



# Preliminary evidence for an association between intake of high-fat high-sugar diet, variations in peripheral dopamine precursor availability and dopamine-dependent cognition in humans

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## Abstract

Obesity is associated with alterations in dopaminergic transmission and cognitive function. Rodent studies suggest that diets rich in saturated fat and refined sugars (HFS), as opposed to diets low in saturated fat and refined sugars (LFS), change the dopamine system independent of excessive body weight. However, the impact of HFS on the human brain has not been investigated. Here, we compared the effect of dietary dopamine depletion on dopamine-dependent cognitive task performance between two groups differing in habitual intake of dietary fat and sugar. Specifically, we used a double-blind within-subject cross-over design to compare the effect of acute phenylalanine/tyrosine depletion on a reinforcement learning and a working memory task, in two groups that are on opposite ends of the spectrum of self-reported HFS intake (low vs high intake: LFS vs HFS group). We tested 31 healthy young women matched for body mass index (mostly normal weight to overweight) and IQ. Depletion of peripheral precursors of dopamine reduced the working memory specific performance on the operation span task in the LFS, but not in the HFS group ( $P = 0.016$ ). Learning from positive- and negative-reinforcement (probabilistic selection task) was increased in both diet groups after dopamine depletion ( $P = 0.049$ ). As a secondary exploratory research question, we measured peripheral dopamine precursor availability (pDAP) at baseline as an estimate for central dopamine levels. The HFS group had a significantly higher pDAP at baseline compared to the LFS group ( $P = 0.025$ ). Our data provide the first evidence indicating that the intake of HFS is associated with changes in dopamine precursor availability, which is suggestive of changes in central dopamine levels in humans. The observed associations are present in a sample of normal to overweight participants (ie, in the absence of obesity), suggesting

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that the consumption of a HFS might already be associated with altered behaviours. Alternatively, the effects of HFS diet and obesity might be independent.

#### KEYWORDS

acute phenylalanine/tyrosine depletion, dopamine, high fat and sugar diet, reinforcement learning, working memory

## 1 | INTRODUCTION

Over recent decades, obesity has become a global health burden, making research on the development and maintenance of obesity more relevant than ever. One of the main drivers of the rapid rise in obesity rates is the increased intake of food products containing high amounts of saturated fat and refined sugars.<sup>1</sup> The question is, what makes people eat beyond their caloric needs, despite negative consequences, such as getting uncomfortably full or the health risks associated with obesity?

Throughout their daily life, people are constantly exposed to food advertisements and easily available food products. Such external food cues have the potential to enhance the motivation to obtain and consume food, even in a satiated state.<sup>2</sup> Recently, it has been shown that people with obesity outperform people with normal weight when learning and tracking the reward predicting value of cues associated with a food reward.<sup>3</sup> In addition, individuals with a higher body mass index (BMI) compared to a lower BMI (normal weight to obese) continue to respond to such food reward cues with the same intensity, despite their decreased motivation to consume the food rewards after devaluation.<sup>4,5</sup> In a meta-analysis, García-García et al<sup>6</sup> showed that people with obesity exhibit hyperactivation in reward-related brain areas and proposed that this enhanced focus on rewards may lead to compulsive-like behaviours. In addition to motivational aspects and behavioural control, obesity is associated with altered decision making and executive functions.<sup>7,8</sup> Adverse decision making might be explained by the inability to integrate negative feedback as shown by impaired reinforcement learning associated with obesity.<sup>9</sup> In a probabilistic reinforcement learning paradigm with monetary rewards, people with obesity chose the correct option less frequently and gained lower overall payout compared to lean participants.<sup>10</sup> Coppin et al<sup>10-13</sup> report similar findings of not only impaired reinforcement learning in obesity, but also impairments of working memory, in line with previous findings.<sup>11,12</sup> The observed alterations of cognitive processes linked to motivation and behavioural control may contribute to the maintenance of obesity and are considered to be a result of alterations in central dopamine pathways.<sup>7</sup> Reinforcement learning and working memory both depend on action of dopamine in the striatum and prefrontal cortex (PFC) and optimal levels are crucial for proper functioning.<sup>14-16</sup>

Although alterations in the dopaminergic system have mainly been associated with body weight in humans,<sup>17-22</sup> studies in rodents suggest that diets high in saturated fat and refined sugar (HFS), as opposed to diets low in saturated fat and refined sugars (LFS), lead to the observed changes, independent of excessive weight: exposure

to high-fat diets reduced dopamine receptor D<sub>2</sub> protein expression levels,<sup>23</sup> affected dopamine synthesis<sup>24,25</sup> and uptake of striatal dopamine in rodents.<sup>26,27</sup> Furthermore, the overconsumption of specifically saturated dietary lipids, predominating in a typical western style diet, reduced dopamine receptor D<sub>1</sub> signalling in rats, as well as impaired dopamine clearance and phasic dopamine release in the nucleus accumbens in mice independent of weight gain.<sup>28,29</sup> Mimicking the effects of hidden sugars in commercial foods and beverages, low-concentration sucrose solutions changed dopamine receptor D<sub>1</sub> and D<sub>2</sub> mRNA and protein expression in the striatum.<sup>30</sup> Furthermore, a high-fat diet down-regulated the expression of striatal dopamine receptor D<sub>1</sub> and D<sub>2</sub> mRNA.<sup>31</sup> However, it is not clear whether the observed alterations of the dopaminergic system are directly caused by HFS or are compensatory adaptations in response to altered dopamine levels.

Taken together, HFS diets may thus be responsible for the observed differences in adaptive behaviour that crucially rely on the neurotransmitter dopamine and that promote the overconsumption of such food products and obesity. However, translating the findings obtained from animal studies to humans has to be carried out with great care because of the large knowledge gap between the fields.<sup>32</sup> To date, a possible relationship between HFS diets and the dopamine system has not been investigated in humans. Here, we aimed to obtain evidence indicating that a high (relative to low) dietary intake of saturated fat and free sugars is associated with alterations of central dopamine and dopamine-dependent cognition, particularly, reinforcement learning and working memory.

Because the synthesis of monoamine neurotransmitters in the brain depends on the availability of their amino acid precursors circulating in the blood [peripheral dopamine precursor availability (pDAP)], central dopamine levels can assumedly be decreased by depleting its precursors tyrosine and phenylalanine relative to the other large neutral amino acids, which competitively share a carrier at the blood-brain barrier.<sup>33</sup> To uncover potential diet-related differences in central dopamine levels and consequently dopamine-mediated cognition, we made use of an acute phenylalanine/tyrosine depletion (APTD) method, which attenuates dopamine synthesis and release in the striatum<sup>34-36</sup> and impairs frontostriatal functional connectivity.<sup>37</sup> The effect of APTD on central dopamine synthesis and release has been shown in human positron emission tomography (PET) studies and is further substantiated by evidence from animal research. APTD increases baseline neuronal firing and amphetamine-induced [<sup>11</sup>C]raclopride binding potential in the striatum, which has been interpreted as reduced dopamine release.<sup>35,36,38</sup> Applying APTD in rats revealed reduced tyrosine

levels in the striatum, frontal cortex and hippocampus, as well as reduced accumulation of the dopamine precursor dihydroxyphenylalanine (DOPA) (synthesised from tyrosine) in the same brain regions.<sup>33</sup> Taken together, the results from human and animal studies suggest that APTD decreases central dopamine synthesis and release. To what extent exactly central dopamine release is decreased by precursor depletion and whether this decrease is of similar magnitude between individuals is not known; higher concentrations of striatal dopamine or presynaptic dopamine synthesis capacity, as shown for women compared to men,<sup>39,40</sup> could serve as buffer for the effects of peripheral depletion. APTD has been shown to modulate reinforcement learning<sup>41,42</sup> and executive functions such as set-shifting and spatial working memory.<sup>37,38,43</sup> These cognitive processes require a certain level of dopamine for optimal performance. Either a decrease or increase in this level will lead to suboptimal performance (ie, dopamine levels relate to cognitive performance in an inverted-U-shaped manner).<sup>16</sup> As such, assessing reinforcement learning and working memory performance after APTD in two groups that differ markedly in their dietary intake of saturated fat and free sugars could reveal potential diet-related differences in the dopamine system.

Our main hypothesis for this study is that APTD differentially affects cognitive performance of two groups that differ in their self-reported intake of saturated fat and refined sugar, as a result of potential diet-associated alterations of the dopaminergic system. As secondary hypothesis, we expect markedly reduced pDAP levels after APTD in both diet groups, indicating effective intervention and causing the changes in cognitive performance analysed in our main hypothesis. Exploratory analyses aimed at characterising the two diet groups with respect to eating behaviour, personality traits, metabolic hormones, and parameters of fat and sugar metabolism.

## 2 | MATERIALS AND METHODS

### 2.1 | Participants

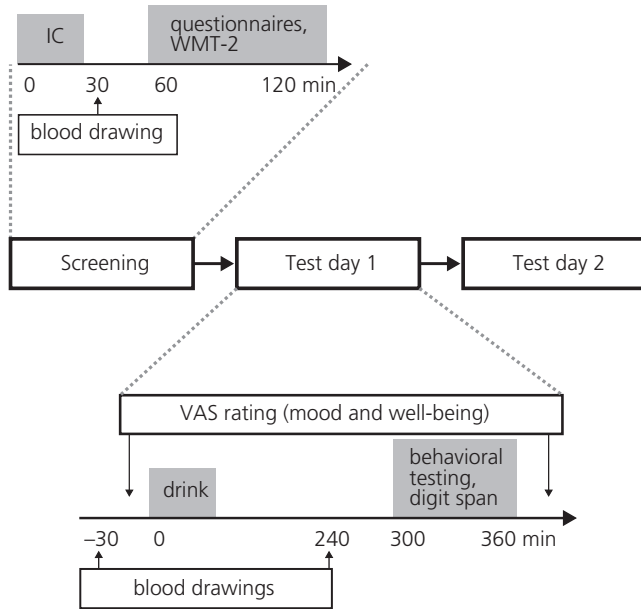
Ninety healthy female participants (mean  $\pm$  SD age, 25.03  $\pm$  3.61 years; BMI = 24.16  $\pm$  5.72 kg m<sup>-2</sup>) were recruited from the internal participant database of the Max Planck Institute for Human Cognitive and Brain Sciences (Leipzig, Germany) and via advertisements placed at university facilities or public spaces. All participants were non-smokers and reported no history of clinical drug or alcohol abuse or neurological disorder, and none had a first-degree relative history of psychiatric disorders. None showed moderate or severe depressive symptoms assessed with the Beck Depression Inventory (BDI), indicated by total scores < 19.<sup>44</sup> We decided to only include female participants because previous studies reported larger behavioural effects of APTD in women compared to men,<sup>42,45</sup> an effect potentially explained by higher striatal dopamine synthesis capacity in women.<sup>40</sup> The study was carried out in accordance with the Declaration of Helsinki and was approved by the Medical Faculty

Ethics Committee of the University of Leipzig (439/16-ek). All participants provided their written informed consent before taking part in the study.

