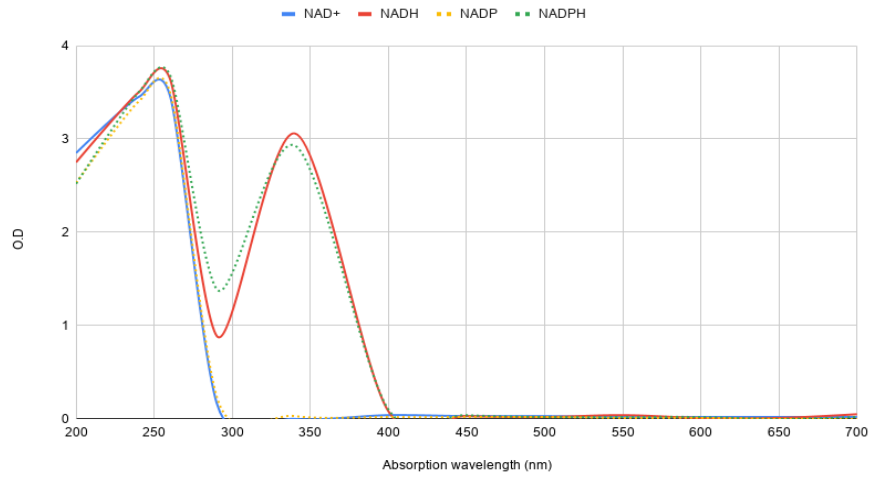
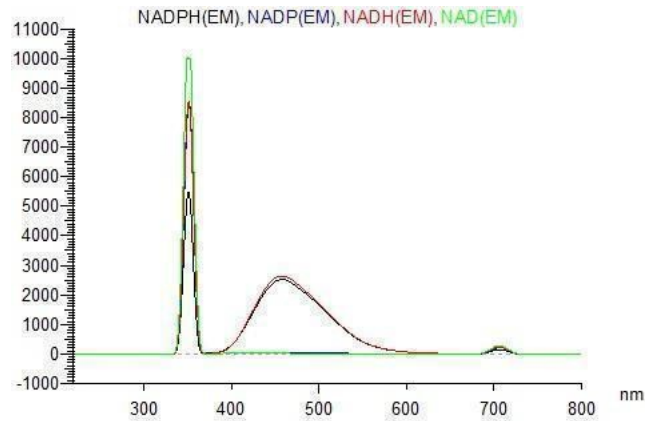


