Supplemental data

ピロロキノリンキノンジナトリウム（PQQ）摂取後の 20 から 65 歳の健康な大人に対する認知機能と安全性の評価

Evaluation of cognitive function and safety in healthy adults aged 20 to 65 years after taking pyrroloquinoline quinone disodium (PQQ)

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インフォームドコンセント

被験者への情報及び同意取得

研究受託機関は試験に先立ち、被験者に対して本試験の内容を十分に説明し、被験者の自由意思に基づいた同意を取得した。

試験責任医師は、初回来院時に被験者本人に対して、倫理審査委員会で承認された下記の内容の同意説明文書に基づいて被験者本人に本試験内容を十分説明し、試験の参加について被験者本人の自由意思に基づいた同意を文書により取得した。

同意取得に際し、試験責任医師は、被験者が質問する機会と試験参加を判断するのに十分な時間を与えるとともに、被験者からの全ての質問に対して被験者が満足できるように答えた。説明を行った試験責任医師は、同意書に署名又は記名捺印し、日付を記入した。なお、試験協力者が補足的に説明を行った場合には、試験協力者も署名又は記名捺印し、日付を記入した。同意する意思のある被験者は、これと同様に同意書に署名又は記名捺印し、日付を記入した。

同意書は実施医療機関が保存した。

【同意説明文書に記載する事項】

① 研究の名称
② 研究機関名称
③ 研究の目的及び意義
④ 研究の方法（研究対象者から取得された試料・情報の利用目的を含む。）及び期間
⑤ 研究対象者として選定された理由
⑥ 研究対象者に生じる負担並びに予測されるリスク
⑦ 研究が実施又は継続されることに同意した場合であっても随時これを撤回できる旨（研究対象者等からの撤回の内容に従った措置を講じることが困難な場合があるときは,その旨及びその理由）
⑧ 研究が実施又は継続されることに同意しないこと又は同意を撤回することによって研究対象者等が不利益な取扱いを受けない旨
⑨ 研究に関する情報公開の方法
⑩ 個人情報等の取扱い（匿名化する場合にはその方法を含む。）
⑪ 試料・情報の保管及び廃棄の方法
⑫ 被験者等及びその関係者からの相談等への対応（連絡窓口）
⑬ 被験者等に経済的負担又は謝礼がある場合には、その旨及びその内容
⑭ 当該研究によって生じた健康被害に対する補償の有無及びその内容
⑮ 研究対象者から取得された試料・情報について、研究対象者等から同意を受ける時点では特定されない将来の研究のために用いられる可能性又は他の研究機関に提供する可能性がある場合には、その旨と同意を受ける時点において想定される内容
⑯ 研究対象者の秘密が保全されることを前提として、モニタリングに従事する者及び監査に従事する者並びに倫理審査委員会が、必要な範囲内において当該研究対象者に関する試料・情報を閲覧する旨
⑰ その他

被験者のプライバシー及び人権保護

試験責任医師、試験協力者及び本試験に関わる全てのものは、「個人情報の保護に関する法律（平成 15 年 5 月 30 日・法律第 57 号）」に則り取り扱った。本試験に関わる全ての情報は、試験に直接関わる者以外、試験責任者の許可なしに開示することはなかった。

だたし、規制当局は、試験責任者の了解なしに試験の記録を調査してもかまわないこととした。

研究受託機関は、検体提供者の個人識別を検体から取り除き、符号化・番号化による連結不可能匿名化を行い、個人の特定ができないようにした。

被験者の意思に影響を与える情報が得られた場合の対応

試験参加の継続に際し、被験者の意思へ影響を与える可能性がある情報が得られた場合には、試験責任医師は、当該情報を速やかに被験者へ伝え、試験参加の継続の意思を確認するとともに、改めて同意を文書により得ることとした。当該試験では、該当する事象は生じなかった。

同意説明文書の改定と試験参加中の被験者の医師確認

被験者の同意に関連し新たな情報が得られた場合には、研究機関又は研究受託機関が速やかに当該情報に基

づき同意説明文書を改訂し、倫理審査委員会の承認を得た後に、再度被験者から同意を取得することとした。
当該試験では、該当する事象は生じなかった。

Table S1 In body による総合身体組成

Variables		**PQQ (N=31)**	**Placebo (N=31)**	***p* -value**
SMM(Skeletal Muscle Mass (kg)	Week 0(baseline)	23.829±5.259	23.11±3.851	0.871M
	Week 8	23.913±5.277	24.203±4.7	0.536M
	Week 12	23.135±4.481	23.777±4.044	0.430M
	Change from baseline	-0.694±7.051	0.668±6.303	0.426T
	p -value	0.600W	0.497W	
全身 ECW/TBW	Week 0(baseline)	0.381±0.006	0.381±0.006	0.606T
	Week 8	0.382±0.005	0.378±0.009	0.044M
	Week 12	0.383±0.006	0.380±0.005	0.018T
	Change from baseline	0.003±0.009	-0.002±0.007	0.040T
	p -value	0.268W	0.235W	

