Adiposity and plasma concentrations of kynurenine pathway metabolites and traditional markers of inflammation

Mengmei E. Wang,[1,2,*] Allison M. Hodge,[2,3,*] Sherly X. Li,[2,3,4] Melissa C. Southey,[1,3,5] Graham G. Giles,[1,2,3] Pierre-Antoine Dugué[1,2,3]

[1] Precision Medicine, School of Clinical Sciences at Monash Health, Monash University, Clayton, VIC, Australia

[2] Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Parkville, VIC, Australia

[3] Cancer Epidemiology Division, Cancer Council Victoria, Melbourne, VIC, Australia

[4] Medical Research Council Epidemiology Unit, University of Cambridge, Cambridge, UK, CB2 0QQ

[5] Department of Clinical Pathology, The University of Melbourne, Parkville, VIC Australia

[*] These authors contributed equally to this work

Correspondence: Dr Pierre-Antoine Dugué, Precision Medicine, School of Clinical Sciences at Monash Health, Monash University, 246 Clayton Road, Clayton VIC 3168, Australia; E-mail: pierre-antoine.dugue@monash.edu

Words: 3,290; **Tables and Figures**: 4; **Supplementary Material**; **References**: 35

Keywords: inflammation, tryptophan kynurenine pathway, body composition, body size, aging

Funding

The MCCS cohort recruitment was funded by VicHealth and Cancer Council Victoria. The MCCS was further augmented by Australian National Health and Medical Research Council grants 209057, 396414, 1074383, and 1106016 and by infrastructure provided by Cancer Council Victoria.

Author Contributions

All authors contributed to the design of the study. MEW and PAD analysed the data. MEW, AMH, SXL and PAD wrote the original draft of the manuscript. All authors reviewed and edited sections of the manuscript. GGG acquired funding. MCS and GGG provided project administration and infrastructure support. AMH, SXL and PAD supervised the study.

Competing Interests

The authors declare no conflict of interest.

ABSTRACT – 230 words

Aim: The kynurenine pathway is increasingly recognised to play a role in inflammation and disease. We assessed the cross-sectional and longitudinal associations of adiposity measures with plasma concentrations of kynurenine pathway metabolites and traditional markers of inflammation.

Methods: We used data from 970 Melbourne Collaborative Cohort Study participants who had plasma markers measured at baseline (median age 59 years) and follow-up (median age 70 years). Linear regression was used to assess cross-sectional and longitudinal associations between four adiposity measures and concentrations of i) nine kynurenine pathway metabolites; ii) two derived markers; iii) eight traditional inflammatory markers.

Results: Cross-sectionally, most kynurenine metabolites were strongly associated with adiposity measures at both time points; associations were generally stronger than for most inflammation markers except CRP (e.g. body mass index at baseline, quinolinic acid (per S.D. β=0.30, 95%CI: 0.24-0.36, $P=10^{-21}$), kynurenine (β=0.25, 95%CI: 0.19-0.31, $P=10^{-16}$) and CRP (β=0.31, 95%CI: 0.25-0.37, $P=10^{-24}$), and these remained largely unchanged after adjustment for confounders. Longitudinally, changes in adiposity measures over approximately a decade were positively associated with changes in kynurenine metabolite concentrations (in particular for, 3-hydroxyanthranilic acid, kynurenine and quinolinic acid), and more strongly so than for other markers of inflammation, including CRP.

Conclusions: In middle-aged and older adults, plasma concentrations of kynurenine metabolites are strongly associated with adiposity, both cross-sectionally and longitudinally. Our study demostrates that kynurenine metabolites may be valuable markers to monitor the adverse consequences of obesity.

1. Introduction

Obesity is a metabolic disorder defined as excessive adipose tissue accumulation and is a major risk factor for poor health [1]. The aetiology of obesity is complex as many environmental, lifestyle, socioeconomic, clinical and genetic factors contribute to dysregulating the balance between fat deposition, energy intake and expenditure.

An important feature of obesity is a chronic, low-grade inflammatory state, which is a key mechanism through which obesity causes diseases such as cancer, atherosclerosis, and type II diabetes [2]. Adipose tissue produces and secretes chronic high concentrations of proinflammatory markers known as adipokines, including interleukins-6 and 8 (IL-6, IL-8), and tumor necrosis factor alpha (TNF-α) [3]. Circulating C reactive protein (CRP) concentration is widely used as an indicator of the low-grade inflammatory process and is strongly increased in people with obesity [4]. Serum concentrations of other biomarkers such as neopterin are modulated by inflammatory cytokines and are associated with inflammation-related conditions such as cardiovascular diseases [5]. Clinically, cystatin C is a biomarker of kidney function, which tends to be altered in people with obesity [6] and is involved in various inflammation-related mechanisms via regulation of macrophage responses to the cytokine interferon-γ (IFN-γ). Higher concentrations of traditional inflammatory markers have been documented in the adipose tissue and plasma of people with obesity, and their levels are reduced after weight loss, which suggests that obesity plays a causal role in inflammation [7].