### 2.2 | Study design

Participants were first invited to the institute for a screening to check for inclusion and exclusion criteria. We used the Dietary Fat and free Sugar Questionnaire (DFS)<sup>46,47</sup> to group our participants into two groups of high and low consumers of saturated fat and refined sugar (HFS vs LFS group). The DFS consists of 24 questions asking how often participants consumed a certain food item on average over the last 12 months (five answer options; from "one per month or less" to "five times or more per week"). Two additional questions ask for the frequency of food consumed outside the home averaged over the last 12 months and the number of spoons of sugar added to food and beverages in the last week. The minimum score possible is 26 (low intake of HFS) and the maximum score is 130 (high intake of HFS). DFS scores were shown to correlate with the percentage energy from saturated fat and free sugar and high intra-class correlations indicate good test-retest reliability.<sup>46,47</sup> Based on the total DFS score, participants were assigned to the LFS group (total score < 54) or the HFS group (total score > 61); participants with DFS scores ranging from 54 to 61 were excluded from the study. Additionally, baseline fasted blood measurements were taken, and participants completed the Viennese Matrices Test 2 (WMT-2) to assess intelligence.<sup>48</sup> They further answered self-reported questionnaires on eating behaviour and personality.

If participants fulfilled all inclusion and none of the exclusion criteria, they underwent two test days with a minimum of 7 days between sessions (mean 11.97 days, maximum 36 days) (Figure 1). A within-subject, double-blind, cross-over design was used to test participants under a dopamine depletion condition (DEP) and a balanced dopamine condition (BAL); the intervention was administered in balanced order. Test sessions were scheduled either at 08.00 AM or 10.00 AM, the two sessions always started at the same time for each participant. Before ingestion of the amino acid drink and at the end of the test session, participants rated their well-being with digital visual analogue scales (VAS) asking for sadness, anxiety, mood, nausea, appetite, hunger, satiety, fullness and urge to move. To monitor success of the APTD intervention, blood samples were drawn before ingestion of the drink and approximately 4 hours post ingestion, prior to behavioural testing. To assess item span, which has been considered a proxy for dopamine,<sup>16</sup> the verbal forward and backward digit span task<sup>49</sup> was administered in a soundproof room immediately before behavioural testing. Behavioural testing was conducted 4.5-5 hours post ingestion (mean: 4 hours 49 minutes, maximum: 5 hours 38 minutes). During the period between ingestion and behavioural testing participants read, watched a movie or worked quietly. Two hours after ingestion participants were provided with a low protein snack, consisting of fruits (apple, banana and grapes) and vegetables (cucumber, carrots and red pepper).



**FIGURE 1** Overview of measurements and timings on screening and test day. Screening: participants gave informed consent (IC) before blood was drawn to measure peripheral dopamine precursor availability (pDAP) and metabolic parameters at baseline. Afterwards, they completed the Dietary Fat and free Sugar Questionnaire (DFS) to assess study eligibility together with questionnaires for eating behaviour and personality traits. Test days: participants completed two test days with different intervention drink. Behavioural testing and the digit span task were conducted approximately 5 h after ingestion of the intervention drink. Blood was drawn prior to ingestion of the drink and prior behavioural testing to measure pDAP pre and post intervention. Visual analogue scales (VAS) were administered prior to ingestion of the drink and at the end of the test session. WMT-2, Viennese Matrices Test 2

## 2.3 | Behavioural testing

Participants performed the probabilistic selection task (PST)<sup>15</sup> and the operation span task (OSPAN)<sup>50</sup> as indirect measures of dopamine function on both test days. The order of PST and OSPAN was different on each test day for individual participants and randomised and counterbalanced across participants within groups. PST and OSPAN were programmed and performed using PRESENTATION, version 16.5 (Neurobehavioral Systems, Inc., Albany, CA, USA).

### 2.3.1 | OSPAN

Working memory performance was examined with a modified version of the automated OSPAN task.<sup>50,51</sup> During the OSPAN task, participants had to mentally solve a presented mathematical problem (eg,  $[4 \times 2] - 7$ ) and then indicate with a mouse click, whether the presented answer is the correct answer to that problem. The time limit for answering was the average time that participants needed to answer the given solutions to mathematical problems in the preceding training phase plus 2.5 SD. Subsequently, a target letter was

presented on the screen, which participants were instructed to remember. After three to seven items (with the number of items per trial varying randomly to prevent participants from anticipating the number of items to be remembered), participants were asked to recall the items by choosing letters from a  $3 \times 4$  matrix containing 12 letters and clicking them in the presented sequence with the mouse. Each length of items was presented three times, adding up to a total of 75 math problems and letters presented. The complete task with training and test phase takes around 30 minutes to finish.

Working memory performance was calculated using the MIS scoring method, a measure that accounts for performance on the distractor task that we have developed previously.<sup>52</sup> In short, the MIS main score (referred to as "MIS score") consists of the working memory related components "number of remembered items" (I) (short-term memory) and "longest contiguous sequence remembered" (S) (relative object placement) and adjusts for performance on the mathematical distractor task (M) on each trial. The MIS score for each trial was calculated using:

$$\text{MIS} = \frac{M_{\text{cor}} - 1}{M_{\text{tot}} - 1} \times \frac{1}{2} \left( \frac{I_{\text{cor}}}{I_{\text{tot}}} + \frac{S_{\text{cor}}}{S_{\text{tot}}} \right)$$

The left side of the multiplication accounts for performance on all mathematical problems (M) except the first one presented, by calculating the ratio of the number of correctly answered problems minus one to the total of mathematical problems minus one ( $\frac{M_{\text{cor}} - 1}{M_{\text{tot}} - 1}$ ). The right side of the multiplication regards short-term memory (I) and relative object placement (S) and is calculated by the ratio of number of correctly recalled items to the total number of items ( $\frac{I_{\text{cor}}}{I_{\text{tot}}}$ ) plus the ratio of the longest contiguous sequence recalled to the total length of the presented sequence ( $\frac{S_{\text{cor}}}{S_{\text{tot}}}$ ). This part of the score is divided by two to weight the distractor and the working memory part of the score equally. The total MIS score for each participant is the sum of all scores per trial; the maximum MIS score possible is 15. The MIS scoring method allows to calculate a subscore only for the working memory components of the OSPAN without the distractor task by only calculating the right side of the multiplication shown above (referred to as the "IS subscore"). The total IS subscore for each participant is the sum of all scores per trial; the maximum IS subscore possible is 15.

### 2.3.2 | PST

The PST consists of a training and a test phase.<sup>15</sup> During the training phase, participants viewed three different pairs of stimuli and had to choose one of the stimuli within each pair. They received positive or negative feedback, depending on whether their choice was correct. Stimuli consisted of six Japanese Hiragana letters (referred to as A-F), always paired as AB, CD and EF. The probabilities for positive feedback for each stimulus were predetermined (A: 80%, B: 20%, C: 70%, D: 30%, E: 60%, F: 40%). The stimulus pairs were presented repeatedly in random order, each pair 20 times in a block

of 60 trials. After each block, learning performance was checked and if a predefined criterion was met (minimum 65% A in AB pair, 60% C in CD pair and 50% E in EF pair), participants advanced to the test phase. If the criterion was not met, participants continued with the next training block, with a maximum of 10 blocks. Participants that did not meet the criterion after 10 training blocks did not advance to the test phase and were excluded from the analysis for this task. During the test phase, the six stimuli were presented in novel pairs and participants were instructed to choose the stimulus that was more likely to have been associated with positive feedback before, although this time no feedback was provided. All 15 possible combinations of stimuli were presented four times each, adding to a total of 60 trials in the test phase. Performance measures from the test phase were learning from positive-feedback ("approach"; choosing A over all other stimuli and C over E and F) and learning from negative feedback ("avoid"; avoiding B in all pairs and D when paired with E or F). Participants that failed to choose A over B in the AB pair two times or more were excluded from the analyses because it was assumed that those participants failed to remember the reward associations of the stimulus pair that was the easiest to discriminate as a result of confusion induced by presentation of novel combinations of stimuli and missing feedback and were thus unable to perform the task properly. The complete task with training and test phase takes around 30 minutes to finish.

## 2.4 | APTD

To first deplete pDAP levels, participants followed a diet low in protein (< 20 g of protein) on the day prior to the test sessions (guidelines provided by a nutritionist) and fasted overnight from 10.00 PM. Drinking water was encouraged and drinking black coffee and tea (without sugar or milk) was allowed in accustomed amounts. On the BAL test days, pDAP levels were replenished by means of ingestion of an amino acid drink containing leucine, isoleucine, lysine, methionine, valine, threonine, tryptophan, tyrosine and phenylalanine. In the DEP condition, a mixture of all aforementioned amino acids except from dopamine's precursors phenylalanine and tyrosine was administered. The composition of amino acid mixtures was based on the formula by McTavish et al.<sup>53</sup> and adapted for three different weight classes to reach ideal dopamine depletion effects with lowest side effects; based on the formula by Frank et al.<sup>54</sup> The three weight classes comprised 50–67 kg, 68–83 kg and higher than 84 kg (maximum weight 146.5 kg) and differed in total amino acid quantity but not their ratio. The amino acid drinks were mixed with lemonade (Fanta or Sprite; Coca-Cola European Partners Plc, Uxbridge, UK) to cover the bitter taste and an anti-foaming agent (Espumisan; BERLIN-CHEMIE AG, Berlin, Germany) for better tolerance. Successful intervention was defined as a positive difference in phenylalanine and tyrosine between post and pre intervention under the balanced condition ( $\text{PheTyr}_{\text{post}} - \text{PheTyr}_{\text{pre}} > 0$ ) and a negative difference between post and pre intervention under the depleted condition ( $\text{PheTyr}_{\text{post}} - \text{PheTyr}_{\text{pre}} < 0$ ).

