by local ethical committees.

2.2. Determination of IDO activity

Estimate of IDO activity, characterized by kynurenine (kyn)/tryptophan (trp) ratio, was determined as described previously [15]. Briefly, reverse-phase high-performance liquid chromatography (HPLC) was used to measure kyn (μmol/l) and trp (mmol/l) concentrations from peripheral blood. Kyn separation was performed with a Hewlett Packard 1100 liquid chromatograph (Palo Alto, CA, USA) using Merck LiChroCart 55–4150 mm cartridge containing a Purospher STAR RP-18 3 μm column (Merck Co, Darmstadt, Germany) followed by determination using ultraviolet absorption at 360 nm wavelength with a Hewlett Packard G13144 detector. Shimadzu liquid chromatograph LC-10AD VP (Shimadzu Co, Kyoto, Japan) using a 50-mm BDS Hypersil C18 5 μm column (Thermo Electron Co, Bellefonte, PA, USA) was used to separate trp followed by fluorescence monitoring using Shimadzu RF-10A XL detector at 266 nm excitation and 366 nm emission wavelengths. Finally, kyn (μmol/l) was divided by trp (mmol/l) to produce an estimate of IDO activity.

2.3. Risk factor data

Atherosclerosis risk factor measurements were conducted in 2007 and 2011. Only participants who had IDO data and complete risk factor data were included in the current analysis. In 2007, the number of female participants was 435 and that of male 342 (age 30–45 for both), and in 2011 the number of female participants was 394 and that of male 312 (age 34–49 for both). BMI was calculated as weight, kg/(height, m)^2, and waist circumference as centimeters (cm) using standardized protocols [14]. Determination of triglyceride and serum cholesterol concentrations was performed enzymatically (Olympus System Reagent; Olympus Diagnostica GmbH, Hamburg, Germany) using a clinical chemistry analyzer (Olympus AU400; Olympus Optical Ltd, Mishima, Japan) [16]. Low-density lipoprotein cholesterol (LDL-C) was precipitated from serum high-density lipoprotein cholesterol (HDL-C) with dextran sulphate 500,000 followed by measurement of HDL-C concentration from serum supernatants [17]. LDL-C levels were calculated indirectly using the Friedewald formula for participants with <4 mmol/L triglycerides [18,19]. Glucose concentrations were determined by standard enzymatic methods using a clinical chemistry analyzer (Olympus AU400). Similarly, serum C-reactive protein (CRP) was analyzed with an automated analyzer (Olympus AU400) with a latex turbidimetric immunoassay kit (CRP-UL assay, Wako Chemicals, Neuss, Germany) [20]. Ultrasound studies were performed for carotid intima-media thickness (IMT) [19], fatty liver [21], and coronary-artery compliance (CAC), Young's elastic modulus (YEM) and stiffness index (SI) [22].

The International Diabetes Federation (IDF) classification was used

Table 1
Baseline characteristics of the study population from 2007 and from 2011.Kyn/trp was measured in 2001.

	2007		2011	
Variable	**Females, n = 435**	**Males, n = 342**	**Females, n = 394**	**Males, n = 312**
Age, years	37.9 ± 4.84	37.89 ± 4.94	41.84 ± 4.84	42.26 ± 5.06
BMI, kg/m^2	25.07 ± 4.96	26.69 ± 4.28	25.78 ± 5.34	26.78 ± 4.35
Waist, cm	82.5 ± 12.38	94.25 ± 12.37	86.8 ± 12.9	97.02 ± 12.51
MetS	0.15 ± 0.36	0.28 ± 0.45	0.17 ± 0.37	0.34 ± 0.48
Glucose, mmol/L	5.15 ± 0.8	5.44 ± 0.79	5.28 ± 1.03	5.54 ± 0.86
Trigycerides, mmol/L	1.09 ± 0.52	1.50 ± 0.74	1.03 ± 0.49	1.43 ± 0.7
HDL-C, mmol/L	1.47 ± 0.33	1.19 ± 0.28	1.46 ± 0.32	1.23 ± 0.28
LDL-C, mmol/L	2.88 ± 0.71	3.28 ± 0.82	3.12 ± 0.77	3.44 ± 0.91
CRP, mg/L	2.00 ± 3.4	1.59 ± 2.9	1.97 ± 2.93	1.76 ± 5.24
IMT (mean), mm	0.6 ± 0.08	0.64 ± 0.12	N/A	N/A
CAC, %/10 mm Hg	2.05 ± 0.74	1.77 ± 0.62	N/A	N/A
YEM, mm Hg - mm	375.56 ± 471.37	437.98 ± 319.87	N/A	N/A
SI	6.29 ± 6.83	6.48 ± 3.81	N/A	N/A
Fatty liver	N/A	N/A	1.08 ± 0.34	1.31 ± 0.56
Kyn/trp (2001), μmol/mmol	26.94 ± 7.02	28.37 ± 7.51	26.94 ± 7.02	28.37 ± 7.51