Values are presentad as mean ± SD

p -value for the comparison of within-group by t-test(T) or Wilcoxon signed rank sum test(W)

p -value for the comparison between two group groups by independent t-test(T), Mann Whitney test(M) or Wilcoxon rank sum test(W)

Table S2 BDNF$^{1)}$

Variables	**PQQ** **（ *n* = 31）**			**Placebo** **(*n* =31)**			**P-value $^{2)}$**
BDNF (pg/mL)							
Week 0	2469.9	±	1896.9	2522.0	±	1522.7	0.318 ^{M}
Week 8	2190.1	±	1172.0	1997.8	±	1119.7	0.477 ^{M}
Week 12	1597.2	±	921.7	2298.1	±	1365.8	0.032 ^{M}
Change from baseline	-872.7	±	1743.5	-223.9	±	1730.0	0.147 ^{T}
P-value $^{3)}$	0.005 ^{W}			0.433 ^{W}			

1) Data are presented as mean ± standard deviation (SD)

2) Independent t-test, Mann-Whitney test or Wilcoxon's rank-sum test were used to compare the differences between two groups.

3) Paired t-test or Wilcoxon signed rank test were used to compare the differences within each group.

Table S3　理学検査結果

	PQQ			Placebo			*p* -value
	N	Mean	SD	N	Mean	SD	
収縮期血圧 **Systolic blood pressure (mmHg)**							
Week 0(baseline)	35	108.143	12.675	35	111.857	11.319	0.200T
Week 8	34	113.382	12.543	33	116.758	11.407	0.254T
Week 12	34	112.971	11.374	32	113.688	11.403	0.837M
Change from baseline	34	4.912	8.750	32	2.344	9.174	0.249T
P-value		0.002	T		0.158	T	
拡張期血圧 Diastolic blood pressure (mmHg)							
Week 0(baseline)	35	64.057	10.386	35	69.514	10.489	0.032T
Week 8	34	68.118	9.831	33	70.515	10.007	0.326T
Week 12	34	68.324	10.755	32	70.500	8.312	0.363T
Change from baseline	34	4.235	8.613	32	1.375	6.932	0.144T
P-value		0.007	T		0.270	T	
Pulse Rate (beats/min)							
Week 0(baseline)	35	67.600	8.197	35	71.971	10.829	0.061T
Week 8	34	69.647	9.711	33	73.000	11.429	0.190M
Week 12	34	72.941	12.577	32	72.281	11.742	0.827T
Change from baseline	34	5.412	11.266	32	0.656	9.248	0.148M
P-value		0.008	T		0.691	T	

Values are presentad as mean with standard deviation(SD)

「*p* -value for the comparison between two group groups by independent t-test(T)，Mann Whitney test(M) or Wilcoxon rank sum test(W)

Table S4 血液学検査結果

	PQQ			Placebo			*p* -value
	N	Mean	SD	N	Mean	SD	
WBC(μL)							
Week 0(baseline)	35	5362.857	1350.618	35	5802.857	1395.894	0.185T
Week 12	34	5214.706	1457.298	32	5540.625	1756.988	0.739M
Change from baseline	34	-220.588	1189.800	32	-237.5	1678.853	0.962T
P-value (0-12 w)		0.288	T		0.430	T	
RBC(10^{4}/μL)							
Week 0(baseline)	35	463.486	45.569	35	459.743	42.835	0.724T
Week 12	34	464.941	43.383	32	457.781	34.780	0.464T
Change from baseline	34	-0.765	20.433	32	-0.531	29.333	0.768M
P-value (0-12 w)		0.829	T		0.919	T	
Hb (g/dL)							
Week 0(baseline)	35	13.986	1.180	35	13.814	1.202	0.549T
Week 12	34	13.85 3	1.269	32	13.534	1.103	0.282T
Change from baseline	34	-0.171	0.636	32	-0.222	0.913	0.480M
P-value (0-12 w)		0.127	T		0.179	T	
Hct(%)							
Week 0(baseline)	35	42.911	3.043	35	42.397	3.557	0.518T
Week 12	34	42.832	3.172	32	42.316	2.996	0.499T
Change from baseline	34	-0.215	1.703	32	0.062	2.603	0.763M
P-value (0-12 w)		0.467	T		0.893	T	
M CV (fL)							
Week 0(baseline)	35	92.929	4.934	35	92.386	4.198	0.622T
Week 12	34	92.368	4.448	32	92.584	4.532	0.845T
Change from baseline	34	-0.391	1.534	32	0.206	1.629	0.130T
P-value (0-12 w)		0.146	T		0.479	T	
MCH (pg)							
Week 0(baseline)	35	30.271	1.809	35	30.120	1.816	0.953M
Week 12	34	29.859	1.769	32	29.613	1.875	0.585T
Change from baseline	34	-0.341	0.714	32	-0.481	0.686	0.420T
P-value (0-12 w)		0.009	T		0	T	
M CHC (%)							
Week 0(baseline)	35	32.571	0.779	35	32.589	0.776	0.694M
Week 12	34	32.315	1.015	32	31.975	0.943	0.165T
Change from baseline	34	-0.238	0.753	32	-0.591	0.739	0.060T
P-value (0-12 w)		0.074	T		0	T	
Plt (10^{4}/μL)							
Week 0(baseline)	35	25.366	6.515	35	26.929	6.484	0.171M
Week 12	34	25.168	7.777	32	27.388	6.803	0.071M
Change from baseline	34	-0.212	3.193	32	0.159	3.066	0.632T
P-value (0-12 w)		0.701	T		0.771	T	