## 2.5 | Self-reported questionnaires

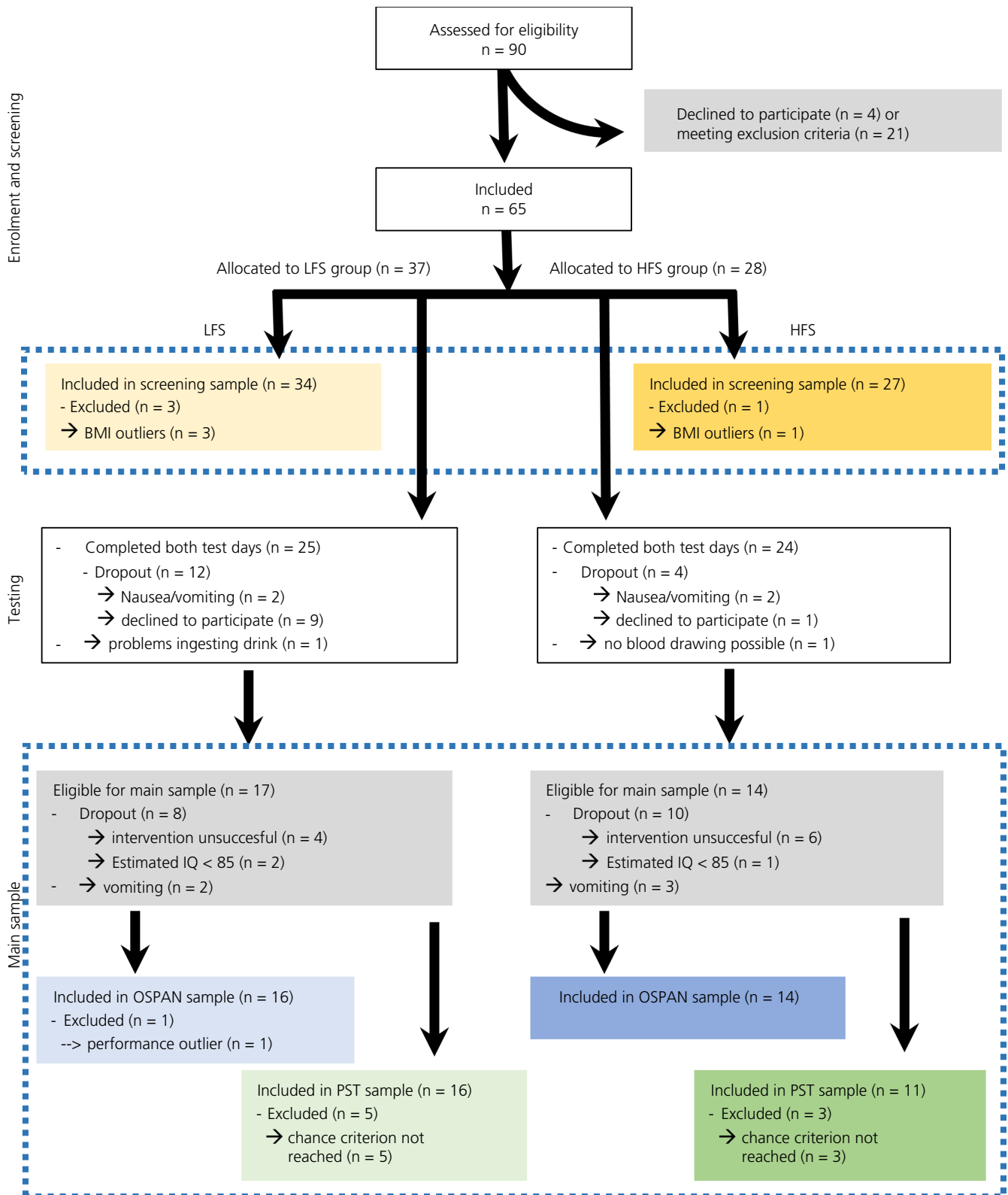
All participants completed the BDI and DFS for inclusion, as well as personality and eating behaviour questionnaires to characterise the two diet groups on the screening day. Feeling of hunger, dietary restraint and disinhibition were assessed using the Three Factor Eating questionnaire (TFEQ).<sup>55,56</sup> Personality measures encompassed the personality traits openness, conscientiousness, extraversion, agreeableness and neuroticism (NEO-FFI),<sup>57</sup> behavioural inhibition and approach system (BIS/BAS)<sup>58</sup> and impulsivity (UPPS).<sup>59</sup>

## 2.6 | Blood measures

Blood samples for the analyses of amino acids were drawn at the screening, as well as prior to ingestion of the drink and prior to behavioural testing on both test days. Blood samples for the analyses of metabolic parameters (cholesterol, triglycerides, glucose, glycated haemoglobin [HbA1c], insulin and leptin) were drawn at the screening and prior to ingestion of the drink on both test days. Insulin resistance was calculated using homeostatic model assessment-insulin resistance. Whole blood samples were drawn using ethylenediaminetetraacetic acid (EDTA) monovettes (2.7-mL EDTA S-monovette; SARSTEDT AG & Co. KG, Nümbrecht, Germany) and kept for 15 minutes at room temperature in an upright position before being stored at  $-80^{\circ}\text{C}$ . Blood serum was drawn using monovettes with clot activator (9-mL S-monovette, SARSTEDT AG & Co. KG), kept for 30 minutes at room temperature in an upright position, centrifuged at 2383 g for 10 minutes at  $15^{\circ}\text{C}$ , and the supernatant stored at  $-80^{\circ}\text{C}$ . Metabolic parameters were analysed with the COBAS 8000 system (Roche Diagnostics, Mannheim, Germany). Glucose was determined by a enzymatic colorimetric photometric assay. HbA1c analysis was performed using a immunoassay (TinaQuant; Roche Diagnostics). Triglycerides and total cholesterol were determined by homogeneous enzymatic colorimetric assays (Roche Diagnostics). Insulin measurement was performed by the fully automated chemiluminescence immunoassay system Liaison (Diasorin, Saluggia, Italy). Leptin concentrations were obtained from a manually processed enzyme-linked immunoassay manufactured by Mediagnost (Kusterdingen, Germany). The analysis of amino acids was performed as reported previously.<sup>60,61</sup> In brief, for protein depletion 10  $\mu\text{L}$  of serum was diluted with methanol containing isotope labelled standards. After centrifugation and derivatisation analysis was performed via tandem mass spectrometry on an API 4500 tandem mass spectrometer (Applied Biosystems, Foster City, CA, USA).

## 2.7 | Study samples

Sixty-five participants completed the screening day (ie, had not to be excluded based on health issues, smoking or drug abuse), including blood drawing and self-reported measures of eating behaviour and personality, and began the test days with dietary intervention.



**FIGURE 2** Flowchart of the study protocol. Enrolled participants were screened for eligibility based on health and diet. Included participants completed two test days varying in intervention drink. Participants with unsuccessful intervention (for details, see Materials and methods) or who vomited/felt nauseous during testing were excluded from the analyses. Task specific criteria were used to define samples for task analyses. Dashed blue frames indicate samples that were used for statistical analyses. BMI, body mass index; LFS, low fat sugar; HFS, high fat sugar

During the course of the study, 16 participants dropped out voluntarily (Figure 2). A further three participants with an estimated IQ lower than 85 and four participants who had to vomit after the ingestion of the amino acid drink on one of the test days were excluded from the analyses. Finally, 11 participants for whom the intervention was unsuccessful had to be excluded from the analyses. Statistical outliers for BMI, based on the 2.2 interquartile range, were included in the analyses to ensure proficient sample size. Thus, the study sample consisted of 31 subjects, 17 in the LFS group and 14 in the HFS group (Table 1). For analyses of the OSPAN task, one subject in the LFS group had to be excluded as a result of poor overall performance (statistical outlier based on 2.2 interquartile range criterion), resulting in a sample of  $n_{LFS} = 16$  and  $n_{HFS} = 14$  participants. During the PST, eight participants did not reach the chance criterion in the test phase, which resulted in a sample of  $n_{LFS} = 12$ ,  $n_{HFS} = 11$  participants available for analyses of this task.

Because a secondary aim of the study was to characterise the two dietary groups with respect to the dopaminergic system, but also metabolic parameters, eating behaviour and personality, we repeated the analyses of measurements obtained at screening day with the remaining participants that completed the screening day but were not eligible for the main sample. The analysed remaining sample consisted of 34 participants, 14 in the HFS group and 20 in the LFS group.