BMI (body mass index), MetS (metabolic syndrome), HDL-C (high-density lipoprotein cholesterol), LDL-C (low-density lipoprotein cholesterol), CRP (C-reactive protein), IMT (carotid intima-media thickness), CAC (coronary-artery compliance), YEM (Young's elastic modulus), SI (stiffness index), Kyn/trp (kynurenine/tryptophan ratio), N/A (non-applicable; data not available due to lack of measurement values).

to define MetS as followed: waist circumference ≥94 cm for males and ≥80 cm for females plus any two of the following four factors: 1) raised triglycerides: >1.695 mmol/l, or specific treatment for this lipid abnormality, 2) reduced HDL-cholesterol: <1.036 mmol/l in males and <1.295 mmol/l in females, or specific treatment for this lipid abnormality, 3) raised blood pressure: blood pressure ≥130/85 mm Hg, or treatment of previously 1 diagnosed hypertension, 4) raised fasting plasma glucose ≥5.6 mmol/L, or previously diagnosed type 2 diabetes [23].

2.4. Statistical analysis

Due to skewed distribution in the cohort, CRP and trigyceride values were logarithmically transformed prior to analysis. Correlation between IDO (kyn/trp ratio) and each risk factor was determined by Pearson correlation coefficient. In addition, correlation was determined as unadjusted and adjusted with age. We also performed multiple testing adjustment with Bonferroni correction for each p-value from correlation analysis to reduce possibility for incorrect conclusions. In practice, we multiplied each p-value by the number of IDO–risk factor pairs in 2007 and 2011 for females and males separately. In order to determine whether IDO increases risk for obesity, we also performed risk ratio analysis using Poisson regression model with robust error variance. Data was adjusted with age, and also with age and BMI (both measured at baseline in 2001). BMI value of 30 was set as a threshold for obesity. Statistically significant results were obtained with $p < 0.05$. All analyses were performed using SAS software (version 9.4, SAS Institute, Cary, NC).

3. Results

3.1. Characteristics of the study participants

Baseline characteristics of the study participants are presented in Table 1. An estimate of IDO activity, characterized by kyn/trp ratio, was measured only in 2001 from 986 participants. In 2001, age of females (n = 544) varied from 24 to 39 years and weight from 40.5 to 134.4 kg. In males (n = 442), age varied from 24 to 39 years and weight from 55.2 to 157 kg. Only participants who had IDO data and complete risk factor data were included in the current analysis. Fatty liver was not determined in 2007, whereas measurements for IMT, CAC, YEM and SI were not performed in 2011. Median BMI values for females in 2007 and 2011 were 23.88 and 24.68, respectively, and for males 26.02 and 26.06, respectively.

3.2. Association between IDO and atherosclerosis risk factors

Pearson correlations between IDO and risk factors for atherosclerosis are shown in Table 2 for female and in Table 3 for male participants. Our aim was to evaluate how an estimate of IDO, measured in 2001, is related to risk factor data from 2007 and from 2011, hence, at different time points.

In unadjusted analyses among females for risk factors in 2007, the strongest correlations were observed for BMI and waist circumference. In addition, statistically significant correlations were identified between IDO and IMT, MetS and logarithmically modified CRP. When the data was adjusted with age, the associations of IDO with BMI, waist and CRP remained significant. Interestingly, IDO also correlated with logarithmically modified triglycerides in the age-adjusted analysis, even though correlation was non-significant in the unadjusted analysis. We continued the analyses by adjusting the results of CRP and triglycerides with cholesterol medication. Based on these analyses, IDO correlated with both CRP ($p = 0.0015$) and triglycerides ($p = 0.0449$) when the data was adjusted for age and cholesterol medication. When female participants from 2011 were examined, statistically significant correlations were discovered in BMI and waist in both unadjusted and age-adjusted analysis. In unadjusted analysis, IDO also correlated with fatty liver. After Bonferroni correction, the correlations of IDO with MetS in unadjusted and with logarithmically modified triglycerides in age-adjusted analyses did not remain statistically significant among females in 2007. Similarly, the associations of IDO with fatty liver in unadjusted and with waist circumference in age-adjusted analyses became non-significant among females in 2011. In addition to risk factors presented on the tables, we also performed correlation analysis with a variety of blood pressure and artery elasticity variables. However, statistically significant correlations were not found in these analyses.