Values are presentad as mean with standard deviation (SD)

p -value for the comparison between two group groups by independent t-test(T), Mann Whitney test(M) or Wilcoxon rank sum test(W)

Table S5 生化学検査結果

	PQQ	Placebo	

	N	Mean	SD	N	Mean	SD	*p* -value
TG (mg/dL)							
Week 0(baseline)	35	61.800	28.956	35	61.029	27.421	0.81M
Week 12	34	61.471	20.316	32	56.375	20.316	0.635M
Change from baseline	34	-0.706	21.400	32	-3.719	18.361	0.969M
P-value (0-12 w)		0.849	T		0.261	T	
T-Cho (mg/dL)							
Week 0(baseline)	35	199.200	28.282	35	191.457	29.993	0.442M
Week 12	34	198.471	34.378	32	194.625	32.371	0.642T
Change from baseline	34	-1.735	17.899	32	4.219	16.293	0.163T
P-value (0-12 w)		0.576	T		0.153	T	
BUN (mg/dL)							
Week 0(baseline)	35	11.074	2.541	35	11.897	3.258	0.290M
Week 12	34	11.909	2.918	32	13.175	2.998	0.087T
Change from baseline	34	0.835	2.162	32	0.978	2.484	0.804T
P-value (0-12 w)		0.031	T		0.033	T	
T-Bil (mg/dL)							
Week 0(baseline)	35	0.831	0.256	35	0.874	0.386	0.957M
Week 12	34	0.750	0.287	32	0.703	0.248	0.702M
Change from baseline	34	-0.091	0.239	32	-0.197	0.251	0.319M
P-value (0-12 w)		0.033	T		0.000	T	
TP (g/dL)							
Week 0(baseline)	35	7.511	0.478	35	7.500	0.473	0.934M
Week 12	34	7.282	0.440	32	7.309	0.333	0.78T
Change from baseline	34	-0.247	0.355	32	-0.175	0.364	0.419T
P-value (0-12 w)		0	T		0.011	T	
Alb (mg/dL)							
Week 0(baseline)	35	4.686	0.328	35	4.649	0.336	0.567M
Week 12	34	4.429	0.304	32	4.434	0.278	0.945T
Change from baseline	34	-0.256	0.249	32	-0.184	0.236	0.236T
P-value (0-12 w)		0.000	T		0.000	T	
ALP (U/L)							
Week 0(baseline)	35	176.914	46.951	35	198.400	45.248	0.055T
Week 12	34	169.971	47.706	32	200.594	51.351	0.015T
Change from baseline	34	-8.765	27.878	32	-1.125	25.828	0.253T
P-value (0-12 w)		0.076	T		0.807	T	
LDH (U/L)							
Week 0(baseline)	35	166.086	25.972	35	175.771	36.901	0.411M
Week 12	34	160.529	26.431	32	164.219	29.783	0.599M
Change from baseline	34	-5.912	10.613	32	-12.594	24.856	0.397M
P-value (0-12 w)		0.003	T		0.007	T	
γ -GTP (U/L)							
Week 0(baseline)	35	20.371	9.369	35	25.000	28.151	0.242M

