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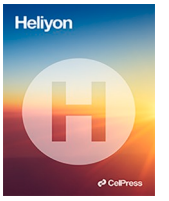

Research article

Middle-aged dogs with low and high Aβ CSF concentrations show differences in energy and stress related metabolic profiles in CSF

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ABSTRACT

Background: Amyloid beta (Aβ) accumulation in the brain is one of the earliest findings in Alzheimer's disease (AD). The dog is a natural animal model for amyloid processing and early brain amyloid pathology. The goal of this study is to examine which differences in metabolomic profiles in cerebrospinal fluid (CSF) could be detected in dogs with a difference in CSF Aβ concentrations before amyloid accumulation occurs.
Method: Metabolic profiling was performed on CSF from 4 to 8 year old dogs with different CSF Aβ concentrations.
Results: Metabolomic profiling of CSF showed differences in brain energy metabolism. More specifically, increases in N-acetylation of amino acids and amino sugars, creatine and pentose metabolism, and a decrease in tricarboxylic acid (TCA) cycle were seen in dogs with a high CSF Aβ concentration. In addition, signs of elevated oxidative stress, higher methionine, lipid and nucleotide metabolism and increased levels of cysteine, myo-inositol and trimethylamine N-oxide were noted in these animals.
Conclusions: Differences in energy metabolism and stress mediated metabolic changes are seen in the brain of dogs with different CSF Aβ concentrations, before any amyloid deposition occurs. Similar metabolic changes, as in the high Aβ dogs, have been described in AD in humans and/or transgenic AD mice, some of them in very early phases.
General significance: The differences observed in metabolomic profiles could help in identifying potential biomarkers for an increased risk of developing amyloid pathology in the brain and open the door to the evaluation of preventive treatments for amyloid pathology in humans.

1. Introduction

One of the earliest findings in Alzheimer's disease (AD) pathology is the accumulation of Aβ peptides in the brain. More than 40 AD associated genetic risk loci are already identified [1]. The diagnosis of prodromal AD is based on changes in CSF $A\beta_{42}$ and phosphorylated tau and/or imaging of plaques when pathology is already present. A healthier lifestyle in the preclinical phase is considered to decrease the risk for developing clinical AD [2], but the identification of early risk factors for AD, preclinical or even before pathology, would increase the chance of a successful outcome of potential treatment strategies.

The Aβ isoform pattern in CSF in humans and dogs is similar [3]. Dogs show amyloid depositions in the brain from approximately 9

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years of age onwards [4]. The plaques in dogs are diffuse and mimic an earlier phase of Aβ deposition [5]. Hence, dogs are considered a good animal model for amyloid pathology in AD [5,6]. Aβ concentration in CSF is a translatable biomarker to evaluate amyloid precursor protein (APP) processing in the brain [3,7,8]. CSF can be sampled at different sites in dogs: lateral ventricle (LV) [9], cisterna magna (CM) and lumbar. The Aβ concentrations in CSF are different between the sample sites, being higher in the CM than in LV [7]. For the current study, dogs were selected based on $A\beta_{42}$ concentrations in CSF from the LV. The age of the animals was between 4 and 8 years, before any amyloid plaques in the brain are expected. The differences in Aβ concentrations in CSF are therefore not a consequence of amyloid deposition in the brain.

Metabolomics of CSF has shown to be valuable in the identification of changes in metabolic processes associated with AD and ageing in humans [10–12]. Metabolic profiling of CSF has also been described in dogs with epilepsy [13]. The aim of this study was to compare metabolic profiles in CSF of dogs with low and high Aβ concentration in CSF. Differences in the performance of dogs with low and high Aβ concentrations in standard cognition tests have previously been described [14].