In males, statistically significant correlation was found between IDO in 2001 and waist circumference in 2007 in an unadjusted analysis. This correlation remained significant when the data was adjusted with age. IDO was also statistically significantly correlated with HDL-cholesterol in age-adjusted analysis, but this correlation did not remain significant when the data was additionally adjusted with cholesterol medication ($p = 0.0615$). When risk factor data from 2011 was analyzed, statistically significant correlation between IDO in 2001 and HDL-cholesterol was observed in both unadjusted and age-adjusted analyses. This correlation remained significant after adjustment with cholesterol medication ($p = 0.0369$). However, in males none of the correlations between IDO and risk factors remained significant after Bonferroni correction.

Table 2
Pearson correlations between IDO and atherosclerosis risk factors in females 2007 and 2011. (A) Unadjusted correlations between IDO and atherosclerosis risk factors in females 2007, (B) Bonferroni corrected *p*-values for panel (A), (C) age-adjusted correlations between IDO and atherosclerosis risk factors in females 2007, (D) Bonferroni corrected *p*-values for panel (C), (E) unadjusted correlations between IDO and atherosclerosis risk factors in females 2011, (F) Bonferroni corrected *p*-values for panel (E), (G) age-adjusted correlations between IDO and atherosclerosis risk factors in females 2011, (H) Bonferroni corrected *p*-values for panel (G). Units for systolic and diastolic BP (blood pressure) were mmHg. BMI (body mass index), MetS (metabolic syndrome), HDL-C (high-density lipoprotein cholesterol), LDL-C (low-density lipoprotein cholesterol), CRP (C-reactive protein), IMT (carotid intima-media thickness), CAC (coronary-artery compliance), YEM (Young's elastic modulus), Log (logarithmically modified), N/A (non-applicable; data not available due to lack measurement values).