Week 12	34	19.353	7.422	32	22.344	22.379	0.371M
Change from baseline	34	-0.912	7.064	32	-3.656	13.074	0.806M
P-value (0-12 w)		0.457	T		0.124	T	
AST (U/L)							
Week 0(baseline)	35	20.600	3.957	35	21.600	5.967	0.859M
Week 12	34	20.471	3.956	32	21.375	6.267	0.634M
Change from baseline	34	-0.235	4.149	32	-0.656	5.620	0.428M
P-value (0-12 w)		0.743	T		0.514	T	
ALT (U/L)							
Week 0(baseline)	35	16.914	7.410	35	16.714	7.614	0.967M
Week 12	34	18.147	9.967	32	16.438	6.525	0.772M
Change from baseline	34	1.088	8.408	32	-0.656	8.361	0.436M
P-value (0-12 w)		0.456	T		0.66	T	
Crea (mg/dL)							
Week 0(baseline)	35	0.690	0.108	35	0.661	0.122	0.307T
Week 12	34	0.684	0.117	32	0.653	0.127	0.31T
Change from baseline	34	-0.007	0.048	32	-0.012	0.044	0.691T
P-value (0-12 w)		0.375	T		0.137	T	
UA (mg/dL)							
Week 0(baseline)	35	4.851	0.818	35	4.883	1.084	0.891T
Week 12	34	4.782	0.985	32	4.731	1.280	0.488M
Change from baseline	34	-0.100	0.537	32	-0.134	0.712	0.825T
P-value (0-12 w)		0.285	T		0.294	T	
LDL - Cho (mg/dL)							
Week 0(baseline)	35	108.971	22.885	35	104.086	25.455	0.503M
Week 12	34	111.765	27.871	32	106.781	26.991	0.705M
Change from baseline	34	1.647	16.021	32	3.563	12.179	0.509M
P-value (0-12 w)		0.553			0.108	T	
HDL - Chol (mg/dL)							
Week 0(baseline)	35	78.914	16.621	35	75.200	12.995	0.302W
Week 12	34	75.794	15.962	32	77.406	14.433	0.669T
Change from baseline	34	-2.912	6.775	32	2.313	7.364	0.004M
P-value (0-12 w)		0.017	T		0.085	T	
Glucose (mg/dL)							
Week 0(baseline)	35	84.257	6.317	35	84.771	10.694	0.86M
Week 12	34	84.941	6.853	32	87.188	10.627	0.52M
Change from baseline	34	0.912	5.457	32	2.281	6.586	0.479M
P-value (0-12 w)		0.337	T		0.059	T	
HbA1c (%)							
Week 0(baseline)	35	5.123	0.372	35	5.091	0.259	0.683T
Week 12	34	5.244	0.341	32	5.231	0.312	0.874T
Change from baseline	34	0.126	0.264	32	0.131	0.191	0.736M
P-value (0-12 w)		0.009	T		0	T	

Values are presentad as mean with standard deviation (SD)

p -value for the comparison between two group groups by independent t-test(T), Mann Whitney test(M) or Wilcoxon rank sum test(W)

Table S6 尿検査結果

	PQQ			Placebo			*p* -value
	N	Mean	SD	N	Mean	SD	
pH							
Week 0(baseline)	35	6.114	0.758	35	5.986	0.636	0.567M
Week 12	34	6.029	0.662	32	6.000	0.684	0.742M
Change from baseline	34	-0.074	0.863	32	0.000	0.823	1M
Specific gravity							
Week 0(baseline)	35	1.014	0.006	35	1.018	0.009	0.088M
Week 12	34	1.016	0.008	32	1.019	0.009	0.159T
Change from baseline	34	0.003	0.008	32	0.001	0.009	*0.585T*
Protein							
Week 0(baseline)	35	0.029	0.169	35	0.229	0.49	0.025M
Week 12	34	0.147	0.359	32	0.281	0.523	0.726M
グルコース							
Week 0(baseline)	35	0	0	35	0	0	–
Week 12	34	0	0	32	0	0	–
ウロビリノゲン							
Week 0(baseline)	35	0	0	35	3 5/0	0	–
Week 12	34	0	0	32	3 2/0	0	–
Occult blood reaction							
Week 0(baseline)	35	0.229	0.843	35	0.143	0.692	0.655M
Week 12	34	0.176	0.716	32	0.344	1.035	0.606M
ビリルビン							
Week 0(baseline)	35	0	0	35	0	0	–
Week 12	34	0	0	32	0	0	–
ケトン							
Week 0(baseline)	35	0.086	0.507	35	0.143	0.601	0.581M
Week 12	34	0	0	32	0	0	–

Values are presentad as mean with standard deviation (SD)

[1] *p* -value for the comparison between two group groups by independent t-test(T), Mann Whitney test(M) or Wilcoxon rank sum test(W)