2. Material and methods

2.1. Selection of animals

The beagle dogs were selected based on their baseline $A\beta_{42}$ levels in CSF, sampled from the LV, over a period of approximately 3 years. Over that period more than 100 animals were sampled repeatedly. A multiplex immunoassay method was used for quantification of $A\beta_{37}$, $A\beta_{38}$, $A\beta_{40}$ and $A\beta_{42}$. The animals selected for this study showed low (<600 pg/ml) or high (>600 pg/ml) $A\beta_{42}$ concentrations in >60 % of the samples, with 600 pg/ml being approximately the median value of the whole population. The $A\beta_{42}$ concentrations were consistent with the other Aβ peptides [14] (Fig. 1a–c, supplementary data). The $A\beta_{42}$ concentrations measured in

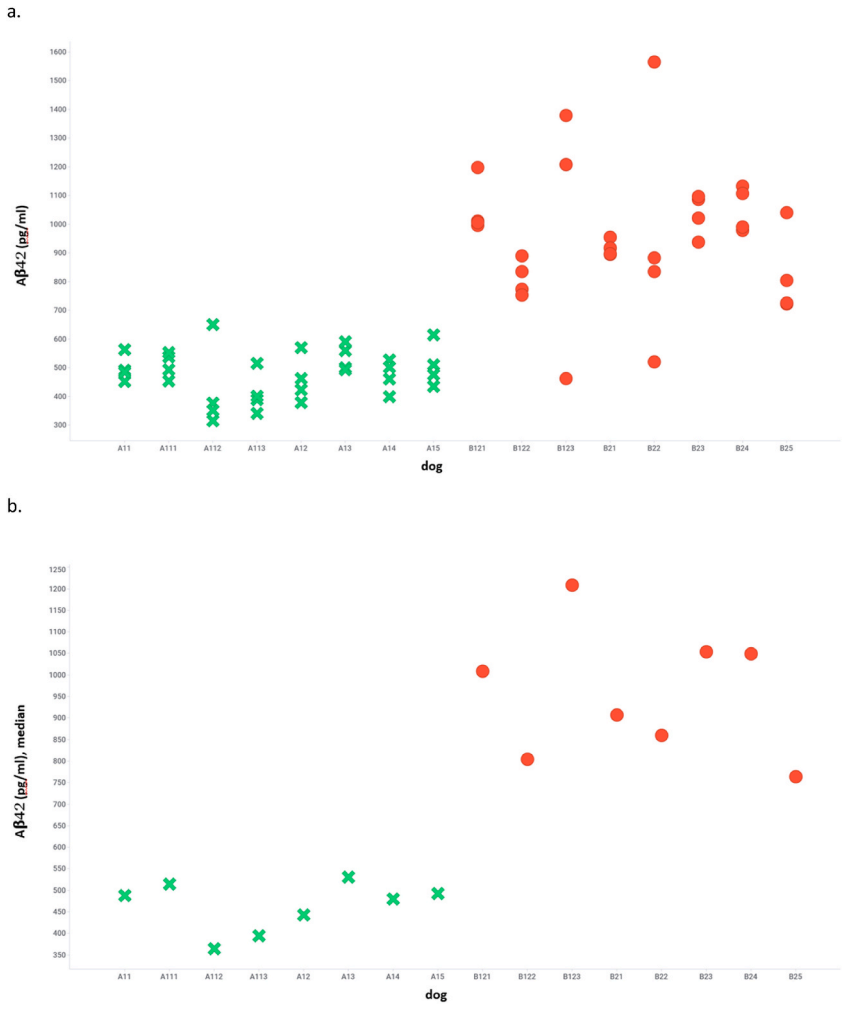

Fig. 1. Individual (a) and median (b) Aβ42 concentrations in CSF of the dogs. Group A (low Aβ animals, green crosses) and B (high Aβ animals, red dots) are the dogs with the low and high Aβ42 respectively. The mean CSF Aβ42 concentration of the colony (n > 70) is 600 pg/ml.

the year before this study are shown in Fig. 1. The mean age was 84 ± 19 months (min 54 – max 102) and 72 ± 8 (min 65 – max 91) and the mean body weight was 11 ± 1 kg (min 9.6 – max 13.3) and 10 ± 1 kg (min 8.4 – max 12.1) for the low and high $A\beta_{42}$ group respectively. Sixteen animals were selected in total, 5 males and 3 females in each group.