	Females 2007				**Females 2011**			
	Age: 30–45				**Age: 34–49**			
Variable	**Pearson correlation (r)**				**Pearson correlation (r)**			
	(A) Unadjusted with IDO (n = 435)	**(B) Multiple testing adjustment**	**(C) Adjusted with age (n = 434)**	**(D) Multiple testing adjustment**	**(E) Unadjusted with IDO (n = 394)**	**(F) Multiple testing adjustment**	**(G) Adjusted with age (n = 384)**	**(H) Multiple testing adjustment**
BMI	0.169, $p = 0.0004$	$p = 0.0052$	0.1598, $p = 0.0008$	$p = 0.0104$	0.192, $p = 0.0001$	$p = 0.0011$	0.1727, $p = 0.0007$	$p = 0.0077$
Waist	0.1734, $p = 0.0003$	$p = 0.0039$	0.1587, $p = 0.0009$	$p = 0.0117$	0.1719, $p = 0.0006$	$p = 0.0066$	0.1393, $p = 0.0063$	$p = 0.0693$
MetS	0.1082, $p = 0.024$	$p = 0.312$	0.0876, $p = 0.0685$	$p = 0.8905$	0.0441, $p = 0.3828$	$p = 4.2108$	0.0118, $p = 0.8175$	$p = 8.9925$
Glucose	0.0897, $p = 0.0617$	$p = 0.8021$	0.0683, $p = 0.1559$	$p = 2.0267$	0.0719, $p = 0.1545$	$p = 1.6995$	0.0721, $p = 0.1591$	$p = 1.7501$
Triglycerides (Log)	0.092, $p = 0.0551$	$p = 0.7163$	0.0948, $p = 0.0488$	$p = 0.6344$	0.0017, $p = 0.9727$	$p = 10.6997$	−0.0351, $p = 0.4935$	$p = 5.4285$
HDL-C	−0.0842, $p = 0.0795$	$p = 1.0335$	−0.069, $p = 0.1513$	$p = 1.9669$	−0.044, $p = 0.384$	$p = 4.224$	−0.0427, $p = 0.4049$	$p = 4.4539$
LDL-C	0.0167, $p = 0.7282$	$p = 9.4666$	−0.0099, $p = 0.8371$	$p = 10.8823$	−0.0308, $p = 0.5422$	$p = 5.9642$	−0.0487, $p = 0.3414$	$p = 3.7554$
CRP (Log)	0.1416, $p = 0.0031$	$p = 0.0403$	0.1535, $p = 0.0014$	$p = 0.0182$	0.0803, $p = 0.1117$	$p = 1.2287$	0.0853, $p = 0.0954$	$p = 1.0494$
IMT (mean)	0.0961, $p = 0.0452$	$p = 0.5876$	0.0447, $p = 0.354$	$p = 4.602$	N/A	N/A	N/A	N/A
Fatty liver	N/A	N/A	N/A	N/A	0.111, $p = 0.0275$	$p = 0.3025$	0.0939, $p = 0.0664$	$p = 0.7304$
Systolic BP	0.0084, $p = 0.8617$	$p = 11.2021$	−0.003, $p = 0.9513$	$p = 12.3669$	0.0899, $p = 0.0747$	$p = 0.8217$	0.0349, $p = 0.4957$	$p = 5.4527$
Diastolic BP	0.0067, $p = 0.8889$	$p = 11.5557$	−0.0032, $p = 0.9464$	$p = 12.3032$	0.0581, $p = 0.2504$	$p = 2.7544$	−0.004, $p = 0.9382$	$p = 10.3202$
CAC	−0.0185, $p = 0.6998$	$p = 9.0974$	0.0385, $p = 0.4244$	$p = 5.5172$	N/A	N/A	N/A	N/A
YEM	0.0497, $p = 0.3009$	$p = 3.9117$	0.0189, $p = 0.6956$	$p = 9.0428$	N/A	N/A	N/A	N/A

3.3. Obesity analysis

To further evaluate the association between IDO and body composition, we analyzed risk ratios (RR) for obesity separately in 2007 and 2011. BMI $\geq$30 kg/m^2 was regarded as threshold for obesity. As the data in Table 4 show, IDO in 2001 had statistically significant associations with obesity in all age-adjusted combinations. We also performed analysis on data adjusted for baseline age and BMI (both measured in 2001), because this allowed more appropriate evaluation on the development of obesity over time. As Table 4 shows, in females IDO in 2001 was related with obesity in 2011 and in males both 2007 and 2011. All these findings remained unchanged when analyses were performed using logarithmically modified IDO.

4. Discussion

We have shown in the present study that estimate of IDO activity associates with obesity in 6- and 10-year follow-up in males and premenopausal females. Statistically significant correlations were found between IDO, and BMI and waist from 2007 and from 2011 female participants after the data was age-adjusted. In risk ratio analysis, estimate of IDO associated with obesity in females after 10 years and in males after 6 and 10 years. Based on risk ratio values, this association was more pronounced in females after 10 years.

To best of our knowledge a connection between IDO, obesity and MetS in longitudinal analysis has not been investigated previously. Many cross-sectional studies have described statistically significant correlations between IDO activity, obesity and MetS [4,5] as well as emphasized influence of IDO activity in promoting BMI and obesity in females [9–11].

Based on our result, estimate of IDO correlated with less risk factors in 2011 compared to 2007 in females. It seems that as females get older, impact of IDO is more directly targeted to BMI and fat accumulation since IDO correlated with less risk factors in 2011 participants than in 2007 participants, and further, after age adjustment only BMI ($p = 0.0007$) and waist ($p = 0.0063$) correlated statistically significantly with IDO. This conclusion was supported by the fact that IDO is highly expressed in abdominal fat content [4], especially in white adipose tissue of females [9]. Recent investigation also demonstrated that IDO has an important role in shaping gut microbiota, which is required to control body weight and insulin resistance [13]. We linked statistically significant correlation of IDO primarily to IDO1 activity, which is expressed in adipose tissue [10], but we cannot exclude the possibility that part of the IDO function, mainly trp catabolism, is also due to IDO2 function, expressed in the liver, kidneys and antigen presenting cells [24], and/or TDO activity, mainly expressed in the liver [25].