Table S7 (1) **Cognitrax 試験結果**

Variables		**PQQ** **(N=31)**	**Placebo** **(N=31)**	***p* -value**
Composite memory **総合記憶**	Week 0(baseline)	97.484±7.234	98.161± 4.981	0.921M
	Week 8	99.452±6.180	97.871± 6.994	0.355M
	Week 12	100.452±5.156	96.194± 5.695	0.003T
	Change from baseline	2.968± 5.288	-1.968± 6.636	0.002T
	p -value	0.006W	0.109T	
Verbal memory **言語記憶**	Week 0(baseline)	51.000 ± 4.531	51.161 ± 3.287	0.538 M
	Week 8	52.645 ± 3.392	52.419 ± 3.810	0.966M
	Week 12	54.097 ± 2.271	51.645 ± 3.272	0.001M
	Change from baseline	3.097 ± 3.664	0.484 ± 3.863	0.014M
	p -value	0.000W	0.524W	
Visual memory **視覚記憶**	Week 0(baseline)	46.484 ± 4.024	47.000 ± 3.540	0.594T
	Week 8	46.806 ± 4.246	45.452 ± 4.632	0.260M
	Week 12	46.355 ± 3.747	44.548 ± 4.081	0.074T
	Change from baseline	-0.129 ± 4.241	-2.452 ± 4.788	0.062M
	p -value	0.867T	0.008T	
Psychomotor speed **認知機能速度**	Week 0(baseline)	188.419 ± 25.971	185.548 ± 18.854	0.620T
	Week 8	194.968 ± 22.863	189.387 ± 17.285	0.283T
	Week 12	193.774 ± 22.253	190.258 ± 17.405	0.491T
	Change from baseline	5.355 ± 10.432	4.710 ± 10.501	0.809T
	p -value	0.008T	0.018T	
Reaction time **反応速度**	Week 0(baseline)	652.516 ± 115.226	661.355 ± 69.333	0.200M
	Week 8	644.742 ± 91.424	673.097 ± 69.288	0.041M
	Week 12	633.935 ± 102.852	643.677 ± 69.157	0.234M
	Change from baseline	-18.581 ± 57.874	-17.677 ± 37.787	0.942T
	p -value	0.063W	0.014T	
Complex attention **総合注意**	Week 0(baseline)	5.742 ± 3.326	5.645 ± 3.322	0.989M
	Week 8	4.742 ± 3.109	5.387 ± 3.490	0.566M
	Week 12	4.903 ± 4.245	5.871 ± 4.209	0.302M
	Change from baseline	-0.839 ± 4.375	0.226 ± 3.631	0.301T
	p -value	0.187W	0.757W	
Cognitive flexibility **認知柔軟性**	Week 0(baseline)	46.452 ± 9.750	44.710 ± 9.303	0.278M
	Week 8	50.097 ± 7.569	47.355 ± 8.110	0.174T
	Week 12	51.871 ± 8.531	48.516 ± 9.241	0.165M
	Change from baseline	5.419 ± 9.705	3.806 ± 8.666	0.374M
	p -value	0.003W	0.021T	

Values are presentad as mean ± SD

p -value for the comparison of within-group by t-test(T) or Wilcoxon signed rank sum test(W)

p -value for the comparison between two group groups by independent t-test(T), Mann Whitney test(M) or Wilcoxon rank sum test(W)

Table S7 (2) **Cognitrax 試験結果**

Variables		PQQ (N=31)	Placebo (N=31)	*p* -value
Processing speed 処理速度	Week 0(baseline)	66.323 ± 11.044	62.613 ± 8.831	0.259M
	Week 8	68.903 ± 11.080	63.677 ± 9.167	0.088M
	Week 12	71.194 ± 12.040	68.000 ± 9.324	0.248T
	Change from baseline	4.871 ± 8.539	5.387 ± 7.297	0.799T
	p -value	0.004T	0.000T	
Executive speed 実行機能	Week 0(baseline)	47.645 ± 9.824	45.774 ± 8.995	0.248M
	Week 8	51.419 ± 7.924	48.419 ± 7.784	0.138T
	Week 12	52.935 ± 8.594	49.613 ± 9.084	0.169M
	Change from baseline	5.290 ± 9.599	3.839 ± 8.263	0.409M
	p -value	0.003W	0.015T	
Social acuity 社会的認知	Week 0(baseline)	7.129 ± 2.513	6.935 ± 2.874	0.779T
	Week 8	7.645 ± 2.858	7.065 ± 2.620	0.408T
	Week 12	8.226 ± 2.578	6.935 ± 2.909	0.070T
	Change from baseline	1.097 ± 2.761	0.000 ± 3.141	0.101M
	p -value	0.035T	1.00T	
Reasoning 論理思考	Week 0(baseline)	6.710 ± 3.900	6.032 ± 4.317	0.519T
	Week 8	6.903 ± 4.110	5.806 ± 4.102	0.297T
	Week 12	8.161 ± 3.522	7.710 ± 3.926	0.640M
	Change from baseline	1.452 ± 4.567	1.677 ± 5.115	0.855T
	p -value	0.069W	0.078T	
Working memory ワーキングメモリー	Week 0(baseline)	11.097 ± 3.673	10.323 ± 3.525	0.248M
	Week 8	12.194 ± 2.822	11.581 ± 3.042	0.399M
	Week 12	11.452 ± 3.974	11.452 ± 3.385	0.761M
	Change from baseline	0.355 ± 2.939	1.129 ± 3.547	0.272M
	p -value	0.181W	0.067W	0.399M
Sustained attention 持続的注意力	Week 0(baseline)	32.129 ± 4.387	30.839 ± 4.525	0.187M
	Week 8	32.903 ± 5.635	33.323 ± 3.219	0.734M
	Week 12	32.645 ± 5.200	32.581 ± 4.493	0.766M
	Change from baseline	0.516 ± 4.836	1.742 ± 4.683	0.262M
	p -value	0.073W	0.019W	
Simple attention 単純注意力	Week 0(baseline)	39.355 ± 1.170	39.710 ± 0.588	0.233M
	Week 8	39.581 ± 0.765	39.581 ± 0.886	0.815M
	Week 12	38.968 ± 2.228	39.065 ± 1.999	0.944M
	Change from baseline	-0.387 ± 2.060	-0.645 ± 1.942	0.573M
	p -value	0.242W	0.109W	
Motor Speed 運動速度	Week 0(baseline)	121.452 ± 18.765	122.129 ± 13.766	0.872T
	Week 8	125.290 ± 15.612	124.645 ± 13.212	0.861T
	Week 12	121.484 ± 14.951	121.258 ± 13.518	0.950T
	Change from baseline	0.032 ± 9.239	-0.871 ± 8.233	0.131M
	p -value	0.985T	0.560T	