The study is approved by the Ethical Committee on Laboratory Animal Testing (ECD, Janssen Beerse) and is performed in consideration of the directive 2010/63/EU of the European parliament and of the council of September 22, 2010 on the protection of animals used for scientific purposes.

2.2. CSF sampling

Samples for metabolomic analysis were taken from the LV and CM once a week for a period of three weeks. At least two days were in between the sample collection from the LV (awake animals) and CM (anesthetized animals with 0.2 ml medetomidine followed by 2 ml propofol intravenously). Each time, approximately 400 μl CSF was sampled. The samples were stored at −80 °C until analysis.

2.3. Data acquisition

Untargeted metabolomic profiling was performed at Metabolon, Inc (Morrisville, NC, USA) as previously described [15]. Briefly, samples were spiked with internal standards, extracted, dried, and reconstituted in solvents compatible with each of the four UPLC-MS methods of the platform. Metabolite detection was accomplished by comparison to a reference library of chemical standards and peak quantification was calculated by area-under-the-curve analysis. Multiple quality control and curation processes were employed to remove background noise/artefacts and ensure accurate biochemical assignment. Statistical analysis was performed on run-day median-scaled and log-transformed data.

2.4. Statistical analysis

Principal component analysis (PCA) and random forest were used to analyse the data. Missing values were imputed with the observed minimum for that particular analyte. Missing values in an untargeted metabolomic dataset are attributed to the signal falling below the limit of detection, which is influenced by several factors including, but not limited to the sample amount available for extraction, analyte ionization efficiency, analyte abundance and analyte reactivity. The statistical analyses were performed on natural log-transformed data using ArrayStudio or programs in R (http://cran.r-project.org/).

Table 1
N-acetylated amino acids and aminosugars.

Biochemical name	High LV/Low LV		High CM/Low CM	
	Fold change	Signif	Fold change	Signif
Amino acids				
N-acetylglycine	1.34	↑	1.08	(↑)
N-acetylserine	1.12	(↑)	1.23	↑
N-acetylthreonine	1.17	↑	1.27	↑
N-acetylasparagine	1.07	[a]NS	1.15	↑
N-acetylglutamate	1.14	NS	1.12	NS
N-acetylglutamine	1.07	NS	1.09	(↑)
N-acetyl-aspartyl-glutamate	1.18	↑	1.28	NS
N-acetylphenylalanine	1.20	↑	1.24	↑
N-acetyltyrosine	1.24	↑	1.31	↑
N-acetyltryptophane	1.07	NS	1.02	NS
N-acetylleucine	0.99	NS	0.97	NS
N-acetylisoleucine	1.07	NS	1.14	(↑)
N-acetylvaline	1.06	NS	1.17	(↑)
N-acetyltaurine	1.46	↑	1.37	↑
N-acetylarginine	1.10	NS	1.27	↑
N-acetylputrescine	1.63	↑	1.08	NS
N-acetyl-beta-alanine	1.2	↑	1.05	NS
N-acetyl-S-ethyl-L-cysteine	1.19	↑	1	NS
N6-acetyllysine	1.43	↑	1.23	(↑)
Aminosugars				
N-acetyl-neuraminate	1.15	NS	1.32	(↑)
N-acetylglucosaminylasparagine	1.17	↑	1.22	↑
N-acetylglucosamine/N-acetyl-galactosamine	1.02	NS	1.32	↑
N-acetamidobutanoate (N4-acetylaminobutanoate)	1.30	↑	1.38	↑
Pyruvate	0.85	NS	0.91	NS

[a] NS = Not Significant.