Appendix 1: Supplementary files for chapter 1 and 2

a

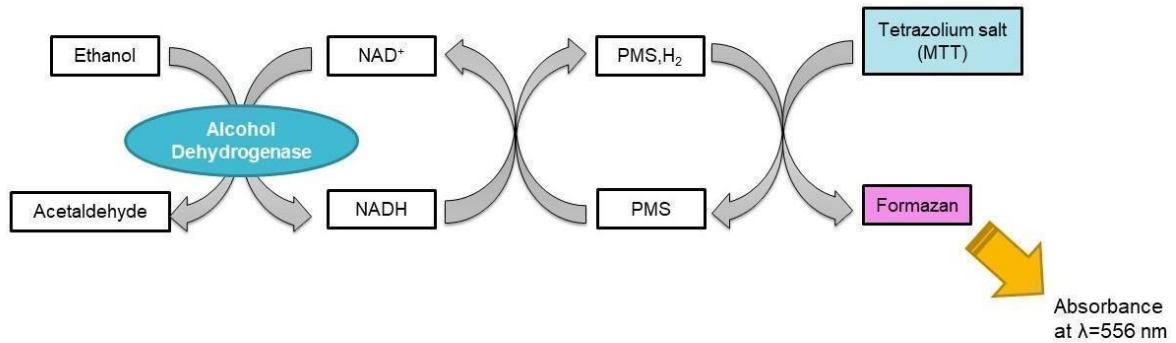


b



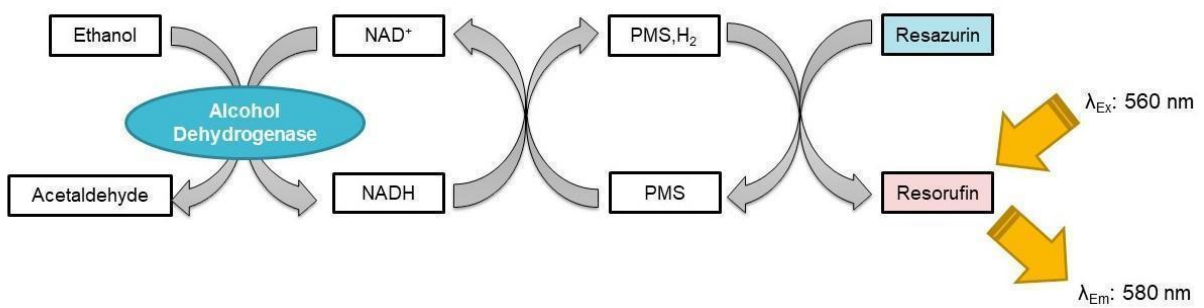
Supplementary figure 1: NAD(H) and NADP(H) absorption and fluorescence spectra. a: NAD⁺, NADH, NADP, NADPH absorption spectra showing the second absorption peak at 340 nm for NADH and NADPH. b: NAD⁺, NADH, NADP, NADPH fluorescence emission spectra resulting from excitation at 350 nm. NADH and NADPH display an emission peak between 450 and 500 nm.

a



Example of an enzymatic colorimetric reaction for NAD quantification: NAD⁺ is reduced into NADH by the alcohol dehydrogenase enzyme. The NADH reduces a tetrazolium salt like MTT, via phenazine methosulfate, to produce the corresponding formazan. The rate of MTT reduction is proportional to the amount of NADH in the solution which can be measured by the absorbance of the purple-colored formazan at 556 nm. PMS: Phenazine Methosulfate. MTT: Methylthiazolyldiphenyl-tetrazolium bromide.

b



Example of an enzymatic fluorometric reaction for NAD quantification: NAD⁺ is reduced into NADH by the alcohol dehydrogenase enzyme. The NADH reduces phenazine methosulfate which in turn reduces resazurin to its fluorescent reduced form resorufin. The fluorescence of resorufin (Ex: 560 nm/Em:580 nm) is proportional to the amount of NADH in the solution. PMS: Phenazine Methosulfate. λ_{Ex}: Fluorescence excitation wavelength. λ_{Em}: Fluorescence emission wavelength.

Supplementary figure 2: Examples of enzyme cycling assays for NAD(H) measurement.

Examples of enzymatic colorimetric (a) and fluorometric (b) assays sharing the first two reactions: Reduction of NAD⁺ by the alcohol dehydrogenase enzyme followed by the reduction of phenazine methosulfate, which is used for the last step for the colorimetric (a) or fluorometric (b) detection.

Supplementary table 1: Medline (Ovid) tissue-focused search query.

1	NAD/
2	nad.ti,ab,kw.
3	Nicotinamide adenine dinucleotide.ti,ab,kw.
4	1 or 2 or 3
5	Exp mammals/
6	(mouse? or mice? or rat? or murine? or pig? or dog? or primate? or ape? or cat?).ti,ab,kw.
7	(human? or homo sapiens or patient).ti,ab,kw.
8	5 or 6 or 7
9	(sampl* adj3 (protocol* or standard* or method*)).ti,ab,kw.
10	(tissue* adj3 (sampl* or collect*)).ti,ab,kw.
11	((animal* or human) adj3 (sampl* or tissue?)).ti,ab,kw.
12	((muscle* or liver* or hepatic* or brain* or neural* or neuron* or kidney* or renal* or fat* or adipose) adj3 (cell* or tissue* or sample*)).ti,ab,kw.
13	9 or 10 or 11 or 12
14	4 and 8 and 13
15	Limit 14 to (english or french)