## 2.8 | Statistical analysis

Statistical analyses were performed in R, version 3.6.1<sup>62</sup> within RSTUDIO,<sup>63</sup> using the packages *car*, *stats*, *pastecs*, *lme4* and *psych*. Group demographics (age, IQ and BMI) and questionnaire data were tested using Welch's t-test for unequal variance. Metabolic

parameters were analysed with linear regression models using the function *lm* of the R package *stats* with diet group (LFS vs HFS) as predictor and BMI as covariate. All data that were measured multiple times (OSPAN and PST performance and reaction times, digit span [forward and backward], pDAP, VAS) were analysed with linear mixed-effect models with random intercepts and fixed slope for the random factor subject, using the function *lmer* of the R package *lme4*, unless stated differently. To test the significance of an effect in question, we compared the full model to a null model without the effect in question with likelihood ratio tests. 95% Confidence intervals (CI) are reported for significant effects to illustrate certainty of this effect (confidence levels displayed as 0.000 are numbers smaller/larger than zero, indicating the CIs do not cross zero). The assumption of normally distributed residuals of the models was checked by visually inspecting the qq-plots and no obvious violations were found. Primary performance measure for the cognitive tasks (OSPAN and PST) was accuracy, secondary performance measure were reaction times (RTs). OSPAN performance was analysed in a model with fixed effects diet group (LFS vs HFS) and intervention (BAL vs DEP) and the diet group  $\times$  intervention two-way interaction, controlled for BMI and test day to account for training effects. Performance in the PST training phase was analysed in an ordinal regression model using the function *clmm* of the R package *ordinal*, with fixed effects diet group (LFS vs HFS) and intervention (BAL vs DEP), and the diet group  $\times$  intervention two-way interaction, controlled for BMI and test day; number of learn blocks were ranked from lowest to highest (1-10) and not reaching the test phase was assigned the highest rank 11. Performance in the PST test phase was analysed in a model with fixed effects diet group (LFS vs HFS), intervention (BAL vs DEP) and task condition (approach vs avoid), the diet group  $\times$  intervention  $\times$  task condition three-way interaction, and all lower levels interactions, controlled for BMI and test

**TABLE 1** Group demographics and metabolic measurements of the study sample

	LFS (n = 17)			HFS (n = 14)			P value
	Mean $\pm$	SD	range	Mean $\pm$	SD	range	
Age (years)	24.7 $\pm$	4.3	19-32	25.8 $\pm$	4.0	21-33	0.452 <sup>b</sup>
Body mass index (kg m <sup>-2</sup> )	26.3 $\pm$	8.5	20.1-50.1	23.1 $\pm$	4.3	18.5-35.9	0.173 <sup>c</sup>
Non-verbal IQ <sup>a</sup>	104.3 $\pm$	9.8	87-118	100.1 $\pm$	8.7	87-113	0.226 <sup>b</sup>
Cholesterol (mmol L <sup>-1</sup> )	4.5 $\pm$	0.8	3.2-6.4	4.6 $\pm$	1.0	3.1-6.7	0.552 <sup>d</sup>
Triglycerides (mmol L <sup>-1</sup> )	1.2 $\pm$	0.6	0.5-3.2	0.9 $\pm$	0.4	0.2-1.6	0.823 <sup>c</sup>
HbA1c (%)	5.0 $\pm$	0.2	4.6-5.4	4.9 $\pm$	0.4	4.4-5.5	0.556 <sup>d</sup>
Glucose (mmol L <sup>-1</sup> )	4.6 $\pm$	0.5	3.5-5.5	4.6 $\pm$	0.5	4.1-5.6	0.662 <sup>d</sup>
Insulin (pmol L <sup>-1</sup> )	57.3 $\pm$	41.5	17.1-179.2	45.8 $\pm$	33.5	13.4-119.1	0.765 <sup>c</sup>
HOMA-IR	2.0 $\pm$	1.4	0.5-5.8	1.6 $\pm$	1.3	0.4-4.8	0.707 <sup>c</sup>
Leptin (ng mL <sup>-1</sup> )	17.7 $\pm$	20.8	0.2-90.2	15.3 $\pm$	16.8	4.6-70.2	0.568 <sup>c</sup>

Abbreviations: LFS, low fat sugar; HFS, high fat sugar; HbA1c, glycated haemoglobin; HOMA-IR, homeostatic model assessment-insulin resistance.

<sup>a</sup>Non-verbal IQ calculated based on the Viennese Matrices Test 2.

<sup>b</sup>Unpaired two-sample t-test.

<sup>c</sup>Wilcoxon signed-rank test.

<sup>d</sup>Analysis of covariance.

day. Forward and backward digit span was analysed in a model with fixed effects diet group (LFS vs HFS) and intervention (BAL vs DEP), and the diet group  $\times$  intervention two-way interaction, controlled for BMI and test day. To check whether the intervention effectively changed pDAP levels, we ran a linear mixed-effects model with the fixed effects diet group (LFS vs HFS), intervention (BAL vs DEP) and time point (pre vs post), the diet group  $\times$  intervention  $\times$  time point three-way interaction, and all lower level interactions, controlled for BMI. We further tested whether pDAP was different between diet groups and at baseline on each experimental day (screening and pre ingestion of the drink at test days) in a model with the fixed effects diet group (LFS vs HFS) and experimental day (screening vs BAL vs DEP) and the diet group  $\times$  experimental day two-way interaction, controlled for BMI. To test whether pDAP differed at screening and intervention days, we contrasted LFS\_screening = 0.5, HFS\_screening = 0.5, LFS\_BAL = -0.25, HFS\_BAL = -0.25, LFS\_DEP = -0.25 and HFS\_DEP = -0.25. To test whether pDAP at baseline of the two intervention days differed, we contrasted LFS\_screening = 0, HFS\_screening = 0, LFS\_BAL = 0.5, HFS\_BAL = 0.5, LFS\_DEP = -0.5 and HFS\_DEP = -0.5. To test diet group differences at screening, we contrasted LFS\_screening = -0.5, HFS\_screening = 0.5, LFS\_BAL = 0, HFS\_BAL = 0, LFS\_DEP = 0 and HFS\_DEP = 0. To test diet group differences at intervention days, we contrasted LFS\_screening = 0, HFS\_screening = 0, LFS\_BAL = -0.5, HFS\_BAL = 0.5, LFS\_DEP = -0.5 and HFS\_DEP = 0.5. Degrees of freedom were estimated using the Kenward-Roger method. Fixed effects for the analysis of VAS were diet group (LFS vs HFS), intervention (BAL vs DEP) and time point at test day (pre vs post), controlled for test day. The significance level alpha was 0.05, unless stated differently when corrected for multiple comparison, using the Holm-Bonferroni method.

Absolute values of amino acid levels were z-transformed as a result of batch differences in the ranges of values of the analysed samples in the laboratory (samples were sent to the laboratory at two different time points). For comparing precursor availability at the different baseline measurements (screening and test days) and pre and post intervention, the absolute values were z-transformed using the mean  $\pm$  SD of all five measurements in each batch. The values of the remaining sample were z-transformed using the mean  $\pm$  SD of each batch at screening day.

### 3 | RESULTS

The present study was designed to investigate the differential effects of a dietary dopamine depletion depending on low vs high self-reported intake of HFS on performance on a working memory and a reinforcement learning task, as indirect measures of dopamine function. First, we checked whether the intervention was successful in our sample by comparing pDAP before and after the intervention on the two different days. This analysis revealed a significant intervention  $\times$  time point interaction ( $\chi^2 = 207.78$ ,  $df = 1$ ,  $P < 0.001$ , 95% CI = -3.141 to -2.677) indicating that the DEP intervention effectively decreased pDAP (Figure 3) The effect of DEP was similar in

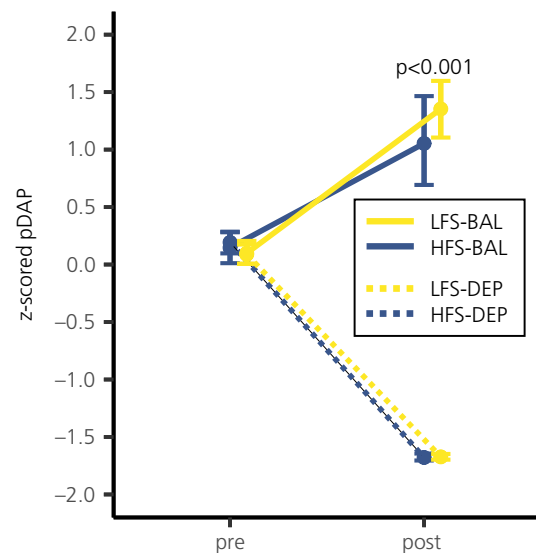
both diet groups, indicated by the nonsignificant diet group  $\times$  intervention  $\times$  time point interaction ( $\chi^2 = 0.95$ ,  $df = 1$ ,  $P = 0.330$ ).

## 3.1 | Effects of dopamine manipulation on cognitive performance

### 3.1.1 | Working memory

On each test day, we measured simple item span with the forward and backward digit span task to account for possible effects of the intervention on diet groups and group differences in short-term memory that might explain different performance on the OSPAN task. The intervention did neither affect forward nor backward item span differently in the two diet groups (diet group  $\times$  intervention interaction, forward  $\chi^2 = 0.01$ ,  $df = 1$ ,  $P = 0.936$ ; backward  $\chi^2 = 0.19$ ,  $df = 1$ ,  $P = 0.661$ ), and neither forward, nor backward item span differed between diet groups and intervention (main effect of diet group, forward  $\chi^2 = 0.18$ ,  $df = 1$ ,  $P = 0.67$ ; backward  $\chi^2 = 0.14$ ,  $df = 1$ ,  $P = 0.711$ ; main effect of intervention, forward  $\chi^2 = 1.25$ ,  $df = 1$ ,  $P = 0.263$ ; backward  $\chi^2 = 0.94$ ,  $df = 1$ ,  $P = 0.331$ ).

Working memory performance was tested with the OSPAN task and scored using the method proposed by Lammert and colleagues.<sup>52</sup> First, we analysed the effects of APTD on the complete MIS score. The diet group  $\times$  intervention interaction was nonsignificant ( $\chi^2 = 1.36$ ,  $df = 1$ ,  $P = 0.243$ ) and both diet groups did not differ in MIS score (main effect of diet group,  $\chi^2 = 0.54$ ,  $df = 1$ ,  $P = 0.464$ ), but were similarly impaired after ingestion of the DEP drink (main effect of intervention,