3. Results

PCA of all samples resulted in a large degree of separation for CM and LV groups, suggesting that the metabolomic profile of CSF is distinct between these two locations. The absence or presence of anesthesia during the sampling is also a difference when comparing both locations, however, the anesthesia was fast and very short and was not expected to have a relevant effect on the metabolic profiles. The same analysis revealed that time (i.e. sampling over a three week period) did not lead to any obvious shifts in the metabolomic profile. PCA was also performed at each location independently to assess the effect of time and Aβ levels. PCA revealed that both locations resulted in notable differences in their metabolomic profiles when comparing low to high Aβ levels. The time of collection did not appear to be driving overt metabolomic shifts within each low to high Aβ cluster.

Next, a machine learning method was used to determine if certain metabolites can distinguish the treatment groups. Random forest is an unbiased and supervised classification technique based on an ensemble of a high number of decision trees. Lists of the top 30 biochemicals, which contributed to the separation of metabolomic profiles was generated and assigned to super-pathway classification for all samples together, CM samples only and LV samples only (Fig. 2a–c, supplementary data). Comparing all low and high Aβ samples together resulted in an overall predictive accuracy of 89 % as compared to 50 % by random chance alone. Comparing low and high Aβ samples from the CM and LV independently resulted in predictive accuracies of 85 % and 92 %, respectively. The details and implications of the metabolites identified with random forest are discussed. In the tables fold change and significance are listed per metabolite. The "↑" represent significantly increased and the "↓" significantly decreased ($p < 0.05$) biochemicals. "(↑)" and "(↓)" represent biochemicals which are trending ($0.05 < p < 0.1$) up or down, respectively.

3.1. N-Acetylation

Most of the metabolites identified with random forest were amino acids, and of the amino acids, there was an enrichment of N-acetyl amino acids. Several other acetyl derivatives were also higher in the high Aβ group. Pyruvate, the precursor of acetyl CoA (acetyl group donor), was not significantly lower in the high Aβ group. The differences were seen in the LV as well as in the CM (Table 1).

3.2. Energy metabolism and carbohydrates

3.2.1. Creatine metabolism

Creatine and creatinine were significantly higher or trending higher under high Aβ conditions in the LV and CM. No changes were seen in creatine phosphate (Table 2).

3.2.2. TCA cycle

Although statistically not significant, most metabolites in the TCA cycle were lower in the high Aβ dogs especially for the metabolites further down the cycle (succinate, fumarate and malate) (Table 2).

3.2.3. Pentose metabolism

Metabolites in pentose metabolism as ribitol, arabinose, arabitol/xylitol, arabonate/xylonate and lyxonate were higher in the high Aβ dogs (Table 2).

Table 2
Metabolites in energy and carbohydrate metabolism.

Biochemical name	High LV/Low LV		High CM/Low CM	
	Fold change	Signif	Fold change	Signif
Creatine metabolism				
creatine	1.17	(↑)	1.31	↑
creatinine	1.15	↑	1.14	↑
creatine phosphate	0.87	NS	1.12	NS
TCA cycle				
citrate	1.01	NS	1.14	NS
aconitate	0.95	NS	1.18	(↑)
α-ketoglutarate	0.87	NS	0.97	NS
succinate	0.87	NS	0.93	NS
fumarate	0.83	NS	0.88	NS
malate	0.77	NS	0.80	NS
Pentose metabolism				
ribitol	1.25	↑	1.12	NS
ribonate	1.17	(↑)	1.24	↑
arabinose	1.16	↑	1.23	↑
arabitol/xylitol	1.18	(↑)	1.30	↑
arabonate/xylonate	1.20	NS	1.26	NS
lyxonate	1.38	(↑)	1.32	(↑)

3.3. Neurotransmitters

3.3.1. Glutamate metabolism

Glutamate, N-acetyl-aspartyl-glutamate and carboxyethyl-GABA were higher in the high Aβ group (Table 3).