A growing body of evidence suggests that obesity-related systemic inflammation is associated with alterations in the catabolism of tryptophan to NAD+ via the kynurenine pathway [8]. During inflammation, IFN-γ activates the indoleamine 2,3-dioxygenase enzyme (IDO), catalyzing the first and rate-determining step of tryptophan catabolism through the kynurenine pathway [9]. There is

also a potential role for kynurenine metabolites and derived markers such as the kynurenine-tryptophan ratio (KTR) and the PAr index (ratio of pyridoxic acid and pyridoxal + pyridoxal-5′-phosphate) in chronic diseases involving inflammatory processes, immune response, and excitatory neurotransmission [10-12]. To date, only a handful of studies have examined the association between obesity and kynurenine pathway metabolites, and these were heterogeneous in terms of study sample size, design, and included population [10, 13-16], for example focusing on people with cardiovascular disease [10] or severe obesity [15] which are highly selected population subgroups, and predominantly used BMI as a measure of adiposity, which does not capture every aspect of obesity. Given the well-established involvement of inflammatory markers in obesity, establishing potentially useful additional markers - such as kynurenine metabolites - requires comparison of their relative strengths of association, but such comparisons have essentially not been made beyond CRP.

We aimed to examine the cross-sectional and longitudinal associations of several adiposity measures with kynurenine pathway metabolites and traditional markers of inflammation.

2. Methods

2.1. Study sample

The Melbourne Collaborative Cohort Study (MCCS) is a prospective cohort of 41,513 men and women, 99.3% of whom were aged between 40 and 69 years at recruitment (1990-1994) [17]. All participants were of white European background, the majority being born in Australia, and 13% in Italy, 11% in Greece, and 6% in the U.K. At recruitment, blood samples were collected and questions covering lifestyle, demographics and medical history were answered using self-

administered questionnaires. A first follow-up was conducted in 1995-1998, and a second follow-up (2003-2007) repeated most baseline measures and collected additional blood samples.

Written informed consent was obtained from all participants. The study was approved by Cancer Council Victoria's Human Research Ethics Committee and performed in accordance with the institution's guidelines.

The details of the selection of participants in this study have been described previously [18, 19]. In brief, 1,100 MCCS participants who attended the second follow-up and had available blood samples at baseline and follow-up were selected for studying longitudinal changes in DNA methylation [20, 21]. Of these, 976 were selected for measurement of an extensive series of plasma markers related to inflammation, the kynurenine pathway and B vitamin status [18, 19].

2.2. Body size and composition measurements

Weight, waist circumference, and hip circumference were measured at baseline and follow-up, whereas height and bioimpedance were measured at baseline only. Waist and hip circumferences were measured to 1mm using a metal anthropometric tape. Weight was measured to 100g using digital electronic scales, height to 1mm using a stadiometer. Single-frequency (50kHz) electric current produced by a BIA-101A RJL system analyzer (RJL systems, Detroit, MI) was used to perform bioelectric impedance analysis. Resistance and reactance were measured with subjects in a supine position and used to calculate fat free mass (FFM) using the formula developed by Roubenoff [22].

2.3. Biochemical analyses

Baseline plasma samples were collected in Lithium-heparin tubes between 1990 and 1994, and second wave samples were collected into ethylenediaminetetraacetic acid (EDTA) tubes between

2003 and 2007. Plasma was stored in liquid nitrogen at -180°C from the time of collection until shipment to the Bevital and IARC laboratories for biochemical analyses. Kynurenine metabolites, including plasma concentrations of tryptophan (TRP), kynurenine (KYN), kynurenic acid (KA), 3-hydroxykynurenine (HK), xanthurenic acid (XA), anthranilic acid (AA), 3-hydroxyanthranilic acid (HAA), picolinic acid (PIC) and quinolinic acid (QA), together with cystathionine, and neopterin, were measured using chromatography-tandem mass spectrometry [23]. C-reactive protein, serum amyloid A (SAA), calprotectin, and cystatin C were measured using matrix-assisted laser desorption/Ionization-time of flight (MALDI-TOF) mass spectrometry [24]. The derived markers kynurenine-tryptophan ratio (KTR) and the PAr index (4-pyridoxic acid / (pyridoxal+ pyridoxal-5′-phosphate)) were calculated. IL-6, IFN-γ and TNF-α were measured using a Meso Scale Discovery 6-Plex kit. Measurement of these biomarkers was shown to be reliable for use in large-scale epidemiological studies [25].

2.4. Covariates

Smoking status was self-reported at baseline and follow-up and classified as never / former / current smoking. Self-reported intakes of beer, wine, and spirits (for the previous week at baseline and for the previous 12 months at follow-up) were combined and converted to grams of alcohol per day. Physical activity information was also self-reported. At baseline, a score ranging from 0 to 16 was calculated based on the weekly frequency at which participants walked or engaged in vigorous (double weight) and less vigorous activity over the last 6 months; at follow-up, total physical activity information was collected using the Long Form International Physical Activity Questionnaire (IPAQ-Long) and converted to metabolic equivalent of tasks per hour [26]. Dietary data from a semi-quantitative food frequency questionnaire (FFQ) were used to calculate a modified Mediterranean Diet Score (MDS), which ranged from 0 (lowest adherence) to 9 (highest

adherence) and was used as a proxy for overall diet quality [27]. Residential postcodes were used to assign participants to a decile of the Socioeconomic Indexes of Areas (SEIFA), an area-based measure of socioeconomic position.