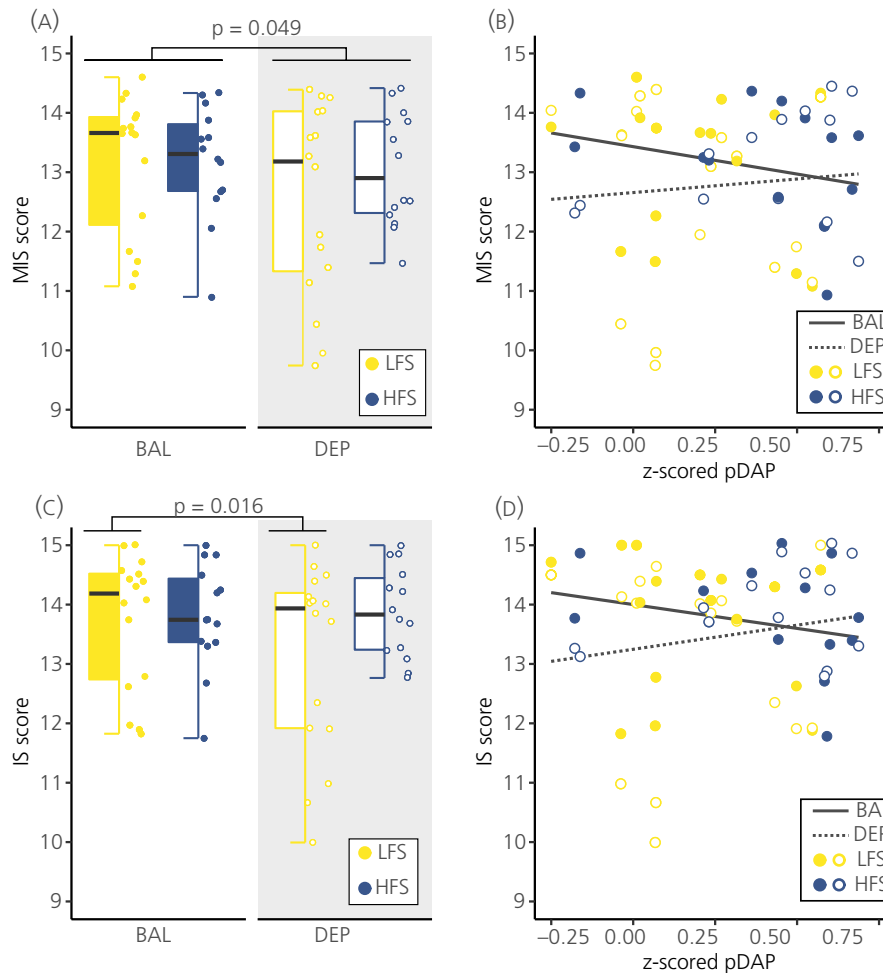
**FIGURE 3** Peripheral dopamine precursor availability (pDAP) pre and post ingestion of the amino acid drinks. Ingestion of the dopamine depletion condition (DEP) drink significantly decreased pDAP compared to the balanced dopamine condition (BAL) drink, confirming successful acute phenylalanine/tyrosine depletion (APTD) intervention (intervention  $\times$  time point interaction,  $\chi^2 = 207.78$ ,  $df = 1$ ,  $P < 0.001$ , 95% CI = -3.141 to -2.677).  $n_{LFS} = 17$ ,  $n_{HFS} = 14$ . LFS, low fat sugar; HFS, high fat sugar



$\chi^2 = 3.86$ ,  $df = 1$ ,  $P = 0.049$ , 95% CI =  $-0.777$  to  $-0.001$ ) (Figure 4A). Analyses of the working memory-specific IS subscore revealed a significant diet group  $\times$  intervention interaction ( $\chi^2 = 5.26$ ,  $df = 1$ ,  $P = 0.022$ , 95% CI =  $0.099$ - $1.172$ ), indicating that the LFS group performed worse after ingestion of the DEP drink, although the HFS group stayed unaffected (post-hoc Tukey's test,  $BAL_{LFS}$  vs  $DEP_{LFS}$ ,  $t_{33.3} = 3.18$ ,  $P = 0.016$ ) (Figure 4C). Reaction times for solving the mathematical problem, evaluating the presented solution and recalling the letter sequence of the OSPAN did not differ significantly between diet groups or intervention (all  $P > 0.189$ ); only when recalling the letter sequence, participants overall tended to be slower on the DEP condition, although this effect was not significant (main effect of intervention,  $\chi^2 = 3.86$ ,  $df = 1$ ,  $P = 0.058$ , 95% CI =  $-2.705$ - $147.89$ ).

### 3.1.2 | Reinforcement learning

Reinforcement learning was tested with the PST, which consists of a training and a test phase.<sup>15</sup> Learning of reward associations during the training phase did not differ between diet groups ( $\chi^2 = 1.26$ ,  $df = 1$ ,  $P = 0.262$ ) or interventions ( $\chi^2 < 0.01$ ,  $df = 1$ ,  $P = 0.952$ ). The diet group  $\times$  intervention interaction was also nonsignificant ( $\chi^2 < 0.01$ ,  $df = 1$ ,  $P = 0.982$ ). Reaction times in the training phase did not differ between diet groups, interventions and there was no significant diet group  $\times$  intervention interaction (all  $P > 0.488$ ). The test phase of the PST tests how well participants learned to approach rewarded stimuli and avoid punished stimuli (referred to as task condition). Analysis of the PST test phase revealed no significant diet group  $\times$  intervention  $\times$  task



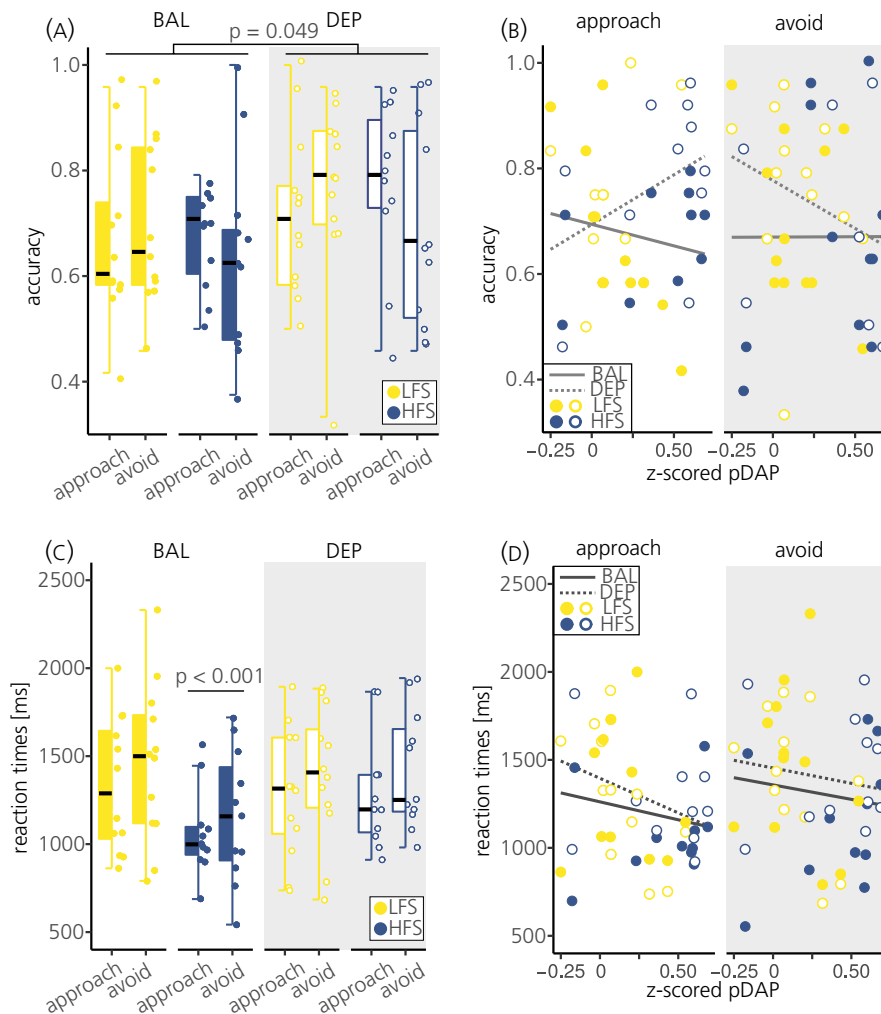
**FIGURE 4** Effects of acute phenylalanine/tyrosine depletion (APTD) on working memory performance measured with the operation span task (OSPAN). (A) MIS score for the two dietary groups under balanced dopamine condition (BAL) and dopamine depletion condition (DEP). Dopamine depletion condition (DEP) trend-significantly decreased working memory performance in both groups (main effect of intervention,  $\chi^2 = 3.86$ ,  $df = 1$ ,  $P = 0.049$ , 95% CI =  $-0.777$  to  $-0.001$ ). (B) Relationship between peripheral dopamine precursor availability (pDAP) at baseline (screening) and MIS score. The effect of intervention remained significant ( $\chi^2 = 3.86$ ,  $df = 1$ ,  $P = 0.049$ , 95% CI =  $-0.777$  to  $-0.001$ ); the pDAP  $\times$  intervention interaction approached significance ( $\chi^2 = 3.37$ ,  $df = 1$ ,  $P = 0.066$ ). (C) IS subscore that stronger represents the working memory component of the OSPAN. The DEP condition impaired working memory performance in the low fat sugar (LFS) group but did not affect performance of the high fat sugar (HFS) group (diet group  $\times$  intervention interaction,  $\chi^2 = 5.26$ ,  $df = 1$ ,  $P = 0.022$ , 95% CI =  $0.099$ - $1.172$ ; post-hoc Tukey test,  $BAL_{LFS}$  vs  $DEP_{LFS}$ ,  $t_{33.3} = 3.18$ ,  $P = 0.016$ ). (D) Relationship between pDAP at baseline (screening) and IS subscore. Significant pDAP  $\times$  intervention interaction indicated that participants with lower pDAP performed better under the BAL condition compared to the DEP condition, in contrast to participants with higher pDAP whose performance appeared to be unaffected by the intervention ( $\chi^2 = 7.59$ ,  $df = 1$ ,  $P = 0.006$ , 95% CI =  $0.441$ - $2.392$ ).  $n_{LFS} = 16$ ,  $n_{HFS} = 14$

condition interaction ( $\chi^2 = 0.26$ ,  $df = 1$ ,  $P = 0.608$ ) or lower level two-way interaction (all  $P > 0.118$ ). The main effects of diet group and task condition were nonsignificant (main effect of diet group,  $\chi^2 = 0.39$ ,  $df = 1$ ,  $P = 0.531$ ; main effect of task condition,  $\chi^2 = 0.08$ ,  $df = 1$ ,  $P = 0.783$ ). The main effect of intervention reached significance ( $\chi^2 = 3.88$ ,  $df = 1$ ,  $P = 0.049$ , 95% CI = 0.000-0.132), indicating that APTD increased accuracy of approach as well as avoid choices in both diet groups (Figure 5A). Analyses of reaction times in the test phase revealed no significant diet group  $\times$  intervention  $\times$  task condition interaction ( $\chi^2 = 0.08$ ,  $df = 1$ ,  $P = 0.771$ ). The diet group  $\times$  intervention interaction was significant ( $\chi^2 = 15.11$ ,  $df = 1$ ,  $P < 0.001$ , 95% CI = 188.64-541.86),

indicating that the HFS group responded faster on the BAL than the DEP day in both task conditions, whereas reaction times were similar for the LFS group on both test days (post-hoc Tukey's test,  $BAL_{HFS}$  vs  $DEP_{HFS}$ ,  $t_{73.2} = -4.65$ ,  $P < 0.001$ ) (Figure 5C).