Values are presentad as mean ± SD, *p* -value for the comparison of within-group by t-test(T) or Wilcoxon signed rank sum test(W), *p* -value for the comparison between two group groups by independent t-test(T), Mann Whitney test(M) or Wilcoxon rank sum test(W)

Supplemental data

パイソンコード

```
from decimal import ROUND_HALF_UP, Decimal
from pathlib import Path

import matplotlib.pyplot as plt
import pandas as pd
import scipy.stats as stats
import seaborn as sns


class DataAnalyzer:
    def __init__(self, file_name, group_column,
results_dir, if_physical_test=False, if_blood_test=False):
        """
        Analyzes data from two groups statistically and
saves the results.

        Args:
            file_name (str): Path to the CSV file for
analysis.
            group_column (str): Name of the column used for
grouping data.
            results_dir (str): Path to the directory where
analysis results will be saved.
            if_physical_test (bool): Flag to indicate if
this is a physical test.
            if_blood_test (bool): Flag to indicate if this
is a blood test.
        """
        self.file_name = file_name
        self.group_column = group_column
        self.df = pd.read_csv(file_name)
        self.results_sub_dir = results_dir /
Path(self.file_name).stem
        self.results_sub_dir.mkdir(exist_ok=True,
parents=True)
        self.if_physical_test = if_physical_test
        self.if_blood_test = if_blood_test

    def _validate_dataframe(self, df):
        """
        Validates if the dataframe has the correct format.

        Args:
            df (pd.DataFrame): The dataframe to be
validated.

        Raises:
            ValueError: If required columns are missing.
        """
        REQUIRED_COLUMNS = ['target', '0w', '8w', '12w']
        if not all(col in df.columns for col in
REQUIRED_COLUMNS):
            raise ValueError(f"Missing required columns:
{REQUIRED_COLUMNS}")

    def _log_warning(self, message):
        with open(self.results_sub_dir/'warning.log', 'a')
as f:
            f.write(message + '¥n')
        print(message)

    def read_data(self):
        encoding_list = ['cp932', 'shift-jis', 'utf-8']
        for encoding in encoding_list:
            try:
                self.df = pd.read_csv(self.file_name,
header=0, encoding=encoding)
                break
            except Exception as e:
                # print(e)
                pass
        self._validate_dataframe(self.df)

    def evaluate_p_value(self, p_value, test_type):
        """
        Evaluates the results of a statistical test based
on p-values.

        Args:
            p_value (float): The p-value obtained from the
statistical test.
            test_type (str): The type of test performed
                             ('normality',
'equal_variance', 'difference test').

        Returns:
            str: Evaluation of the p-value.

        Examples:
            >>> analyzer = DataAnalyzer('dummy.csv',
'target', 'results')
            >>> analyzer.evaluate_p_value(0.01,
'normality')
            'Non-normal distribution'
        """
        if p_value is None or pd.isna(p_value):
            return 'No data'
        if p_value == 1.0:
            return 'Test not applicable: data may lack
variability'
        if test_type == 'normality':
            return 'Normal distribution' if p_value > 0.05
else 'Non-normal distribution'
        elif test_type == 'equal_variance':
            return 'Equal variances' if p_value > 0.05 else
'Unequal variances'
        else:
            result = 'No significant improvement' if p_value
> 0.05 else 'Significant improvement'
            return result

    def _analyze_within_group(self):
        """
        Performs within-group analysis (paired t-test or
Wilcoxon signed-rank test)
        for '0w' and '12w' columns within each group ('A'
and 'B').

        Logs warnings if there are missing values and
performs the analysis
        on the valid data.

        For physical tests and blood tests, always uses
paired t-test.
        """
        for label in ['A', 'B']:
            group_data = self.df[self.df[self.group_column]
== label][['0w', '12w']]

            # Remove rows with missing values
            group_data_valid = group_data.dropna()

            # Check if there were any missing values
```

```
            if len(group_data) != len(group_data_valid):
                warning_message = f'Warning: Missing values
are present in the data for Group {label}. Missing values
will be excluded in the one-sample test calculation.'
                self._log_warning(warning_message)
            group_data_0w = group_data_valid['0w']
            group_data_12w = group_data_valid['12w']

            # Test for normality
            if len(group_data_0w) >= 3 and
len(group_data_12w) >= 3:
                p_value_0w = stats.shapiro(group_data_0w,
nan_policy='omit')[1]
                test_name = 'Shapiro-Wilk'
                evaluation =
self.evaluate_p_value(p_value_0w, 'normality')