2.5. Statistical analysis

Six participants were excluded from the analysis due to having missing values in more than 50% of the markers. The proportion of missing values in any other individual variable was very low (**Table S1**), and the random forest method *missForest* [28] was used to impute any missing values to obtain a complete dataset of baseline and follow-up.

Baseline and follow-up measures of inflammatory markers were: i) log-transformed to obtain distributions closer to Gaussian, ii) winsorized at +/- 3 standard deviations from the mean to minimize the potential influence of extreme outliers, and iii) re-scaled to z-scores for easier comparisons of effect size estimates. Adiposity measures were also rescaled to z-scores for easier comparisons. Pearson correlations between adiposity measures were calculated using height-adjusted measures (obtained by regressing each variable on height). Linear regression models were used to obtain coefficient estimates and 95% confidence intervals (CI) for the cross-sectional and longitudinal associations between adiposity measures and plasma markers.

2.5.1. Cross-sectional analyses

Cross-sectional associations were assessed separately at baseline and follow-up. We first examined the association of markers in the kynurenine pathway with each of the following adiposity measures: BMI, waist circumference, waist-hip ratio (WHR) and fat-mass ratio (FMR) (the latter at baseline only). Model 1 adjusted for age, sex, country of birth, and height. Model 2 additionally adjusted for socioeconomic status, alcohol consumption, smoking, and MDS. Sensitivity analyses

were performed by fitting two additional models: i) Model 3 additionally adjusted for cystatin C to control for the effect of renal function on kynurenine concentrations; ii) Model 4 included Model 2 variables and additional adjustment for CRP to determine whether associations were independent of general inflammation. The same covariates as in Model 2 were used to assess associations of adiposity measures with traditional inflammation markers including CRP, IL-6, IFN- γ, serum amyloid A, TNF-α, cystatin C, calprotectin, and neopterin. To further compare the strengths of association across adiposity measures, we extracted the R^2 statistics from baseline and follow-up regression models.

2.5.2. Longitudinal analyses

We assessed the associations of changes in BMI, waist circumference and WHR from baseline to follow-up with plasma concentrations of kynurenine pathway metabolites and general inflammatory markers at follow-up, adjusting for baseline levels of these. The models were adjusted for age, sex, country of birth, height, baseline smoking status, alcohol consumption, socioeconomic status, MDS, physical activity, and time between baseline and follow-up. Baseline adiposity measures were adjusted for in the models as appropriate.

3. Results

The median age of participants was 59 years at baseline and 70 years at follow-up. Participants generally had normal BMI or were slightly overweight (at baseline median [interquartile range] BMI: 26.4 [24.2-28.9]; at follow-up: 26.8 [24.3-29.4]), **Table 1**. The correlations (height-adjusted, at baseline) of BMI with waist circumference, WHR and FMR were 0.83, 0.49 and 0.50, respectively; of waist circumference with FMR: 0.26; and of WHR with FMR: -0.11 (**Table 2**).

3.1 Cross-sectional analyses

At baseline, QA had the strongest association with all measures of adiposity (e.g., BMI: β=0.30, 95%CI: 0.24-0.36, P=$5x10^{-23}$, Model 2), **Figure 1** and **Tables S2-S3**. Associations were also strong for KYN and KA (β=0.25, P=$7x10^{-16}$ and β=0.23, P=$4x10^{-13}$, respectively), and positive for other kynurenine metabolites including TRP, HAA, HK, AA (P<0.05, β coefficients ranging from 0.07 to 0.20). PIC was negatively associated with all measures of adiposity, albeit weakly (e.g., for BMI β=-0.10, 95%-CI: -0.16 to -0.04, P=0.001). The derived marker KTR showed a strong positive association (e.g., for BMI β=0.19, 95%CI: 0.13-0.25, P=$4x10^{-10}$) whereas the association for the PAr index was weaker (β=0.09, P=0.007). Several traditional inflammatory markers were strongly associated with adiposity, including CRP (BMI: β=0.33, 95%CI: 0.27-0.39), SAA (β=0.25, 95%CI: 0.19-0.31), and IL-6 (β=0.20, 95%CI: 0.13-0.26). Associations were positive but weaker (β≤0.11) for cystatin C, neopterin, calprotectin, TNF-α and IFN-γ (**Figure 1**, **Tables S2-S3**). The variance explained was greatest for models of BMI for 8/19 markers, FMR for 8/19 markers, waist circumference for 4 markers and WHR for one marker (**Table S6**).

Adjustment for confounders in Model 2 at baseline made virtually no difference to the results (e.g. fat mass ratio: QA in Model 1: β=0.35, p=$7x10^{-19}$; QA in Model 2: β=0.34, p=$3x10^{-18}$), **Figure S7**. The same was observed for other inflammatory markers (e.g., fat mass ratio – CRP, Model 1: β=0.40, and Model 2: β=0.39) and across adiposity measures.

Model 3 further adjusted for cystatin C, a marker of kidney function and strong determinant of kynurenine marker concentrations. This resulted in small attenuation of regression coefficients, for instance, β coefficient for BMI and QA decreased from 0.30 to 0.28 (**Table S4, Figure S7**).