It is noteworthy to emphasize that MetS did not have an impact for IDO in age-adjusted analysis. This could be explained by the premenopausal stage of females. At young age estrogen maintains elasticity of arteries, thereby protecting females. Further, this could also explain why artery elasticity variables did not correlate significantly with IDO. Based on statistically significant correlation between BMI and waist with IDO, it is likely that these females are in the process of developing MetS, which, however, is not in clinical stage yet. A previous cross-sectional

Table 3
Pearson correlations between IDO and atherosclerosis risk factors in males 2007 and 2011. (A) Unadjusted correlations between IDO and atherosclerosis risk factors in males 2007, (B) Bonferroni corrected *p*-values for panel (A), (C) age-adjusted correlations between IDO and atherosclerosis risk factors in males 2007, (D) Bonferroni corrected *p*-values for panel (C), (E) unadjusted correlations between IDO and atherosclerosis risk factors in males 2011, (F) Bonferroni corrected *p*-values for panel (E), (G) age-adjusted correlations between IDO and atherosclerosis risk factors in males 2011, (H) Bonferroni corrected *p*-values for panel (G). BMI (body mass index), MetS (metabolic syndrome), HDL-C (high-density lipoprotein cholesterol), LDL-C (low-density lipoprotein cholesterol), CRP (C-reactive protein), IMT (carotid intima-media thickness), Log (logarithmically modified), N/A (non-applicable; data not available due to lack measurement values).

	Males 2007				**Males 2011**			
	Age: 30–45				**Age: 34–49**			
Variable	**Pearson correlation (r)**				**Pearson correlation (r)**			
	(A) Unadjusted with IDO (n = 342)	**(B) Multiple testing adjustment**	**(C) Adjusted with age (n = 342)**	**(D) Multiple testing adjustment**	**(E) Unadjusted with IDO (n = 312)**	**(F) Multiple testing adjustment**	**(G) Adjusted with age (n = 300)**	**(H) Multiple testing adjustment**
BMI	0.1037, p = 0.0554	p = 0.4986	0.0872, p = 0.1079	p = 0.9711	0.0698, p = 0.2188	p = 1.7504	0.072, p = 0.2144	p = 1.7152
Waist	0.1317, p = 0.0148	p = 0.1332	0.1132, p = 0.0367	p = 0.3303	0.0686, p = 0.2273	p = 1.8184	0.0692, p = 0.2326	p = 1.8608
MetS	−0.0146, p = 0.7883	p = 7.0947	−0.0264, p = 0.6273	p = 5.6457	−0.0095, p = 0.8671	p = 6.9368	−0.0162, p = 0.7799	p = 6.2392
Glucose	0.0063, p = 0.907	p = 8.163	−0.0155, p = 0.7762	p = 6.9858	−0.0579, p = 0.3082	p = 2.4656	−0.0544, p = 0.3485	p = 2.788
Triglycerides (Log)	−0.0167, p = 0.7585	p = 6.8265	−0.0256, p = 0.6375	p = 5.7375	0.0218, p = 0.7014	p = 5.6112	0.0265, p = 0.6479	p = 5.1832
HDL-C	−0.0943, p = 0.0816	p = 0.7344	−0.1067, p = 0.0489	p = 0.4401	−0.1153, p = 0.0418	p = 0.3344	−0.1221, p = 0.0348	p = 0.2784
LDL-C	0.0225, p = 0.6789	p = 6.1101	0.0058, p = 0.915	p = 8.235	−0.0029, p = 0.9595	p = 7.676	−0.0127, p = 0.8273	p = 6.6184
CRP (Log)	0.0091, p = 0.8675	p = 7.8075	−0.0009, p = 0.9869	p = 8.8821	0.0438, p = 0.4411	p = 3.5288	0.0471, p = 0.4174	p = 3.3392
IMT (mean)	0.0434, p = 0.4239	p = 3.8151	0.0121, p = 0.8242	p = 7.4178	N/A	N/A	N/A	N/A

Table 4
Risk ratio analysis for obesity.