                self.statistics.append([f'{label} 0w',
test_name, group_data_0w.mean(), p_value_0w, evaluation])

                p_value_12w = stats.shapiro(group_data_12w,
nan_policy='omit')[1]
                test_name = 'Shapiro-Wilk'
                evaluation =
self.evaluate_p_value(p_value_12w, 'normality')
                self.statistics.append([f'{label} 12w',
test_name, group_data_12w.mean(), p_value_12w, evaluation])

                if self.if_physical_test or
self.if_blood_test or all([p_value_0w > 0.05, p_value_12w >
0.05]):
                    # Paired t-test
                    test_stat_two_sided, p_value_two_sided
= stats.ttest_rel(group_data_12w, group_data_0w,
alternative='two-sided', nan_policy='omit')
                    test_name = 'Paired t-test'
                    evaluation_two_sided =
self.evaluate_p_value(p_value_two_sided, 'difference test')
                else:
                    # Wilcoxon signed-rank test
                    test_stat_two_sided, p_value_two_sided
= stats.wilcoxon(group_data_12w, group_data_0w,
alternative='two-sided', zero_method='wilcox')
                    test_name = 'Wilcoxon signed-rank test'
                    evaluation_two_sided =
self.evaluate_p_value(p_value_two_sided, 'difference test')

                self.statistics.append([f'{label} 0w vs 12w
(two-sided)', test_name, test_stat_two_sided,
p_value_two_sided, evaluation_two_sided])
            else:
                test_name = 'No data'
                self.statistics.append([f'{label} 0w',
test_name, None, None, 'No data'])
                self.statistics.append([f'{label} 12w',
test_name, None, None, 'No data'])
                self.statistics.append([f'{label} 0w vs 12w
(two-sided)', test_name, None, None, 'No data'])
                self._log_warning(f"Skipping paired test
for {label} 0w vs 12w due to insufficient data points.")

    def _analyze_between_groups(self, analyze_column):
        """
        Perform between-group analysis.

        Tests the difference between Group A and B for the
given column.

        Args:
            analyze_column (str): The column to analyze.
        """
        group_a_data = self.df[self.df[self.group_column]
== 'A'][analyze_column]
        group_b_data = self.df[self.df[self.group_column]
== 'B'][analyze_column]

        # Note: Exception handling for change rate (%)
        if analyze_column == '%':
            # If 0w contains zero, the change rate cannot
be calculated.
            # Set NaN where division by zero would occur.
            self.df['%'] = self.df.apply(
                lambda row: (row['12w'] - row['0w']) /
row['0w'] * 100 if row['0w'] != 0 else float('nan'),
                axis=1)

        # Remove rows with missing values
        group_a_data_valid = group_a_data.dropna()
        group_b_data_valid = group_b_data.dropna()

        # Check if there were any missing values
        if len(group_a_data) != len(group_a_data_valid):
            warning_message = f'Warning: Missing values
exist in {analyze_column} data of Group A. Calculations will
be performed excluding missing values.'
            self._log_warning(warning_message)
        if len(group_b_data) != len(group_b_data_valid):
            warning_message = f'Warning: Missing values
exist in {analyze_column} data of Group B. Calculations will
be performed excluding missing values.'
            self._log_warning(warning_message)

        # Check if there is enough data
        if len(group_a_data_valid) == 0 or
len(group_b_data_valid) == 0:
            warning_message = f'Warning: No data left for
analysis in {analyze_column} column for either Group A or
Group B.'
            self._log_warning(warning_message)
            return  # skip the rest of the processing


        # Test for normality
        if all(1.0 > stats.shapiro(data,
nan_policy='omit')[1] > 0.05 for data in
[group_a_data_valid, group_b_data_valid]):
            equal_var = stats.levene(group_a_data_valid,
group_b_data_valid)[1] > 0.05
            test_name = 'T-test' if equal_var else "Welch's
T-test"
            test_func = stats.ttest_ind
            kwargs = {'equal_var': equal_var}
        else:
            test_name = 'Mann-Whitney U-test'
            test_func = stats.mannwhitneyu
            kwargs = {}

        alternative = 'two-sided'
        test_stat, p_value = test_func(group_a_data_valid,
group_b_data_valid, alternative=alternative, **kwargs)
        self.statistics.append([f'Group A vs Group B
{analyze_column} ({alternative})',
                                test_name,
                                test_stat,
                                p_value,
```

```
self.evaluate_p_value(p_value, 'difference test')])
        '''
        self._plot_histogram(sample_1={'data':
group_a_data_valid, 'label': 'Group A'},
                            sample_2={'data':
group_b_data_valid, 'label': 'Group B'},
                            title=f'{analyze_column}
({test_name})')
        self._plot_qq_plot(sample_1={'data':