The attenuation after adjustment for CRP (Model 4, **Table S5, Figure S7**) was somewhat more substantial, for example the β coefficient for BMI and QA decreased from 0.30 to 0.24. Most

kynurenine markers nevertheless remained strongly associated with adiposity measures in CRP-adjusted models: KYN: β=0.20 ($P=10^{-10}$), HK: β=0.16 ($P=10^{-6}$), KA: β=0.24 ($P=10^{-13}$), XA: β=0.14 ($P=10^{-5}$), HAA: β=0.09 ($P=10^{-3}$), QA: β=0.24 ($P=10^{-14}$), KTR: β=0.24 ($P=10^{-14}$).

The overall patterns and strengths of associations observed at the follow-up visit were very similar to baseline results (**Figure S8** and **Tables S9-S12**). QA remained the marker most strongly associated with adiposity measures (e.g. BMI in Model 1, β=0.29) and very strong associations were observed for KA. KYN, HK, XA, and HAA (BMI: β: 0.24-0.28). Differences with the baseline analysis included weaker or null association for TRP, neopterin and IFN-γ. The variance explained was greatest for 11/19 markers using the BMI variable, and for 5 and 3 markers using waist circumference and WHR, respectively (**Table S13**). Sensitivity analyses with adjustment for cystatin C or CRP did not materially change the strengths of associations (**Tables S11-S12**).

3.2. Longitudinal analysis

Figure 3 and **Table S14** show the β coefficients for the association between change (Δ) in waist circumference, BMI and WHR from baseline to follow-up and concentrations of kynurenine and inflammation markers at follow-up. Several kynurenine metabolites showed strong longitudinal associations, although these were generally weaker than in cross-sectional analyses. For example, for ΔBMI, HAA: β=0.22, 95%CI: 0.16-0.28 ($P=10^{-13}$); KYN: β=0.18 ($P=10^{-10}$); QA: β=0.17 ($P=10^{-11}$), KA: β=0.15 ($P=10^{-7}$), XA: β=0.15 ($P=10^{-6}$), which was greater than for CRP (β=0.11, 95%CI: 0.05-0.16, $P=10^{-4}$). Positive associations were also found for HK (β=0.11), TRP (β=0.09) and KTR (β=0.08). Traditional inflammatory markers other than CRP showed weak or null longitudinal association with adiposity measures (β<0.05). Longitudinal associations appeared somewhat stronger for BMI than for other adiposity measures (**Table S14**).

Discussion

Most kynurenine metabolites showed strong positive associations (to a varying degree across markers) with adiposity measures, with generally similar strength and pattern at baseline (median age 59 years) and follow-up (median age 70 years). In both the cross-sectional and longitudinal analysis, the associations observed for kynurenine metabolites were generally greater than for other markers of inflammation and remained strong after adjustment for CRP or cystatin C.

Our study had a sample size larger than most previous studies [13-16, 29] and provided reasonably accurate estimates of association. Nevertheless, larger studies would be useful to shed more light on some of our findings because the confidence intervals obtained across markers, adiposity measures and time points were somewhat overlapping, and some inconsistent results were observed, for example when contrasting results from baseline and follow-up analyses. Interestingly, although the correlations between adiposity measures were only moderate or weak (except that of BMI with waist circumference), all were strongly associated with marker concentrations. Analyses were sequentially adjusted for a comprehensive set of confounders, which explained little of the associations. A major strength of our study was the wide range of markers we investigated, including 19 plasma markers or derived markers relevant to inflammatory processes. This allowed the comparison of the relative strengths of association observed for kynurenine metabolites and traditional markers of inflammation, which was largely not made in previous studies. Our findings therefore offer a comprehensive insight into the relation between inflammation-related markers and different facets of obesity.

Several limitations should be acknowledged. Although the measurement of the markers included in our study is considered reliable for large-scale epidemiological studies, external studies using the same technologies reported intraclass correlation coefficients ranging between 0.5 to 0.8 [30].

This issue is not specific to our study and would have resulted in estimates biased toward the null if measurement error was non-differential [31]. The longitudinal analysis was based on only two-time points; additional time points would be useful to provide insights into the temporality and stability of changes in adiposity and marker concentrations. Our study design also could not rule out reverse causation, i.e. the possibility that changes in marker concentrations would be a driver of obesity. Lastly, although there were no evident departures from linearity (as assessed using scatter plots, not shown), models allowing for non-linearity might provide better data fits and be of interest in future studies.

Our findings are overall consistent with the few previous studies on obesity and the kynurenine pathway. The case-control study by Cussotto et al. [15] measured CRP, IL-6, KTR and TRP in 127 adults with obesity; BMI was positively associated with KTR but negatively associated with TRP. Although we found a strong positive association between BMI and KTR (both cross-sectionally and longitudinally), we found a weak positive association of TRP with BMI at baseline and no association at follow-up. The study by Mangge et al. (N=527 adolescents and adults) found no association of TRP with overweight/obesity [14]. Different participant characteristics might explain discrepant findings as Cussotto et al. included participants with severe obesity who had been selected for bariatric surgery due to pre-existing medical conditions, whereas the participants in Mangge et al. and our study were healthier. The Hordaland Health Study [32] has participants' characteristics similar to ours in terms of age, ancestry and health profile, and also reported a weakly positive association of BMI with TRP. Given that TRP is derived from the diet, cross-country differences in dietary habits may also have contributed inconsistent findings. While their study also observed strong associations for KYN, KA, HK, HAA and KTR, other markers such as QA, PIC, CRP or IL-6 were not evaluated. Favennec et al. included 836 randomly selected

participants (mean BMI=27.8 kg/m^2) from the DESIR cohort (Data from an Epidemiological Study on the Insulin Resistance Syndrome) [16] and obtained findings consistent with ours, including strong positive associations of BMI with KYN and KTR and weaker with TRP. Other anthropometric measures such as waist circumference, hip circumference and WHR surprisingly showed no associations with these markers. Other kynurenine metabolites and traditional inflammatory markers were not evaluated. We also found that AA had positive associations with all measures of adiposity at baseline and follow-up, unlike two other studies finding no association [10, 32]. These were relatively weak compared with other kynurenine metabolites in both the cross-sectional and longitudinal analysis.