3.4. Oxidative stress

Oxidized glutathione (GSSG), cysteine-glutathione disulfide, methionine sulfoxide, and kynurenine, which are all markers of oxidative stress, were higher in the high Aβ group, especially in the CM (Table 4).

3.5. Cysteine

Cysteine levels were higher in dogs with the high Aβ levels, especially in the CM (Table 5).

3.6. Polyamines

5-methylthioadenosine (MTA) level in CSF was higher in the high Aβ dogs (Table 6).

3.7. Methionine metabolism

Methionine metabolites were higher in the high Aβ dogs (Table 7).

3.8. Inositol metabolism

Myo-inositol was higher in the CM of the high Aβ group (Table 8).

3.9. Lipidomics

Differences in lipidomic metabolites were observed across several nodes of lipid metabolism. Numerous species were higher in the high Aβ group, primarily in the CM. Higher values of multiple sphingomyelins and plasmalogens were also seen in the LV of the high Aβ dogs (Table 9).

3.10. Nucleotide metabolism

Higher levels of multiple metabolites in nucleotide metabolism (purines and pyrimidines) were observed in the high Aβ group (Table 10).

4. Discussion

The difference is Aβ concentrations in the animals selected for this study was seen in CSF sampled from the lateral ventricle. However, this is a site, which can only be sampled after placement of a canula in the skull. It was decided to collect CSF from the lateral ventricle and cisterna magna to capture metabolomic profiles in multiple parts of the brain. Metabolic waste products from the brain parenchyma are drained via the glymphatic systems. The lateral ventricle collects solutes drained from the surrounding brain structures whereas the cisterna magna which is more distal from the brain collects solutes from all parts of the brain, including the cortex [16,17]. It is known that different metabolites can have different concentration gradients between LV and CM. A sampling site caudally from the brain is also more comparable with humans, where CSF is sampled from the lumbar area.

Concentrations of N-6 acetyllysine, multiple N-acetyl amino acids, and several amino sugars were higher in the CSF of high Aβ animals, both in LV as well as in CM. Acetylation is one of the major post-translational protein modifications in the cell. The acetyl group can be attached to either the α-amino group of the N-terminus of proteins or to the ε-amino group of lysine residues. In case of lysine acetylation, the reaction is enzymatically reversible [18]. Conversely, acetylation of an N-terminal (Nt) amino group is an irreversible modification. Nt-acetylation has many functions in the cell. It can target proteins for polyubiquitination and proteasomal degradation and protect proteins against degradation. Nt-acetylation can also be required for a correct folding of proteins,

Table 3
Metabolites in glutamate metabolism.

Biochemical name	High LV/Low LV		High CM/Low CM	
	Fold change	Signif	Fold change	Signif
glutamate	1.33	↑	1.21	NS
N-acetyl-aspartyl-glutamate (NAAG)	1.18	↑	1.28	NS
carboxyethyl-GABA	1.29	↑	1.52	↑

Table 4
Metabolites linked to oxidative stress.

Biochemical name	High LV/Low LV		High CM/Low CM	
	Fold change	Signif	Fold change	Signif
Glutathione, oxidized (GSSG)	0.89	NS	17.18	↑
Cysteine-glutathione disulfide	1.05	NS	1.40	NS
Methionine sulfoxide	1.16	↑	1.15	↑
kynurenine	1.14	NS	1.52	↑

Table 5
Cysteine.

Biochemical name	High LV/Low LV		High CM/Low CM	
	Fold change	Signif	Fold change	Signif
cysteine	1.11	NS	1.29	↑

Table 6
5-methylthioadenosine.

Biochemical name	High LV/Low LV		High CM/Low CM	
	Fold change	Signif	Fold change	Signif
5-methylthioadenosine	1.14	(↑)	1.30	↑

Table 7
Metabolites in methionine metabolism.