### 3.1.3 | The effects of dopamine-depletion on mood and well-being

To check whether dopamine depletion affects potential confounders such as mood and well-being differentially in the two diet groups,



**FIGURE 5** The effect of acute phenylalanine/tyrosine depletion (APTD) on reinforcement learning. (A) Performance in the test phase of probabilistic selection task (PST). APTD increased accuracy of approach as well as avoid choices in both diet groups (main effect of intervention,  $\chi^2 = 3.88$ ,  $df = 1$ ,  $P = 0.049$ , 95% CI = 0.000-0.132). (B) Relationship between peripheral dopamine precursor availability (pDAP) at baseline (screening) and accuracy. Significant pDAP  $\times$  intervention  $\times$  task condition interaction ( $\chi^2 = 4.16$ ,  $df = 1$ ,  $P = 0.041$ , 95% CI = -0.888 to -0.018). Approach condition: participants with lower pDAP performed similarly under the balanced dopamine condition (BAL) and the dopamine depleted condition (DEP), whereas participants with higher pDAP performed better under the DEP than the BAL condition. Avoid condition: participants with lower pDAP performed better under the DEP than the BAL condition, performance was unchanged for participants with higher pDAP. (C) Reaction times (RTs). Significant diet group  $\times$  intervention interaction ( $\chi^2 = 15.11$ ,  $df = 1$ ,  $P < 0.001$ , 95% CI = 188.64-541.86), indicating that the high fat sugar (HFS) group responded faster on the BAL than the DEP day in both task conditions, whereas RTs were similar for the low fat sugar (LFS) group on both test days (post-hoc Tukey's test,  $BAL_{HFS}$  vs  $DEP_{HFS}$ ,  $t_{73.2} = -4.65$ ,  $P < 0.001$ ) (D) Relationship between RTs and pDAP at baseline (screening). APTD increased RTs irrespective of pDAP and task condition (main effect of intervention,  $\chi^2 = 5.29$ ,  $df = 1$ ,  $P = 0.021$ , 95% CI = 17.66-214.33) and all participants responded slower in the avoid than the approach condition (main effect of task condition,  $\chi^2 = 5.05$ ,  $df = 1$ ,  $P = 0.028$ , 95% CI = 14.78-209.77).  $n_{LFS} = 12$ ,  $n_{HFS} = 11$

we analysed scores on visual analogue scales that were employed before the ingestion of the drink and at the end of each test day. For anxiety the diet group  $\times$  time point interaction was significant ( $\chi^2 = 6.452$ ,  $df = 1$ ,  $P = 0.011$ , 95% CI = 0.273-2.064, adjusted alpha level with Holm-Bonferroni method: 0.036), indicating that, in the LFS group, anxiety decreased from start to the end of the test days, whereas, in the HFS group, anxiety increased. Furthermore, the intervention  $\times$  time point interaction was nominal significant for anxiety, but not when correcting for multiple comparison ( $\chi^2 = 3.87$ ,  $df = 1$ ,  $P = 0.049$ , 95% CI = -1.785 to -0.003, adjusted alpha level with Holm-Bonferroni method: 0.045), indicating that, on the BAL day, anxiety decreased over the course of the test day, but not on the DEP day. The analyses revealed a nominal significant diet group  $\times$  intervention interaction for nausea, but not when correcting for multiple comparison ( $\chi^2 = 4.06$ ,  $df = 1$ ,  $P = 0.044$ , 95% CI = -0.38, adjusted alpha level with Holm-Bonferroni method: 0.042), indicating that nausea was higher on the DEP day than on the BAL day in the LFS group and vice versa in the HFS group. Mood was significantly decreased from start to end of both test days in both diet groups (main effect of time point,  $\chi^2 = 4.84$ ,  $df = 1$ ,  $P = 0.028$ , 95% CI = 0.061-1.029, adjusted alpha level with Holm-Bonferroni method: 0.038). Fullness, satiety and urge to move were significantly elevated at the end of both test days in both diet groups (fullness,  $\chi^2 = 17.44$ ,  $df = 1$ ,  $P < 0.001$ , 95% CI = -2.028 to -0.765, adjusted alpha level with Holm-Bonferroni method: 0.029; satiety,  $\chi^2 = 7.79$ ,  $df = 1$ ,  $P = 0.005$ , 95% CI = -1.514 to -0.273, adjusted alpha level with Holm-Bonferroni method: 0.033; urge to move,  $\chi^2 = 17.19$ ,  $df = 1$ ,  $P < 0.001$ , 95% CI = -2.111 to -0.789, adjusted alpha level with Holm-Bonferroni method: 0.031).

### 3.2 | pDAP

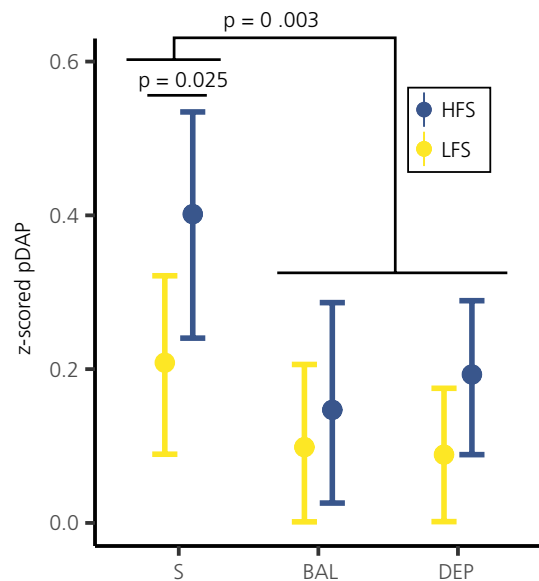
We assessed group differences in pDAP at baseline (screening, prior ingestion of amino acid drinks on test days) as a proxy for the status of central dopamine release in the two dietary groups, because constantly higher levels of dopamine could induce the alterations of dopaminergic transmission observed in rodents after HFS interventions.<sup>23-28,30,31</sup> Overall, pDAP was significantly higher at the screening day compared to the baseline at both test days (main effect of test day,  $t_{66.3} = 4.26$ ,  $P = 0.003$ , 95% CI = 0.069-0.277, adjusted alpha level with Holm-Bonferroni method: 0.029) (Figure 6). Baseline levels of pDAP did not differ between the two intervention days (main effect of test day,  $t_{66.3} = -0.39$ ,  $P = 0.701$ ). The HFS group had significantly higher pDAP than the LFS group at screening, when the diet was not manipulated (main effect of group,  $t_{81.7} = 2.71$ ,  $P = 0.025$ , 95% CI = 0.006-0.219, adjusted alpha level with Holm-Bonferroni method: 0.031). This group difference disappeared at test days, when both groups had similar diets the day before (main effect of group,  $t_{47.6} = 1.58$ ,  $P = 0.242$ ).

Because pDAP and item span measured with the digit span task have both been associated with aspects of central dopamine transmission (release and levels), we tested the correlation between these

two measures. pDAP at baseline did not correlate with mean (BAL and DEP) forward or backward digit span (all  $P = 0.976$ ), and pDAP prior to ingestion of the intervention drink did not correlate with forward or backward digit span on that test day (BAL and DEP: all  $P > 0.372$ ).

### 3.3 | Self-reported eating behaviour and personality traits

Because the preference for HFS might be influenced by general differences in eating behaviour, we investigated potential group differences on the three factor eating questionnaire. The HFS group showed significantly lower restrained eating ( $t_{18.76} = 3.34$ ,  $P = 0.003$ ,  $r = 0.611$ , adjusted alpha level with Holm-Bonferroni method: 0.029), higher disinhibition ( $t_{21.69} = -2.41$ ,  $P = 0.025$ ,  $r = 0.460$ , adjusted alpha level with Holm-Bonferroni method: 0.033) and higher hunger feeling ( $t_{25.59} = -2.51$ ,  $P = 0.019$ ,  $r = 0.444$ , adjusted alpha level with Holm-Bonferroni method: 0.031).



**FIGURE 6** Peripheral dopamine precursor availability (pDAP) measured at the screening day (S) and pre ingestion of the intervention drinks: balanced dopamine condition (BAL) and the dopamine depleted condition (DEP). The low protein diet the day before test days reduced pDAP compared to the screening day with normal diet the day before ( $t_{66.3} = 4.26$ ,  $P = 0.003$ , 95% CI = 0.069-0.277, adjusted alpha level with Holm-Bonferroni method: 0.029). Baseline levels of pDAP did not differ between the two intervention days (main effect of test day,  $t_{66.3} = -0.39$ ,  $P = 0.701$ ). The HFS group had significantly higher pDAP at the screening (main effect of group,  $t_{81.7} = 2.71$ ,  $P = 0.025$ , 95% CI = 0.006-0.219, adjusted alpha level with Holm-Bonferroni method: 0.031). This group difference disappeared at test days, when both groups had similar diets the day before (main effect of group,  $t_{47.6} = 1.58$ ,  $P = 0.242$ ).  $n_{LFS} = 17$ ,  $n_{HFS} = 14$ . LFS, low fat sugar; HFS, high fat sugar

The two diet groups did not show significant differences on any of the subscales of the NEO-FFI, BIS/BAS or UPPS questionnaire (all  $P > 0.08$ ).

### 3.4 | Metabolic blood parameters

We analysed parameters of the fat and sugar metabolism and eating related hormones to check if the dietary preference of the groups is reflected in physiological measurements. The two dietary groups did not differ in any parameter of fat (cholesterol and triglycerides) or sugar metabolism (glucose and HbA1c), as well as leptin, insulin and insulin resistance (all  $P > 0.503$ ) (Table 1).

### 3.5 | Further characterisation of diet groups (remaining sample)

Because an aim of the present study was to characterise the two dietary groups with respect to the dopaminergic system, but also metabolic parameters, eating behaviour and personality, we repeated the analyses of measurements obtained at screening day with the remaining participants that completed the screening day and were not excluded based on health issues, but were also not eligible for the main sample (see Supporting information, Table S1).