A major finding of our study is that several kynurenine metabolites such as HAA, QA, KTR, KA, KYN, HK had stronger associations with adiposity measures than most traditional inflammation-related markers, except CRP. Such comparison was not possible in most previous studies because only a few other markers were considered. Zahed et al. [33], using general population participant data (88% with BMI<30 and 53% with BMI<25), measured kynurenine metabolites along with CRP, neopterin and the PAr index and the findings were similar to ours. Taken together, the existing data suggest that kynurenine metabolites may be better indicators of obesity-related changes in metabolism than most established inflammation-related markers.

To our knowledge, hardly any studies have assessed longitudinal associations. The study by Christensen et al. [13] followed 37 patients with severe obesity who underwent bariatric surgery and assessed kynurenine metabolites, CRP, neopterin and KTR at baseline, month 3 and month 12. Although somewhat difficult to compare because of its small sample size and different participants' health profile, the findings were overall consistent with ours in showing: i) a decrease in KYN,

KA, HK, XA, HAA, KA, CRP and KTR after surgery-related weight loss and ii) a small decrease or no change for AA and neopterin.

The fact that the associations between kynurenines and adiposity measures were still strong after adjusting for CRP and cystatin C suggests the existence of alternative mechanisms in addition to obesity causing inflammation and IDO influencing TRP metabolism. Cussotto et al. [15] suggested parallel pathways from obesity to TRP metabolism via inflammation and the gut microbiota, where change in the gut microbiota composition in obesity could also alter TRP metabolism and thus induce inflammation, independently of other routes involving, e.g. CRP and cystatin C. The attenuation of coefficients from Model 1 to Model 2, 3 or 4 was no greater than 20%, suggesting that the link between adiposity measures and kynurenine metabolites is essentially independent of unhealthy lifestyle factors, poor renal function or systemic chronic inflammation (as assessed by CRP only). While all adiposity measures showed a similar pattern of association, further investigation is required to understand how different aspects of body size and composition are physiologically associated with tryptophan metabolism, including their combined effect or using optimised tools to assess obesity [34].

The strengths of associations observed in our study, in particular for QA, KYN, KA, HK, HAA, XA, and KTR, suggest these markers should be further explored as clinical tools for monitoring metabolic conditions as they are likely indicators of an inflammatory state and other altered pathways mediating risk of obesity-related conditions. Previous research using the same data showed associations of kynurenine metabolites with overall, cancer and cardiovascular mortality [18], in line with other studies showing increased risks of cardiovascular disease, type II diabetes, and cancer [5, 10, 35]

Our findings demonstrate that tryptophan catabolism is an important pathway in obesity, which may reflect obesity-induced inflammation and possibly other mechanisms by which obesity causes adverse health outcomes. These findings contribute to establishing kynurenine metabolites as valuable markers to monitor the adverse consequences of obesity.

REFERENCES

[1] Gonzalez-Muniesa P, Martinez-Gonzalez MA, Hu FB, Despres JP, Matsuzawa Y, Loos RJF, et al. Obesity. Nat Rev Dis Primers. 2017;3:17034.

[2] Hruby A, Hu FB. The Epidemiology of Obesity: A Big Picture. Pharmacoeconomics. 2015;33:673-89.

[3] Rodriguez-Hernandez H, Simental-Mendia LE, Rodriguez-Ramirez G, Reyes-Romero MA. Obesity and inflammation: epidemiology, risk factors, and markers of inflammation. Int J Endocrinol. 2013;2013:678159.

[4] Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity and inflammation: the linking mechanism and the complications. Arch Med Sci. 2017;13:851-63.

[5] Zuo H, Ueland PM, Ulvik A, Eussen SJ, Vollset SE, Nygard O, et al. Plasma Biomarkers of Inflammation, the Kynurenine Pathway, and Risks of All-Cause, Cancer, and Cardiovascular Disease Mortality: The Hordaland Health Study. American journal of epidemiology. 2016;183:249-58.

[6] Hall JE, do Carmo JM, da Silva AA, Wang Z, Hall ME. Obesity, kidney dysfunction and hypertension: mechanistic links. Nature reviews nephrology. 2019;15:367-85.

[7] Esposito K, Pontillo A, Di Palo C, Giugliano G, Masella M, Marfella R, et al. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. Jama. 2003;289:1799-804.

[8] Cervenka I, Agudelo LZ, Ruas JL. Kynurenines: Tryptophan's metabolites in exercise, inflammation, and mental health. Science. 2017;357.

[9] Taylor MW, Feng G. Relationship between interferon-γ, indoleamine 2, 3-dioxygenase, and tryptophan catabolism. The FASEB Journal. 1991;5:2516-22.