Outcome	**Age-adjusted, RR (95% CI)**	**Age- and BMI-adjusted, RR (95% CI)**
Female		
Obesity in 2007	1.037 (1.012–1.062), p = 0.0033	0.996 (0.965–1.028), p = 0.8057
Obesity in 2011	1.057 (1.037–1.077), p < 0.0001	1.026 (1.005–1.047), p = 0.0147
Male		
Obesity in 2007	1.027 (1.008–1.046), p = 0.0044	1.019 (1.005–1.033), p = 0.0091
Obesity in 2011	1.024 (1.005–1.044), p = 0.0148	1.015 (1.001–1.03), p = 0.0404

Female and male risk ratio was determined separately in 6- and 10-year follow-up. The data was adjusted for age, and also for age and BMI (all measured at baseline in 2001). RR (risk ratio), CI (confidence interval).

study verified statistically significant correlation between elevated IDO activity and MetS [5] giving further evidence that these two components may be linked.

In female risk ratio analysis, estimate of IDO activity was not increasing risk for obesity in 2007 when the data was adjusted for age and BMI even though IDO correlated with BMI and waist. Since we used BMI value of 30 as a threshold for obesity, it may be that the majority of females still have BMI-value below this threshold even though fat accumulation has already begun. In 2011, however, the risk for obesity was increased by IDO activity. It could be that the borderline age when IDO begins to affect female obesity is 34–49 years. Until then female physiology may be able to diminish the impact of IDO. Alternatively, IDO may not be fully activated until female physiology changes as females begin to approach menopausal stage.

In males, weak correlations were found between estimate of IDO, waist and HDL in 2007 participants, and IDO and HDL in 2011 participants. Lack of strong correlation for further risk factors, especially BMI and waist, was unexpected and we were unable to evaluate whether IDO influences fat accumulation in males. In Pertovaara and co-workers investigation that used the same participant cohort as the present study, IDO correlated with both BMI and waist (p = 0.05 and 0.02, respectively) in males in 2001 [7]. In contrast, we observed a completely opposite outcome in male risk ratio analysis, where IDO activity increased risk for obesity already after a 6-year follow-up. IDO clearly influenced BMI and consequently obesity in males but the mechanism appeared to be different compared to females. Impact was not transmitted via risk factors but seemed to be directly related to weight. We speculate that this phenomenon is explained by differences in physiology between sexes. More specifically, due to low estrogen levels, males may not have the same protection capability as females, thus, IDO begins to influence weight in their early life consequently increasing risk for obesity. These observations are in line with CVD morbidity outcomes between sexes: adverse CVD outcomes appear later in females compared to males.

Understanding underlying mechanisms through which IDO influences obesity and consequently development of atherosclerosis is still of importance. An interesting finding in this regard is an investigation by Laurans et al. who recently elucidated previously undiscovered interactions between IDO and obesity in mice, showing that deletion or inhibition of IDO actually improves insulin sensitivity, preserves gut mucosal barrier, decreases endotoxemia and chronic inflammation and regulates lipid metabolism in the liver and adipose tissues. Authors speculated that obesity is associated with an increase of intestinal IDO activity, which shifts tryptophan metabolism from indole derivate and

interleukine 22 production towards kynurenine production [13]. Obesity could therefore be defined as a state of intestinal dysbiosis with increased intestinal permeability *versus* bacterial composants. It is clear that these important observations provide new insights for the role of IDO in obesity. Future investigations are likely to provide further evidence on whether these results are replicable in human cohorts.

Furthermore, it seems that NAFLD and high IDO activity share common clinical and biological outcomes [6,7]: central abdominal obesity along with other components such as hypertriglyceridemia and components of insulin resistance [6]. Up to 80% of patients with NAFLD are obese, defined as a body mass index (BMI) > 30 kg/m^2. It is plausible that high IDO activity is one of the risk factors of NAFLD in females, although we have only indirect evidence of NAFLD in this study. This should be further investigated in prospective studies.

Since our study was based on data from the Cardiovascular Risk in Young Finns Study cohort, a definite strongpoint was a longitudinal, long-term follow-up of a well-characterized and widely examined population along with an extensive array of risk factors used in the analysis. These facts warrant a possibility for further and in-depth examinations. In terms of other limitations, we were unable to perform survival analysis due to lack of time-event data. Secondly, IDO activity was measured from this cohort only in 2001. Later IDO measurements in 2007 and 2011 would have demonstrated how IDO activity changes as the population ages. Thirdly, further follow-up of participants would have provided evidence on how many participants are actually developing MetS and in this regard elucidated the role of IDO in this process. Lastly, despite the fact that HPLC ultraviolet detection in some cases lack selectivity compared to current gold standard method, liquid chromatography-mass spectrometry [26,27], we think that the method used in this study is nevertheless sensitive and specific enough to produce a reliable estimate of IDO activity. We base this opinion on the fact that HPLC ultraviolet detection method allows measurement of kyn and trp concentrations with high sensitivity and without loss of specificity [15], and also on the fact that extraction efficiencies for kyn and trp were similar regardless of whether HPLC ultraviolet detection or liquid chromatography-mass spectrometry was used [26].