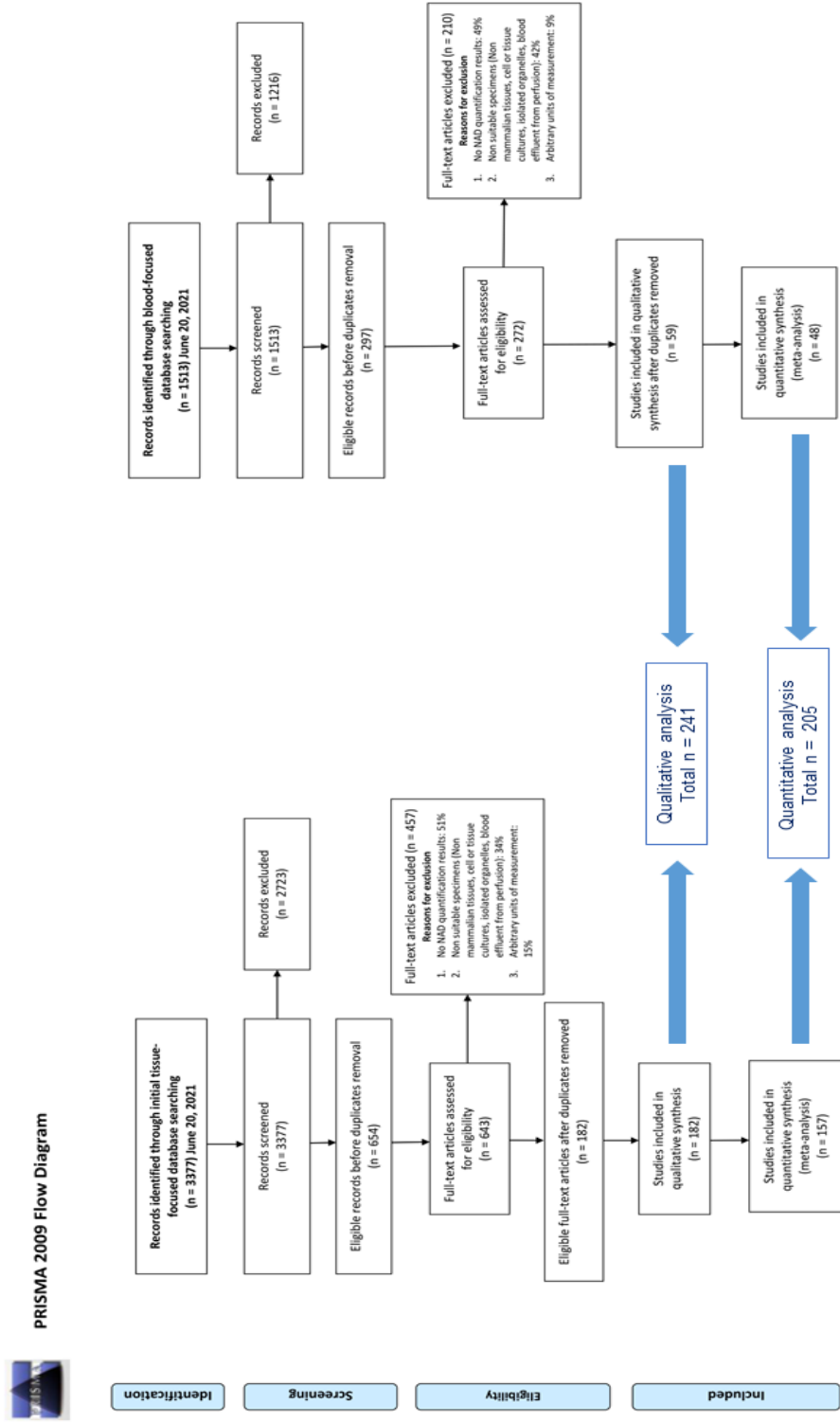
/: subject heading, ab: abstract, ti: title, kw: keyword heading (Author keywords), exp: search for the term and any narrower subject term, ?: optional wildcard (keyword substitutions), *: focus on the term as main topic, adj3: adjacency, in any order, up to 3 words in between.

Supplementary table 2: Medline (Ovid) search query focused on blood related samples.