[10] Rebnord EW, Strand E, Midttun O, Svingen GFT, Christensen MHE, Ueland PM, et al. The kynurenine:tryptophan ratio as a predictor of incident type 2 diabetes mellitus in individuals with coronary artery disease. Diabetologia. 2017;60:1712-21.

[11] Ulvik A, Midttun Ø, McCann A, Meyer K, Tell G, Nygård O, et al. Tryptophan catabolites as metabolic markers of vitamin B-6 status evaluated in cohorts of healthy adults and cardiovascular patients. The American journal of clinical nutrition. 2020;111:178-86.

[12] Zuo H, Tell GS, Ueland PM, Nygard O, Vollset SE, Midttun O, et al. The PAr index, an indicator reflecting altered vitamin B-6 homeostasis, is associated with long-term risk of stroke in the general population: the Hordaland Health Study (HUSK). The American journal of clinical nutrition. 2018;107:105-12.

[13] Christensen MHE, Fadnes DJ, Rost TH, Pedersen ER, Andersen JR, Vage V, et al. Inflammatory markers, the tryptophan-kynurenine pathway, and vitamin B status after bariatric surgery. PloS one. 2018;13:e0192169.

[14] Mangge H, Summers KL, Meinitzer A, Zelzer S, Almer G, Prassl R, et al. Obesity-related dysregulation of the tryptophan-kynurenine metabolism: role of age and parameters of the metabolic syndrome. Obesity (Silver Spring). 2014;22:195-201.

[15] Cussotto S, Delgado I, Anesi A, Dexpert S, Aubert A, Beau C, et al. Tryptophan Metabolic Pathways Are Altered in Obesity and Are Associated With Systemic Inflammation. Frontiers in immunology. 2020;11:557.

[16] Favennec M, Hennart B, Caiazzo R, Leloire A, Yengo L, Verbanck M, et al. The kynurenine pathway is activated in human obesity and shifted toward kynurenine monooxygenase activation. Obesity (Silver Spring). 2015;23:2066-74.
[17] Milne RL, Fletcher AS, MacInnis RJ, Hodge AM, Hopkins AH, Bassett JK, et al. Cohort Profile: The Melbourne Collaborative Cohort Study (Health 2020). International journal of epidemiology. 2017;46:1757-i.
[18] Dugué PA, Hodge AM, Ulvik A, Ueland PM, Midttun O, Rinaldi S, et al. Association of markers of inflammation, the kynurenine pathway and B vitamins with age and mortality, and a signature of inflammaging. The journals of gerontology Series A, Biological sciences and medical sciences. 2021.
[19] Li SX, Hodge AM, MacInnis RJ, Bassett JK, Ueland PM, Midttun O, et al. Inflammation-Related Marker Profiling of Dietary Patterns and All-cause Mortality in the Melbourne Collaborative Cohort Study. The Journal of nutrition. 2021;151:2908-16.
[20] Dugué PA, Jung CH, Joo JE, Wang X, Wong EM, Makalic E, et al. Smoking and blood DNA methylation: an epigenome-wide association study and assessment of reversibility. Epigenetics : official journal of the DNA Methylation Society. 2020;15:358-68.
[21] Dugué PA, Wilson R, Lehne B, Jayasekara H, Wang X, Jung CH, et al. Alcohol consumption is associated with widespread changes in blood DNA methylation: Analysis of cross-sectional and longitudinal data. Addiction biology. 2021;26:e12855.
[22] Roubenoff R. Applications of bioelectrical impedance analysis for body composition to epidemiologic studies. The American journal of clinical nutrition. 1996;64:459S-62S.
[23] Midttun Ø, Hustad S, Ueland PM. Quantitative profiling of biomarkers related to B-vitamin status, tryptophan metabolism and inflammation in human plasma by liquid chromatography/tandem mass spectrometry. Rapid Communications in Mass Spectrometry. 2009;23:1371-9.
[24] Gao J, Meyer K, Borucki K, Ueland PM. Multiplex immuno-MALDI-TOF MS for targeted quantification of protein biomarkers and their proteoforms related to inflammation and renal dysfunction. Analytical chemistry. 2018;90:3366-73.
[25] Midttun O, Townsend MK, Nygard O, Tworoger SS, Brennan P, Johansson M, et al. Most blood biomarkers related to vitamin status, one-carbon metabolism, and the kynurenine pathway show adequate preanalytical stability and within-person reproducibility to allow assessment of exposure or nutritional status in healthy women and cardiovascular patients. The Journal of nutrition. 2014;144:784-90.
[26] van Roekel EH, Dugué PA, Jung CH, Joo JE, Makalic E, Wong EEM, et al. Physical Activity, Television Viewing Time, and DNA Methylation in Peripheral Blood. Med Sci Sports Exerc. 2019;51:490-8.
[27] Hodge A, English D, Itsiopoulos C, O'dea K, Giles G. Does a Mediterranean diet reduce the mortality risk associated with diabetes: evidence from the Melbourne Collaborative Cohort Study. Nutrition, Metabolism and Cardiovascular Diseases. 2011;21:733-9.
[28] Stekhoven DJ, Bühlmann P. MissForest—non-parametric missing value imputation for mixed-type data. Bioinformatics. 2012;28:112-8.
[29] Carayol M, Leitzmann MF, Ferrari P, Zamora-Ros R, Achaintre D, Stepien M, et al. Blood Metabolic Signatures of Body Mass Index: A Targeted Metabolomics Study in the EPIC Cohort. J Proteome Res. 2017;16:3137-46.