The two groups in the remaining sample did not differ in age ( $t_{22.44} = 1.89$ ,  $P = 0.071$ ), BMI ( $t_{31.20} = 0.22$ ,  $P = 0.827$ ) and IQ ( $t_{30.55} = 0.43$ ,  $P = 0.667$ ). In this remaining sample, there was no significant group difference in pDAP ( $F_{1,31} = 0.91$ ,  $P = 0.348$ ). The LFS group showed lower levels of cholesterol at the edge of significance ( $F_{1,31} = 4.13$ ,  $P = 0.051$ , 95% CI =  $-1.131$ - $0.002$ ), significantly lower HbA1c ( $F_{1,31} = 10.19$ ,  $P = 0.003$ , 95% CI =  $-0.395$  to  $-0.087$ ) and trend significant higher levels of insulin ( $F_{1,31} = 3.79$ ,  $P = 0.061$ , 95% CI =  $-0.787$  to  $34.236$ ) and insulin resistance ( $F_{1,31} = 3.58$ ,  $P = 0.068$ , 95% CI =  $-0.045$ - $1.203$ ). Triglycerides, glucose and leptin did not differ between the two diet groups (all  $P > 0.535$ ). The HFS group still showed lower restraint eating ( $t_{30.72} = 2.92$ ,  $P = 0.006$ ,  $r = 0.466$ , adjusted alpha level with Holm-Bonferroni method: 0.029) and higher hunger feeling ( $t_{28.77} = -2.60$ ,  $P = 0.015$ ,  $r = 0.436$ , adjusted alpha level with Holm-Bonferroni method: 0.033). The higher disinhibition of the HFS relative to the LFS group was not observed in this sample ( $t_{29.56} = -0.71$ ,  $P = 0.484$ ). All personality measures stayed nonsignificant (all  $P > 0.172$ ), only the neuroticism subscale of the NEO-FFI was significantly different between groups, with higher neuroticism in the HFS group ( $t_{29.72} = -2.42$ ,  $P = 0.022$ ,  $r = 0.406$ , adjusted alpha level with Holm-Bonferroni method: 0.031).

### 3.6 | The effect of pDAP on dopamine-dependent cognition

The two diet groups differed significantly in pDAP at screening (Figure 6), when measurements should reflect pDAP levels

associated with participants' regular diet. Because pDAP can be considered a proxy for central dopamine release<sup>35,36,38</sup> and chronically higher release of dopamine might induce adaptive changes in the dopaminergic system, such as higher sensitivity of receptors,<sup>64</sup> we included pDAP at screening as predictor in our models for cognitive performance (OSPAN and PST) and simple span (digit span) instead of diet group.

#### 3.6.1 | Working memory

Similar to the original analyses we first tested the effect of pDAP on forward and backward digit span to identify possible confounding effects of short-term memory for the analyses of the OSPAN task. The pDAP  $\times$  intervention interaction (forward  $\chi^2 = 0.08$ ,  $df = 1$ ,  $P = 0.771$ ; backward  $\chi^2 = 0.27$ ,  $df = 1$ ,  $P = 0.603$ ) as well as the main effect of pDAP on digit span were nonsignificant (forward  $\chi^2 = 2.82$ ,  $df = 1$ ,  $P = 0.09$ ; backward  $\chi^2 = 1.86$ ,  $df = 1$ ,  $P = 0.173$ ).

Analyses of the full MIS score revealed that pDAP did not have a significant effect in line with the previous analyses with diet group as factor (main effect of pDAP,  $\chi^2 = 0.13$ ,  $df = 1$ ,  $P = 0.723$ ) (Figure 4B). The pDAP  $\times$  intervention interaction approached significance, but still remained nonsignificant ( $\chi^2 = 3.37$ ,  $df = 1$ ,  $P = 0.066$ ). The effect of intervention remained unchanged (main effect of intervention,  $\chi^2 = 3.86$ ,  $df = 1$ ,  $P = 0.049$ , 95% CI =  $-0.777$  to  $-0.001$ ). Compared to the original analyses of the IS subscore, the pDAP  $\times$  intervention interaction was even more significant ( $\chi^2 = 7.59$ ,  $df = 1$ ,  $P = 0.006$ , 95% CI =  $0.441$ - $2.392$ ), indicating that participants with lower pDAP performed better under the BAL condition compared to the DEP condition, in contrast to participants with higher pDAP whose performance seemed to be unaffected by the intervention (Figure 4D). Levels of pDAP did not affect RTs for solving the mathematical problem, evaluating the presented solution and recalling the letters (pDAP  $\times$  intervention interaction and main effect of pDAP, all  $P > 0.198$ ).

#### 3.6.2 | Reinforcement learning

Acquisition of stimulus-reward associations measured in the PST training phase were not influenced by pDAP ( $\chi^2 = 0.64$ ,  $df = 1$ ,  $P = 0.425$ ), the intervention ( $\chi^2 = 1.31$ ,  $df = 1$ ,  $P = 0.252$ ) and there was no pDAP  $\times$  intervention interaction ( $\chi^2 = 1.78$ ,  $df = 1$ ,  $P = 0.182$ ). Reaction times in the training phase were not associated with pDAP ( $\chi^2 = 0.74$ ,  $df = 1$ ,  $P = 0.390$ ) and there was no pDAP  $\times$  intervention interaction ( $\chi^2 = 0.35$ ,  $df = 1$ ,  $P = 0.553$ ). Analysis of performance in the test phase revealed a significant pDAP  $\times$  intervention  $\times$  task condition interaction ( $\chi^2 = 4.16$ ,  $df = 1$ ,  $P = 0.041$ , 95% CI =  $-0.888$  to  $-0.018$ ), indicating that in the approach condition participants with lower pDAP performed similarly under the BAL and the DEP condition, whereas participants with higher pDAP performed better under the DEP than the BAL condition (Figure 5B). In the avoid condition, participants with lower pDAP performed worse under

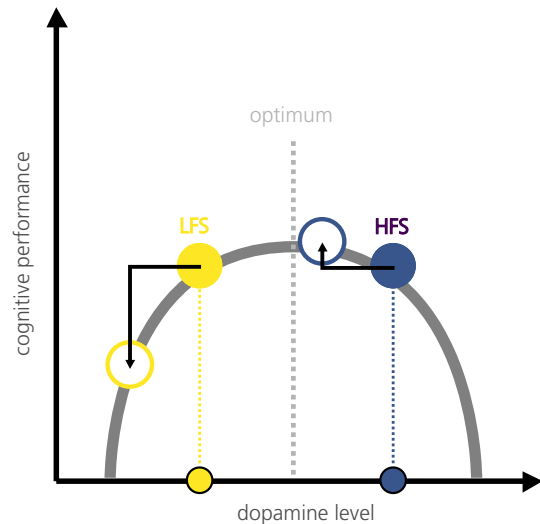
the BAL than the DEP condition and performance was unchanged for participants with higher pDAP. Analyses of reaction times in the test phase revealed no significant pDAP  $\times$  intervention  $\times$  task condition interaction or any of the lower two-way interactions and no significant main effect of pDAP (all  $P > 0.118$ ) (Figure 5D). The DEP intervention increased reaction times irrespective of pDAP and task condition (main effect of intervention,  $\chi^2 = 5.29$ ,  $df = 1$ ,  $P = 0.021$ , 95% CI = 17.66–214.33) and all participants responded slower in the avoid than the approach condition (main effect of task condition,  $\chi^2 = 5.05$ ,  $df = 1$ ,  $P = 0.028$ , 95% CI = 14.78–209.77).

## 4 | DISCUSSION

We aimed to provide first evidence indicating that habitual dietary intake of saturated fat and added sugar is associated with alterations of the dopaminergic system in humans. For this purpose, we grouped participants based on their self-reported fat and sugar intake into a low and a high consumer group (LFS vs HFS). In a within-subjects design, we investigated the diet-dependent effects of acute phenylalanine and tyrosine depletion on dopamine-mediated cognitive performance in a working memory task (OSPAN) and a reinforcement learning task (PST). Furthermore, the groups were characterised in terms of pDAP (a potential proxy for central dopamine release), metabolic parameters, eating behaviour and personality traits at baseline.

The main findings of this study are (i) different levels of pDAP at baseline and (ii) the differential effect of APTD on working memory performance (the IS subscore) and reaction times in a reinforcement learning task in the two diet groups. More specifically, we show that reduction of peripheral dopamine precursor availability led to decreased working memory performance in the LFS group, whereas performance was unaffected in the HFS group.

Work by Cools and D'Esposito<sup>16</sup> suggests the existence of an inverted-U-shaped relationship between dopamine levels and human working memory, with an optimum level of dopamine for performance. Along those lines, the observed differential effect of APTD on working memory may indeed indirectly reflect underlying group differences in dopamine transmission. Given the observation that APTD reduces dopamine release in the human brain,<sup>35,36,38</sup> together with reduced synthesis reported in rats,<sup>34</sup> it can be assumed that, ultimately, APTD reduces central dopamine levels and shifts both groups on the proposed inverted-U function, either further away from or closer to the optimum dopamine level, which is reflected in task performance (Figure 7). Performance of the LFS group is reduced after APTD, which suggests the LFS group is located on the left-hand side of the inverted-U-function. Performance of the HFS group at a similar level as the LFS group under the BAL condition with no change in performance after depletion suggests that the HFS group may have started on the right-hand side of the optimum. Thus, the reduction of pDAP, that was similar in both groups, might have shifted the HFS to a less steep part of the inverted-U, resulting in no measurable change in performance. Possible molecular