Biochemical name	High LV/Low LV		High CM/Low CM	
	Fold change	Signif	Fold change	Signif
N-formylmethionine	1.1	NS	1.16	(↑)
Methionine sulfoxide	1.16	↑	1.15	↑
5-methylthioribose	1.27	↑	1.13	NS
2.3-diOH-5-methylthio-4-pentenoate (DMTPA)	1.19	↑	1.23	↑
2-OH-4-(methylthio)butanoic acid	1.28	↑	1.18	↑

Table 8
Myo-inositol.

Biochemical name	High LV/Low LV		High CM/Low CM	
	Fold change	Signif	Fold change	Signif
myo-inositol	1.02	NS	1.47	↑

protein-protein interactions (PPis) in protein complex formation and targeting proteins for membranes [19]. Importantly, acetylation of proteins impedes oligomerization and could be a natural defence mechanism against Aβ-oligomerization [20] resulting from a higher Aβ concentration [21]. Elevated acetylation requires a higher supply of pyruvate, the source for the donor of the acetyl group, acetyl-CoA (Ac-CoA), which would explain the lower levels of pyruvate in the high Aβ group. Besides protein acetylation, Ac-CoA is mainly channelled into the TCA cycle for energy production [22]. The lower TCA metabolites in the high Ab group could be explained by increased use of Ac-CoA for protein acetylation. Aβ peptides are also known to accumulate in mitochondria and impair mitochondrial energy metabolism. The disturbance of the TCA cycle can result in an export of citrate from the mitochondria, which is converted to Ac-CoA in the cytosol resulting in increased acetylation [23]. Differences in the metabolism of the neurotransmitters glutamate and GABA could also result from an effect on energy metabolism, which has been observed in the APPswe/PSE1dE9 mouse model before amyloid plaque deposition [24]. Changes in excitatory signalling resulting from regional adaptations in astrocyte metabolism in the early phases of amyloid pathology have been shown in the 5xFAD mouse model [25,26]. A higher pentose and creatine metabolism in the high Aβ group reflects also differences in energy metabolism. The higher pentose metabolites could originate from intestinal microflora [27]. Creatine is an essential molecule that serves to maintain ATP levels in tissues with high metabolic demand by acting as a high energy buffer system with creatine phosphate. Creatine is synthesized mostly in the liver and kidney and is transported through the blood to target tissues. Most creatine in the brain is taken up continuously through the blood-brain barrier, although certain brain cells have the capacity for endogenous creatine biosynthesis. A perturbed energy state

Table 9
Metabolites in lipid metabolism.

Pathway Biochemical name	High LV/Low LV		High CM/Low CM	
	Fold change	Signif	Fold change	Signif
Phospholipid metabolism				
glycerophosphorylcholine	1.13	NS	3.89	↑
Phosphatidylcholine (PC)				
1-stearoyl-2-oleoyl-GPC	0.93	(↓)	1.63	(↑)
Phosphatidylethanolamine (PE)				
1-stearoyl-2-arachidonoyl-GPE	1.05	NS	6.7	(↑)
Phosphatidylserine				
1-stearoyl-2-arachidonoyl-GPE	1.07	NS	5.53	(↑)
Phosphatidylinositol				
1-stearoyl-2-arachidonoyl-GPI	0.96	NS	2.01	↑
Trimethylamine N-oxide	1.34	(↑)	1.41	(↑)
Lysophospholipids				
1-palmitoleoyl-GPC	0.70	NS	1.79	↑
1-stearoyl-GPE	0.77	NS	3.33	(↑)
1-oleoyl-GPE	0.64	NS	3.03	(↑)
1-stearoyl-GPI	0.92	NS	2.46	↑
Sphingolipids/Sphingomyelins				
palmitoyl sphingomyelin	1.21	NS	2.20	(↑)
stearoyl sphingomyelin	1.02	↑	1.46	(↑)
sphingomyelin (d18:1/14:0, d16:1/16:0)	1.04	NS	1.79	↑
sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0)	1.06	NS	2.26	↑
sphingomyelin (d18:1/18:1, d18:2/18:0)	1.14	(↑)	1.62	(↑)
sphingomyelin (d18:1/20:0, d16:1/22:0)	1.10	↑	1.46	(↑)
sphingomyelin (d18:1/20:1, d18:2/20:0)	1.01	NS	1.61	(↑)
sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)	1.31	↑	1.62	(↑)
sphingomyelin (d18:2/24, d18:1/24:2)	1.29	(↑)	2.07	NS
Plasmalogens				
1-(1-enyl-palmitoyl)-2-oleoyl-GPE	0.88	(↓)	1.61	NS
1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (P-16:0/20:4)	1.13	NS	3.39	↑
1-(1-enyl-palmitoyl)-2-oleoyl-GPC	1.00	↑	1.23	NS
1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (P-18:0/20:4)	1.02	↑	3.95	↑
1-(1-enyl-oleoyl)-2-oleoyl-GPE	0.90	↓	1.53	NS