[30] Bevital. https://www.bevital.no/summary.html. 2021.
[31] Armstrong BKW, E.; Saracci, R. Principles of Exposure Measurement in Epidemiology. Oxford: Oxford University Press; 1992.
[32] Theofylaktopoulou D, Midttun O, Ulvik A, Ueland PM, Tell GS, Vollset SE, et al. A community-based study on determinants of circulating markers of cellular immune activation and kynurenines: the Hordaland Health Study. Clinical and experimental immunology. 2013;173:121-30.
[33] Zahed H, Johansson M, Ueland PM, Midttun O, Milne RL, Giles GG, et al. Epidemiology of 40 blood biomarkers of one-carbon metabolism, vitamin status, inflammation, and renal and endothelial function among cancer-free older adults. Scientific reports. 2021;11:13805.
[34] James F, Wootton S, Jackson A, Wiseman M, Copson E, Cutress R. Obesity in breast cancer–what is the risk factor? European journal of cancer. 2015;51:705-20.
[35] Huang JY, Larose TL, Luu HN, Wang R, Fanidi A, Alcala K, et al. Circulating markers of cellular immune activation in prediagnostic blood sample and lung cancer risk in the Lung Cancer Cohort Consortium (LC3). International journal of cancer Journal international du cancer. 2020;146:2394-405.

Table 1. Characteristics of the participants in the Melbourne Collaborative Cohort Study (MCCS) at baseline and follow-up visits included in this sub-study.

	Baseline (1990-1994)	**Follow-up (2003-2007)**
Sex, N (%)		
Female	306 (32%)	
Male	664 (68%)	
Median age, years (IQR)[1]	59 (51-64)	70 (62-76)
Country of birth, N (%)		
Aust/N.Z./other	749 (77%)	
Greece	37 (4%)	
Italy	79 (8%)	
U.K./Malta	105 (11%)	
Smoking status, N (%)		
Never smoker	493 (51%)	492 (51%)
Current smoker	104 (11%)	55 (6%)
Former smoker	373 (39%)	423 (44%)
Median physical activity score (IQR)[2]	4 (1.5-6)	19 (7.4-35)
Median alcohol consumption (g/d) (IQR)[3]	7 (0-19)	10 (0-22)
Median MDS score (IQR)[4]	4 (3-6)	5 (4-6)
Median socioeconomic status (SEIFA) decile (IQR)[5]	7 (3-9)	7 (4-9)
Body size and composition variables, median (IQR)		
Height (cm)	169.5 [162.5-176.0]	169.5 [162.5-176.0]
Weight (kg)	76.3 [67.7-83.8]	76.9 [68.4-85.9]
Body mass index (kg/m^2)	26.4 [24.2-28.9]	26.8 [24.3-29.4]
Waist circumference (cm)	89.1 [80.0-96.0]	95.0 [86.5-102.2]
Waist-hip ratio	0.89 [0.81-0.94]	0.92 [0.86-0.96]
Fat mass ratio	0.32 [0.27-0.37]	-

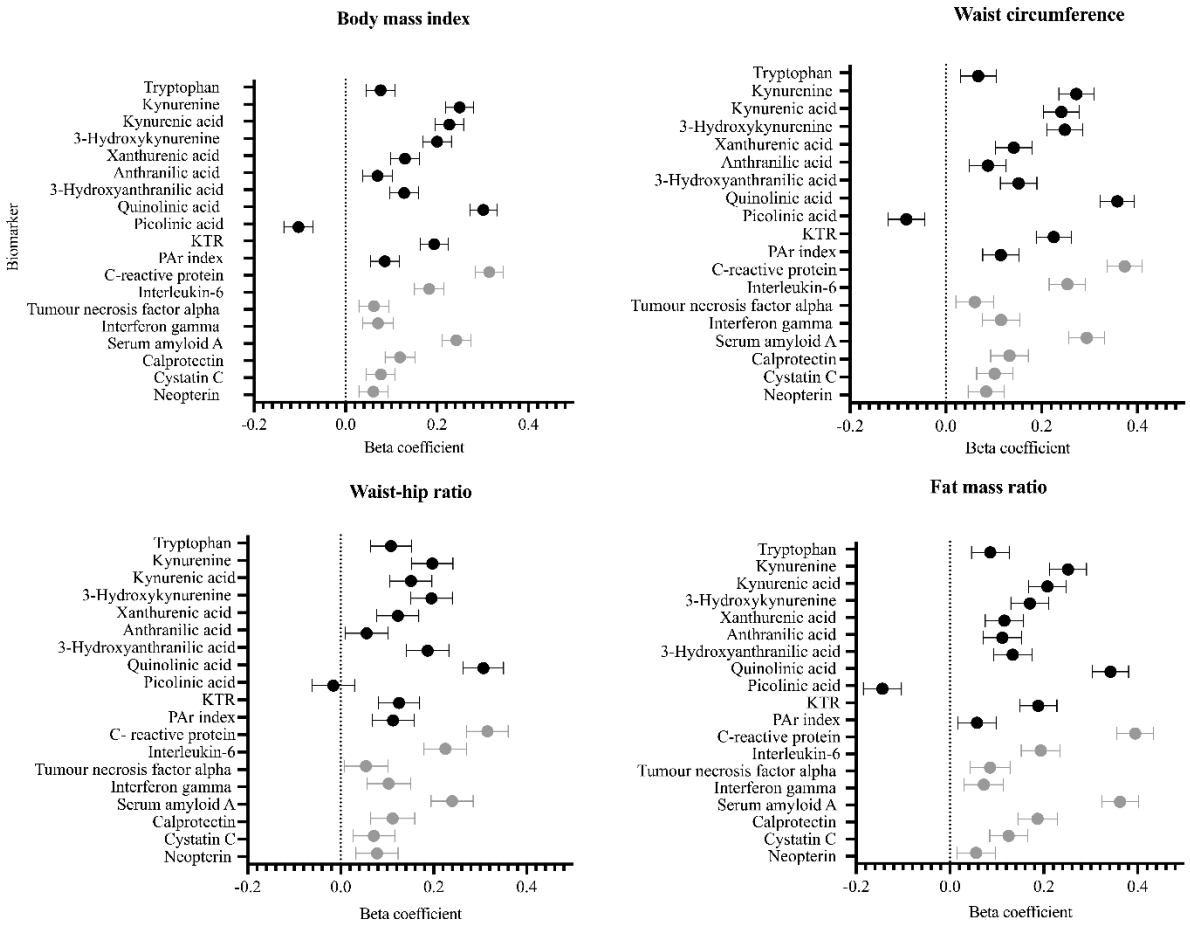
[1] IQR: interquartile range; [2] Physical activity scores reflecting the total equivalent of tasks, using different questionnaires at baseline and follow-up; [3] At baseline, total alcohol consumption per day in the last week; at follow up, total alcohol consumption per day in the last 12 months; [4] MDS: Mediterranean diet score ranged 0-9. [5] SEIFA: Socio-Economic Indexes for Areas.