**FIGURE 7** Proposed dopamine levels of the two dietary groups under balanced dopamine condition (BAL) and dopamine depletion condition (DEP). Based on the differential effect of acute phenylalanine/tyrosine depletion (APTD) on working memory performance (IS subscore) we propose higher central dopamine levels in the high fat sugar (HFS) group at baseline. APTD shifts both groups on the inverted-U-shaped curve of performance to lower dopamine levels. Performance in the low fat sugar (LFS) group was decreased after APTD (ie, the group was shifted away from the optimum), which puts the LFS group on the left side of the inverted-U curve. Performance of the HFS group was unchanged when dopamine levels were decreased, either because the inverted-U has a plateau around the optimum and this group was shifted on that plateau or the group was shifted beyond the optimum and ended up where the LFS group started

explanations for the absence of an effect of the depletion in the HFS group could be that the putatively higher dopamine level of the HFS group (i) induces compensatory structural changes at dopaminergic synapses, like altered receptor or transporter expression, (ii) expressed receptors become more sensitive to binding ligands<sup>64</sup> or (iii) the HFS group has a higher central capacity to buffer dopamine and hence withstand peripheral depletion. Interestingly, performance on the digit span task was not affected by the intervention, even though it is proposed to be associated with central dopamine.<sup>16</sup> This could be explained by the different forms of memory that simple and complex span tasks rely on,<sup>65</sup> and that these might be differentially affected by APTD. As a result of the applied intervention (ie, manipulation of diet prior to the APTD intervention and ingestion of the drinks), we unfortunately cannot make any statement about or draw inferences from dopamine-dependent cognition at baseline. Successful intervention required participants in both groups to adhere to the same low-protein diet prior to the test days which differed from their normal diet. This might temporarily have rendered the groups more similar, in contrast to being tested on their normal diet and has to be considered as a limitation of our study. Such short-term effects might be possible to account for by adjusting the preparatory diet to the groups (ie, similar in protein content, but still differing in fat and sugar content). Thus, it is difficult to compare

our findings with the only other study investigating the association of HFS with PFC-related executive function in humans, by Francis and Stevenson,<sup>66</sup> who found no differences between LFS and HFS groups. It was argued that the effects of diet on PFC may be too subtle to detect with the administered tasks. Thus, we propose that further studies with more dopamine-sensitive tasks are warranted to investigate possible baseline differences in dopamine-dependent cognition associated with HFS.

APTD affected performance in the test phase of the PST equally in both diet groups, leading to higher accuracy after APTD in the approach and avoid condition of the task. The HFS group responded significantly faster under the BAL condition, still achieving the same accuracy as the LFS group. The fact that we observed no significant association of diet with performance on the PST, nor an interaction between diet and the intervention, has to be interpreted with care. One important limitation of the present study is the small sample that we could analyse for this particular task, which negatively affects the power to detect diet-related differences. Furthermore, the retest reliability of the PST, together with other tasks tapping into self-regulation, has recently been called into question by a large scale literature review and empirical study by Enkavi et al,<sup>65</sup> which is highly relevant for within-subject designs as ours. Theoretically, subtle differences in variance in task performance explained by the interaction between diet and dopamine depletion could therefore have been masked by the random variance inherent to the task.

Interestingly, the observation of higher pDAP in the HFS relative to the LFS group also points to the potential existence of diet-related group differences in central dopamine release. Studies applying APTD showed that peripheral availability of the two dopamine precursor amino acids phenylalanine and tyrosine correlated with dopamine release in the brain.<sup>35,36</sup> Given the additional observation that a 24-h low protein diet prior to the test days reduced pDAP, our findings raise the alternative hypothesis that diet-related differences in central dopamine, as observed in animals after HFS intervention, may very well be the result of acute dietary effects on peripheral amino acid levels. Such short-term effects have been shown for the ratio of carbohydrates to protein in a standardised breakfast.<sup>68</sup> It should be noted that the present study did not include a direct measure of central dopamine such as PET and can therefore not confirm our hypothesis. In addition, long-term dietary interventions are required to confirm an effect of HFS diets on amino acid level availability and dopamine-mediated cognition, preferably including PET-measurements of central dopamine levels in humans. Another possible explanation for the observed group difference in pDAP could be that the LFS and HFS group differ in the absorption or metabolism of phenylalanine and tyrosine compared to the other LNAAs and the resulting differences in central dopamine in turn could influence the preference for high-caloric food. Such a mechanism could explain why the groups still differed slightly in pDAP after they consumed a comparable diet for 24 hours. This hypothesis is highly speculative, however, because all LNAAs, including phenylalanine and tyrosine, are transported by the same transporter in the gut<sup>69</sup> and altered amino acid metabolism can lead to severe diseases

such as phenylketonuria.<sup>70</sup> To our knowledge, only one study has looked at the relationship of central dopamine transmission and food preference in humans. Wallace et al<sup>71</sup> combined a food rating paradigm, asking for wanting and perceived healthiness of various food items, with PET to measure striatal dopamine synthesis binding. They report higher preference for perceived healthy, but not objectively healthy food items in people with lower striatal dopamine synthesis, supporting the hypothesis that endogenous dopamine is indeed related to food preference.

Based on our aforementioned hypothesis that chronically higher central dopamine levels might change the dopaminergic system, we used pDAP instead of diet group as predictor in the models for the two cognitive tasks in an exploratory analysis. The effect of the intervention on working memory was strongest in individuals with lower pDAP and attenuated with higher pDAP levels (Figure 4D). As a result of constantly higher pDAP and thus potentially increased dopamine synthesis capacity, those individuals might have a larger central dopamine storage to buffer externally induced depletion of dopamine precursors and consequently reduced capability for synthesis. Approach learning was improved by APTD in individuals with higher pDAP, whereas avoid learning improved in individuals with lower pDAP (Figure 5B).

In our main sample, as well as in the remaining sample, we found group differences in eating behaviour and personality traits. In line with Francis and Stevenson,<sup>66</sup> who used similar dietary groups, the LFS group reported significantly higher dietary restraint, and lower hunger than the HFS group. Additionally, the HFS group in our study reported significantly higher disinhibition, though only in the main sample. Whether eating behaviour itself influences dopamine dependent cognitive performance has only been investigated in one study so far. Sadler et al<sup>72</sup> reported lower working memory capacity measured with the n-back task for participants with higher self-reported dietary restraint and between group differences in reward and punishment sensitivity measured with the PST. To disentangle possible effects of diet and eating behaviour, diet intervention studies with two groups differing in any of the TFEQ subscales are needed. Our two dietary groups also differed in the two subscales neuroticism and agreeableness of the NEO-FFI and the urgency subscale of the UPPS. Food preference and dietary style have been associated with personality traits before: in line with our finding, higher neuroticism is associated with higher preference for and consumption of sweet foods.<sup>73,74</sup> However, it is still debatable whether personality traits influence food consumption<sup>75</sup> or whether more basal factors such as genetic predisposition are stronger contributors.<sup>76</sup>

Note that the dopamine depletion effects have to be interpreted with care and await future replication. It also should be considered that some of the findings in the main sample could not be replicated in the second sample of screened participants. Specifically, the higher pDAP observed in the HFS group of the main sample has to be interpreted with care because this finding was not significant in the remaining screening sample. We also could not replicate the differences in higher disinhibition in eating

behaviour in this second screening sample. This calls for replication in different study populations. The small sample size is a major limitation of our study and the results have to be interpreted with caution because of the concomitant low statistical power. In consequence, our findings have to be considered as preliminary, requiring replication with a higher sample size to provide sufficient statistical power for detecting smaller effects, which have to be assumed when studying diet. The size of the main sample was low due to an unusually high dropout rate (Figure 1) compared to other studies that administered APTD.<sup>36,41</sup> Nausea or vomiting is a common side-effect of APTD, likely because of its unpalatable taste<sup>42,45,77</sup>; however, our administration of the APTD intervention differed in the sense that we mixed the amino acid drink with lemonade instead of syrup and also that syrup might have a stronger flavor to disguise the bitter taste of amino acids. Furthermore, other studies administered the unpleasant amino acids such as methionine separately from the dissolved mixture<sup>78,79</sup> to reduce risk of nausea. We recommend that future APTD studies follow these precautions. Additionally, we recognised that female participants on average report more nausea or regurgitating, in contrast to male participants.<sup>36,54,78,80</sup> Furthermore, the generalisability of our findings is limited because we only included young healthy women in this study. Dopamine availability in the striatum appears to depend on gender<sup>81</sup> and cortical plasticity is influenced by levels of sex hormones,<sup>82</sup> factors that might determine the strength of putative diet-induced changes of the dopaminergic system. Additionally, it should be noted that, unfortunately, we were not able to control analyses of the cognitive tasks for menstrual cycle as a result of missing cycle data for some of the participants because the levels of the sex hormone oestradiol have been shown to affect dopamine-dependent cognition such as working memory and reinforcement learning.<sup>83-85</sup> We are also not able to make any statement about possible interaction effects of HFS and obesity because our sample mainly included participants from the normal to overweight range. We are also aware of the fact that the genetic background influences baseline dopamine transmission parameters and cognitive function,<sup>86</sup> which we cannot account for in our study. Future studies, including men and women, focusing on a more narrow range of BMI and with a sample size sufficiently large to consider genotypic variation affecting dopaminergic transmission, are needed to shed further light on the association of HFS and the dopaminergic system in humans.

## 5 | CONCLUSIONS

The present study provides the first evidence indicating that the amount of saturated fat and refined sugars habitually consumed is associated with the different availability of dopamine precursors in humans that could potentially explain differential effects of a dietary dopamine manipulation. We provide first evidence indicating that (i) the effect of a dietary dopamine depletion on working memory (but not reinforcement learning) performance and (ii) peripheral

availability of dopamine precursors, a proxy for central dopamine release,<sup>35,36</sup> differed between two groups reporting high relative to low intake of high fat and sugar food products. It has to be stated explicitly, however, that any conclusions drawn from the present study are limited by the low sample size and statistical power and thus await future replication.

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## CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

## AUTHOR CONTRIBUTIONS

**Hendrik Hartmann:** Data curation; Formal analysis; Project administration; Validation; Visualisation; Writing – original draft; Writing – review & editing. **Larissa K Pauli:** Conceptualisation; Data curation; Formal analysis; Investigation; Project administration; Writing – review & editing. **Lieneke Katharina Janssen:** Conceptualisation; Formal analysis; Supervision; Writing – original draft; Writing – review & editing. **Sebastian Huhn:** Methodology; Writing – review & editing. **Uta Ceglarek:** Formal analysis; Methodology; Resources; Writing – review & editing. **Annette Horstmann:** Conceptualisation; Funding acquisition; Investigation; Project administration; Resources; Supervision; Writing – review & editing.

## PEER REVIEW

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## DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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