Table 10
Metabolites in nucleotide metabolism.

PathwayBiochemical name	High LV/Low LV		High CM/Low CM	
	Fold change	Signif	Fold change	Signif
Purine metabolism (Hypo)xanthine/Inosine containing				
N1-methylinosine	1.18	(↑)	1.29	↑
allantion	1.41	↑	1.29	↑
1-methylhypoxanthine	1.39	↑	1.46	↑
Purine metabolism Adenosine containing				
Adenosine 5'monophosphate (AMP)	1.35	NS	6.36	NS
adenosine	1.06	NS	1.42	(↑)
adenine	1.07	↑	1.27	↑
N6-carbamoylthreonyl- adenosine	1.27	↑	1.15	NS
N6-succinyladenosine	1.22	(↑)	1.27	(↑)
Purine metabolismGuanine containing				
7-methylguanine	1.22	↑	1.10	NS
N2,N2-dimethylguanosine	1.15	↑	1.15	↑
Pyrimidine metabolismUracil containing				
pseudouridine	1.22	↑	1.25	↑
5.6-dihydrouridine	1.20	↑	1.23	↑
3-ureidopropionate	1.58	↑	1.30	(↑)
N-acetyl-beta-alanine	1.20	↑	1.05	NS
3-(3-amino-3-carboxypropyl) uridine	1.28	↑	1.38	↑
Pyrimidine metabolismCytidine containing				
5-methylcyclidine	1.24	↑	1.09	NS
5-hydroxymethylcyclidine	1.43	(↑)	1.15	NS