4.1. Conclusions

In this study, we demonstrated that estimate of IDO activity associate with obesity in 6- and 10-year follow-up in males and premenopausal females. Multiple studies have already shown an interaction between IDO and obesity even though the exact mechanism of action of IDO is still not fully understood. It is unclear whether IDO activity has a key function in promoting obesity, especially visceral obesity and NAFLD in females, and therefore adverse events leading to atherosclerosis, or whether its activation is rather a consequence from other obesity-related events such as chronic inflammation and endothelial dysfunction. In this case, it is also possible that obesity in fact boosts further IDO activation, thus, worsening the situation on a cellular level.

We also cannot exclude the possibility that IDO may have a dual role in humans; under certain conditions IDO activity may protect a person from the initiation of atherosclerosis, whereas in others it may promote adverse events. Should this be a possibility, it could mean that IDO activity is both the reason for and the consequence of obesity.

In terms of clinical relevance, our results do not support the use of IDO as a therapeutic target per se. More human studies and data are required to reveal mechanisms by which IDO could forecast CVD, and also whether targeted IDO treatments would have beneficial outcomes on initiation and/or progression of obesity.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CRediT authorship contribution statement

Petri Niinisalo: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing, Visualization, All authors reviewed, edited and approved the manuscript. **Olli T. Raitakari:** Methodology, Validation, Investigation, All authors reviewed, edited and approved the manuscript. **Mika Kähönen:** Validation, All authors reviewed, edited and approved the manuscript. **Mikko Hurme:** Methodology, All authors reviewed, edited and approved the manuscript. **Terho Lehtimäki:** Validation, All authors reviewed, edited and approved the manuscript. **Costan Magnussen:** Validation, All authors reviewed, edited and approved the manuscript. **Jorma Viikari:** Methodology, Validation, Investigation, All authors reviewed, edited and approved the manuscript. **Markus Juonala:** Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing, All authors reviewed, edited and approved the manuscript. **Risto Kaaja:** Conceptualization, Writing – original draft, Writing – review & editing, All authors reviewed, edited and approved the manuscript.

Appendix A. Supplementary data

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References

[1] A.L. Mellor, D.H. Munn, Tryptophan catabolism and regulation of adaptive immunity, J. Immunol. 170 (2003) 5809–5813.
[2] A.L. Mellor, D.H. Munn, Ido expression by dendritic cells: tolerance and tryptophan catabolism, Nat. Rev. Immunol. 4 (2004) 762–774.
[3] G. Brandacher, E. Hoeller, D. Fuchs, et al., Chronic immune activation underlies morbid obesity: is Ido a key player? Curr. Drug Metabol. 8 (2007) 289–295.
[4] H. Mangge, K.L. Summers, A. Meinitzer, et al., Obesity-related dysregulation of the tryptophan-kynurenine metabolism: role of age and parameters of the metabolic syndrome, Obesity 22 (2014) 195–201.
[5] N.H. Mallmann, E.S. Lima, P. Lalwani, Dysregulation of tryptophan catabolism in metabolic syndrome, Metab. Syndr. Relat. Disord. 16 (2018) 135–142.
[6] S. Milic, D. Lulic, D. Stimac, Non-alcoholic fatty liver disease and obesity: biochemical, metabolic and clinical presentations, World J. Gastroenterol. 20 (2014) 9330–9337.
[7] M. Pertovaara, A. Raitala, M. Juonala, et al., Indoleamine 2,3-dioxygenase enzyme activity correlates with risk factors for atherosclerosis: the Cardiovascular Risk in Young Finns Study, Clin. Exp. Immunol. 148 (2007) 106–111.
[8] L. Young, L. Cho, Unique cardiovascular risk factors in women, Heart 105 (2019) 1656–1660.
[9] I. Wolowczuk, B. Hennart, A. Leloire, et al., Tryptophan metabolism activation by indoleamine 2,3-dioxygenase in adipose tissue of obese women: an attempt to maintain immune homeostasis and vascular tone, Am. J. Physiol. Regul. Integr. Comp. Physiol. 303 (2012) R135–R143.
[10] M. Favennec, B. Hennart, R. Caiazzo, et al., The kynurenine pathway is activated in human obesity and shifted toward kynurenine monooxygenase activation, Obesity 23 (2015) 2066–2074.
[11] M. Groer, D. Fuchs, A. Duffy, et al., Associations among obesity, inflammation, and tryptophan catabolism in pregnancy, Biol. Res. Nurs. 20 (2018) 284–291.