Table 2. Pearson correlations between height-adjusted adiposity measures.

	Height-adjusted measures[1]	Weight	Waist circumference	Waist-hip ratio	Fat mass ratio
Baseline	**BMI**	0.99	0.83	0.49	0.50
	Weight		0.82	0.48	0.49
	Waist circumference			0.81	0.26
	Waist-hip ratio				-0.11
Follow-up	**BMI**	0.99	0.86	0.48	
	Weight	1	0.86	0.48	
	Waist circumference		1	0.78	

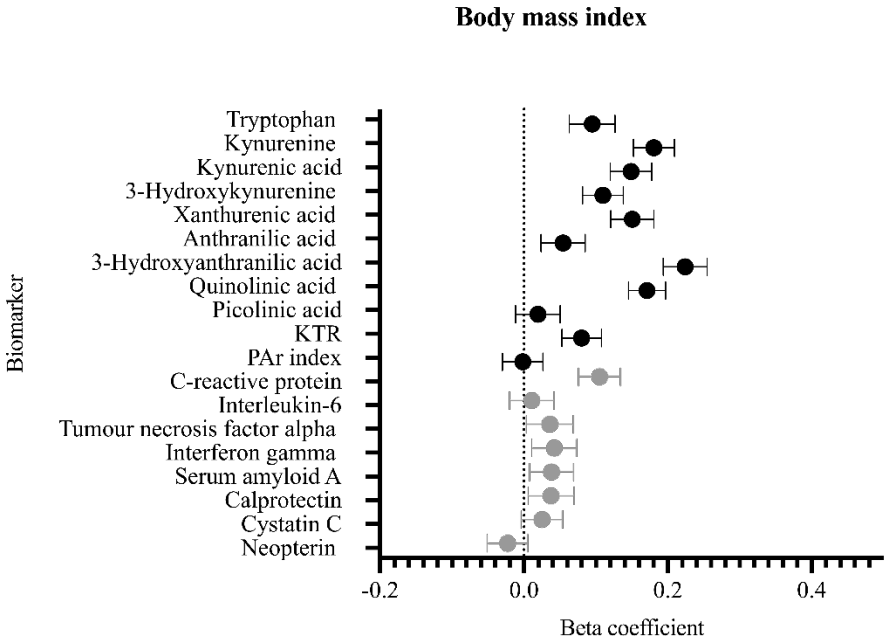
[1] Correlations with height were at baseline / follow-up: BMI:-0.07/-0.07; weight: 0.58/0.54; waist circumference: 0.38/0.33; waist-hip ratio: 0.40/0.39; fat mass ratio: -0.34/NA; Each measure was regressed on height to obtain anthropometric measures that were independent of height.

Figure 1. Associations between adiposity measures and concentrations of circulating concentrations of markers of the kynurenine pathway and inflammation at baseline (1990-1994, 970 participants, median age 59 years). †



† Model adjusted for age, sex, height, and country of birth, socioeconomic status (seifa-10), physical activity, smoking status, Mediterranean diet score and alcohol consumption

Figure 2. Associations between change in body mass index (between baseline and follow-up) and concentrations of circulating markers of the kynurenine pathway and inflammation at follow-up [†]



[†] Model adjusted for age, sex, height, and country of birth, socioeconomic status (seifa-10), physical activity, smoking status, Mediterranean diet score, and alcohol consumption