associated with changes in creatine metabolism have been implicated in AD [28]. Focal deposits of creatine have been detected in APP transgenic mice and in the brain of AD patients [29]. Markers of oxidative stress, oxidized glutathione (GSSG), cysteine-glutathione disulfide, methionine sulfoxide and kynurenine, were higher in dogs with high Aβ levels. Oxidative stress is considered to contribute to increased Aβ production [30]. The kynurenine pathway is activated by oxidative stress, which can also impair mitochondrial function and disrupt the cellular energy metabolism [31,32]. An increase in methionine sulfoxide is shown in CSF of AD patients [33]. Methionine sulfoxide is increased in asymptomatic persons carrying familial AD mutations, which are known to have an overproduction of Aβ [34]. Methionine sulfoxide is subject to reduction by the methionine sulfoxide reductase (Msr) system. Ms reductase A deficient APP (APP(+)/MsrAKO) mice exhibit higher levels of soluble Aβ in brain compared to APP(+) mice [35]. Active immunization with methionine sulfoxide-rich protein antigen showed protective effects against the development of AD in transgenic (APP/PS1) mice [36]. Dogs with high Aβ levels in CSF also showed high cysteine levels. An increase in cysteine levels could be a reaction to the high Aβ levels since cysteine has been shown to inhibit amyloid fibril formation and protect cells against amyloid induced cytotoxicity [37]. Elevated cysteine levels have been seen in the brains of AD transgenic mouse models [38] and intrahippocampal administration of N-acetyl cysteine alleviated Aβ induced deficits in an AD rat model [39]. Regarding the polyamine metabolism, a higher level of 5-methylthioadenosine was measured in the high Aβ dogs. Neuroprotective properties have been attributed to 5-methylthioadenosine [40], which has been shown to be involved in the methionine metabolism [41]. Multiple metabolites of methionine metabolism were higher in the high Aβ group, including 2,3-Dihydroxy-5-methylthio-4-pentenoic acid (DMTPA), a metabolite that can be synthesized from 5-methylthioadenosine [42]. Numerous species of lipid metabolism were higher in the high Aβ group. These lipidomic changes could be consistent with perturbations in membrane lipid composition in the brain. Amyloid β disturbs membranes properties in living cells [43], which in turn, could affect the amyloidogenic processing of amyloid precursor protein [44,45]. Lipid profiling of brain tissue from AD patients revealed perturbations of several lipid pathways [46]. Trimethylamine N-oxide, a metabolite originated from intestinal bacterial phospholipid metabolism [47], was higher in the high Aβ group. An elevation in trimethylamine N-oxide in CSF has been described in AD [48]. Myo-inositol is stored in kidney, brain and liver and is necessary for functions such as signal transduction, metabolic flux, insulin signalling, regulation of ion-channel permeability and stress response [49]. A significantly higher level of myo-inositol was measured in the CSF of high Aβ dogs. It has been shown that myo-inositol levels in brain are elevated already at asymptomatic stages of AD [50]. Similar to what was observed in the lipidomic profile, multiple significant differences across nodes of nucleotide metabolism (purines and pyrimidines) were observed. Increases in nucleotide metabolites in CSF are seen when the brain is stressed [51,52]. Alterations for numerous purine and pyrimidine metabolites were found in all brain regions of APP/PS1 mice [53].

5. Conclusion

Metabolomic analysis of CSF from middle-aged beagle dogs with low and high Aβ concentrations in CSF showed differences in multiple pathways. Increased N-acetylated amino acids and amino sugars, elevated creatine and pentose metabolites, and decreased TCA cycle metabolites suggest key differences in brain energy metabolism in high Aβ dogs. In addition, the CSF of high Aβ dogs showed signs of higher oxidative stress, increased lipid and nucleotide metabolism, and higher levels of methionine, cysteine, myo-inositol and trimethylamine N-oxide. A limitation to this study is that it has not been shown yet that the animals with a high Aβ concentration will develop more amyloid pathology in the brain with aging. Further follow up of the animals is ongoing until they are old enough to evaluate amyloid deposition in the brain. Another limitation is that this study is not able to show if differences precede or are secondary to the differences in CSF Aβ concentrations. The goal of this study was to examine which differences in metabolomic profiles could be detected in animals with a clear difference in Aβ CSF concentrations before amyloid pathology in the brain occurs. Although similar metabolic changes have been described in AD and/or AD transgenic mice, it is too early to conclude if and which differences are most relevant as predictors for an increased risk of developing amyloid pathology in the brain with aging. More research is needed. However, the current findings could hopefully contribute in the search for reliable predictive biomarkers or combinations of, which would open the door to the evaluation of preventive treatments of amyloid pathology, which is a main trigger for AD in humans.

CRediT authorship contribution statement

Herman Borghys: Writing – original draft. **Andrew Schwab:** Writing – review & editing. **Brian Keppler:** Writing – review & editing.

Data availability statement

The MS1 data supporting the findings of this study are available in Metabolights under study number MTBLS2420.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing

interests:Herman Borghys reports financial support was provided by Janssen Pharmaceutica NV. Herman Borghys reports a relationship with Janssen Pharmaceutica NV that includes: employment and equity or stocks.

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Appendix A. Supplementary data

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