[12] K.A. Polyzos, O. Ovchinnikova, M. Berg, et al., Inhibition of indoleamine 2,3-dioxygenase promotes vascular inflammation and increases atherosclerosis in Apoe-/- mice, Cardiovasc. Res. 106 (2015) 295–302.
[13] L. Laurans, N. Venteclef, Y. Haddad, et al., Genetic deficiency of indoleamine 2,3-dioxygenase promotes gut microbiota-mediated metabolic health, Nat. Med. 24 (2018) 1113–1120.
[14] O.T. Raitakari, M. Juonala, T. Rönnemaa, et al., Cohort profile: the cardiovascular risk in Young Finns Study, Int. J. Epidemiol. 37 (2008) 1220–1226.
[15] A. Laich, G. Neurauter, B. Widner, et al., More rapid method for simultaneous measurement of tryptophan and kynurenine by HPLC, Clin. Chem. 48 (2002) 579–581.
[16] M. Juonala, J.S. Viikari, N. Hutri-Kähönen, et al., The 21-year follow-up of the Cardiovascular Risk in Young Finns Study: risk factor levels, secular trends and east-west difference, J. Intern. Med. 255 (2004) 457–468.
[17] K.V. Porkka, O.T. Raitakari, A. Leino, et al., Trends in serum lipid levels during 1980–1992 in children and young adults. The Cardiovascular Risk in Young Finns Study, Am. J. Epidemiol. 146 (1997) 64–77.
[18] W.T. Friedewald, R.I. Levy, D.S. Fredrickson, Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge, Clin. Chem. 18 (1972) 499–502.
[19] O.T. Raitakari, M. Juonala, M. Kähönen, et al., Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood – the Cardiovascular Risk in Young Finns Study, J. Am. Med. Assoc. 290 (2003) 2277–2283.
[20] J. Koskinen, M. Kähönen, J.S. Viikari, et al., Conventional cardiovascular risk factors and metabolic syndrome in predicting carotid intima-media thickness progression in young adults: the cardiovascular risk in young Finns study, Circulation 120 (2009) 229–236.
[21] E. Suomela, M. Oikonen, J. Virtanen, et al., Prevalence and determinants of fatty liver in normal-weight and overweight young adults. The Cardiovascular Risk in Young Finns Study, Ann. Med. 47 (2015) 40–46.
[22] M. Juonala, M.J. Järvisalo, N. Mäki-Torkko, et al., Risk factors identified in childhood and decreased carotid artery elasticity in adulthood: the Cardiovascular Risk in Young Finns Study, Circulation 112 (2005) 1486–1493.
[23] K.G. Alberti, P. Zimmet, J. Shaw, IDF Epidemiology Task Force Consensus Group. The metabolic syndrome–a new worldwide definition, Lancet 366 (2005) 1059–1062.
[24] L.M.F. Merlo, J.B. DuHadaway, J.D. Montgomery, et al., Differential roles of Ido1 and Ido2 in T and B cell inflammatory immune responses, Front. Immunol. 11 (2020) 1861.
[25] H.J. Ball, A. Sanchez-Perez, S. Weiser, et al., Characterization of an indoleamine 2,3-dioxygenase-like protein found in humans and mice, Gene 396 (2007) 203–213.
[26] Y. Huang, A. Louie, Q. Yang, et al., A simple LC–MS/MS method for determination of kynurenine and tryptophan concentrations in human plasma from HIV-infected patients, Bioanalysis 5 (2013) 1397–1407.
[27] C.G. Adu-Gyamfi, D. Savulescu, J.A. George, et al., Indoleamine 2, 3-dioxygenase-mediated tryptophan catabolism: a leading star or supporting act in the tuberculosis and HIV Pas-de-Deux? Front. Cell. Infect. Microbiol. 9 (2019) 372.