group_a_data_valid, 'label': 'Group A'},
                        sample_2={'data':
group_b_data_valid, 'label': 'Group B'},
                        title=f'{analyze_column}
({test_name})')
        '''

    def _plot_histogram(self, sample_1, sample_2, title):
        """
        Plot a histogram of the two samples.

        Args:
            sample_1 (dict): The first sample data and
label.
            sample_2 (dict): The second sample data and
label.
            title (str): The title of the plot.
        """
        sns.set_theme(style='white')
        plt.rcParams['font.size'] = 14
        fig,ax = plt.subplots()
        ax.hist(sample_1['data'], alpha=0.5, bins=15,
label=sample_1['label'])
        ax.hist(sample_2['data'], alpha=0.5, bins=15,
label=[sample_2['label']])
        ax.set_title(title)
        ax.legend()
        plt.savefig(self.results_sub_dir /
f'histgram_{title}.png', dpi=300, bbox_inches='tight')
        plt.close()

    def _plot_qq_plot(self, sample_1, sample_2, title):
        """
        Plot a QQ plot of the two samples.

        Args:
            sample_1 (dict): The first sample data and
label.
            sample_2 (dict): The second sample data and
label.
            title (str): The title of the plot.
        """
        sns.set_theme(style='white')
        plt.rcParams['font.size'] = 14
        fig,ax = plt.subplots()
        stats.probplot(sample_1['data'].dropna(),
dist="norm", plot=ax)
        ax.set_title(title)
        plt.savefig(self.results_sub_dir /
f'qqplot_{title}_{sample_1["label"]}.png', dpi=300,
bbox_inches='tight')
        plt.close()

        fig,ax = plt.subplots()
        stats.probplot(sample_2['data'].dropna(),
dist="norm", plot=ax)
        ax.set_title(title)
        plt.savefig(self.results_sub_dir /
f'qqplot_{title}_{sample_2["label"]}.png', dpi=300,
bbox_inches='tight')
        plt.close()

    def _add_descriptive_statistics(self, analyze_column):
        """
        Add descriptive statistics (mean and standard
deviation) for the given column.

        Args:
            analyze_column (str): The column to analyze.
        """

        for label in ['A', 'B']:
            data = self.df[self.df[self.group_column] ==
label][analyze_column]
            self.descriptive_stats.append([f'{label}
{analyze_column}', 'Mean', data.mean()])
            self.descriptive_stats.append([f'{label}
{analyze_column}', 'Std', data.std()])

    def analyze(self):
        """
        Perform the data analysis.

        Reads the data, analyzes within and between groups,
and saves the results.
        """
        self.read_data()
        self.statistics = []
        self.descriptive_stats = []

        # Continuous variable
        for idx, column in enumerate(['0w', '8w', '12w',
'd(12-0)', '%']):
            if idx == 0:
                self._analyze_within_group()
            self._analyze_between_groups(column)
            self._add_descriptive_statistics(column)

        self.save_statistics('statistics.csv',
self.statistics)
        self.save_statistics('descriptive_statistics.csv',
self.descriptive_stats)
        print('')

    def save_statistics(self, filename, data):
        """
        Save the statistical results to a CSV file.

        Args:
            filename (str): The name of the file to save the
results to.
            data (list): The statistical results to save.
        """
        def format_number(item):
            if isinstance(item, (int, float)):
                try:
                    return
str(Decimal(str(item)).quantize(Decimal('0.001'),
rounding=ROUND_HALF_UP))
                except:
                    return str(item)
            elif pd.isna(item):
                return ''
            else:
```

```
                return item

        if 'descriptive' in filename:
            columns = ["Data", "Measure", "Value"]
        else:
            columns = ["Comparison", "Test", "Statistic",
"P-value", "Evaluation"]

        formatted_data = [[format_number(item) for item in
row] for row in data]

        df = pd.DataFrame(formatted_data, columns=columns)
        df.to_csv(self.results_sub_dir/filename,
index=False, encoding='utf-8-sig')

def main():
    # setting
    group_column = 'target'
    file_dir = Path('../data/検査結果_生物学検査_理学検査
_processed')

    # analyze
    file_paths = file_dir.glob('*.csv')
    results_dir = Path('../results') / f'{file_dir.name}_解
析結果_20240902'
    results_dir.mkdir(exist_ok=True, parents=True)

    if_physical_test = '理学検査' in file_dir.name
    if_blood_test = '血液学検査' in file_dir.name

    for file_path in file_paths:
        file_name = str(file_path)
        print(f'Processing file: {file_name}')
        analyzer = DataAnalyzer(file_name=file_name,
                                group_column=group_column,
                                results_dir=results_dir,

if_physical_test=if_physical_test,

if_blood_test=if_blood_test)
        analyzer.analyze()
if __name__ == "__main__":
    main()
```