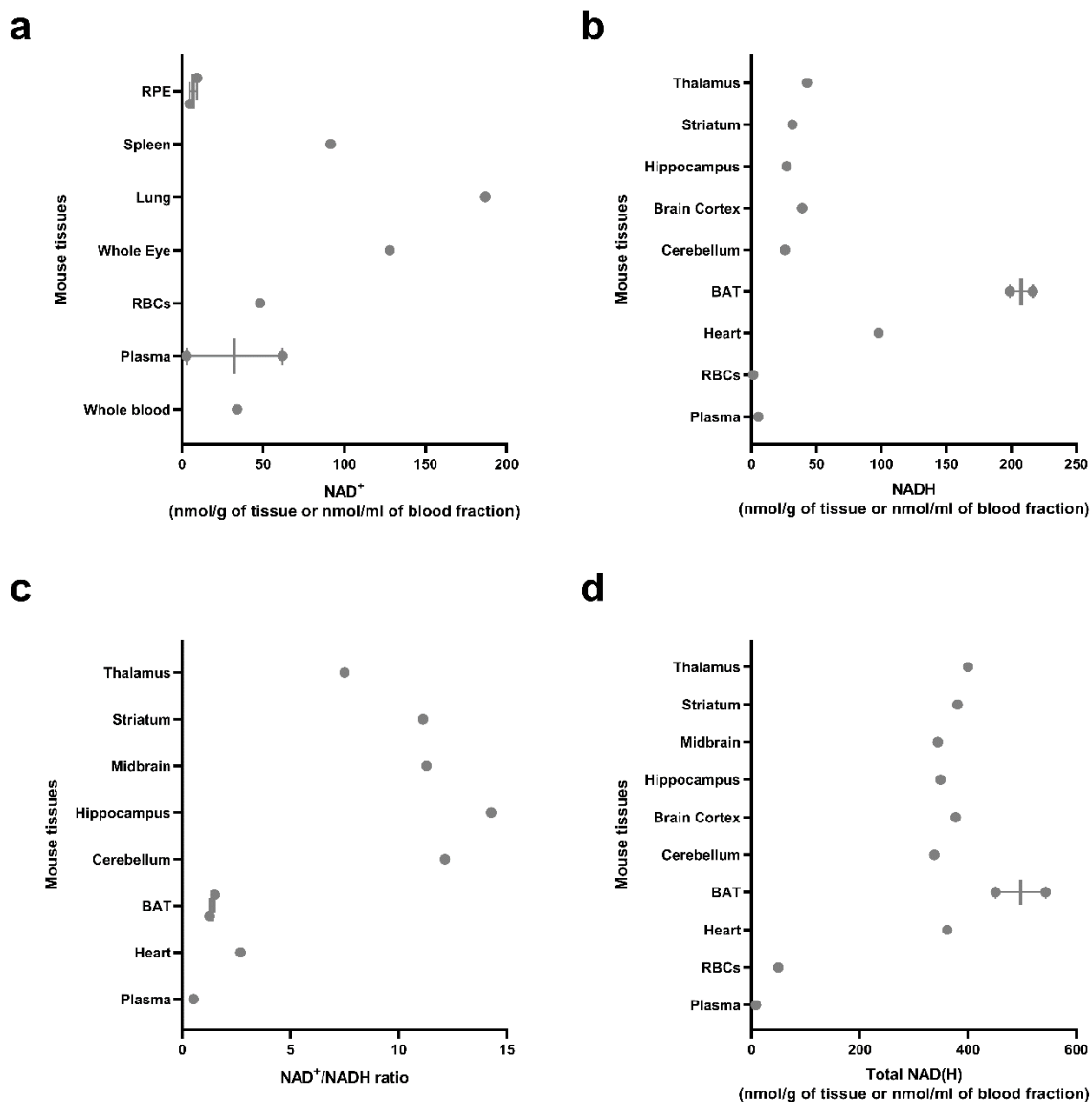
1	NAD/
2	nad.ti,ab,kw.
3	Nicotinamide adenine dinucleotide.ti,ab,kw.
4	1 or 2 or 3
5	Exp mammals/
6	(mouse? or mice? or rat? or murine? or pig? or dog? or primate? or ape? or cat?).ti,ab,kw/
7	(human? or homo sapiens? or patient?).ti,ab,kw.
8	5 or 6 or 7
9	(sampl* adj3 (protocol* or standard* or method*)).ti,ab,kw.
10	(tissue* adj3 (sampl* or collect*)).ti,ab,kw.
11	((animal* or human* or patient*) adj3 (sampl* or tissue?)).ti,ab,kw.
12	((Blood* or plasma* or serum* or PBMC* or PRBC* or Platelets* or lymphocytes* or leucocytes* or hematocytes*) adj3 (cell* or sample*)).ti,ab,kw.
13	9 or 10 or 11 or 12
14	4 and 8 and 13
15	Limit 14 to (english or french)

/: subject heading, ab: abstract, ti: title, kw: keyword heading (Author keywords), exp: search for the term and any narrower subject term, ?: optional wildcard (keyword substitutions), *: focus on the term as main topic , adj3: adjacency, in any order, up to 3 words in between.

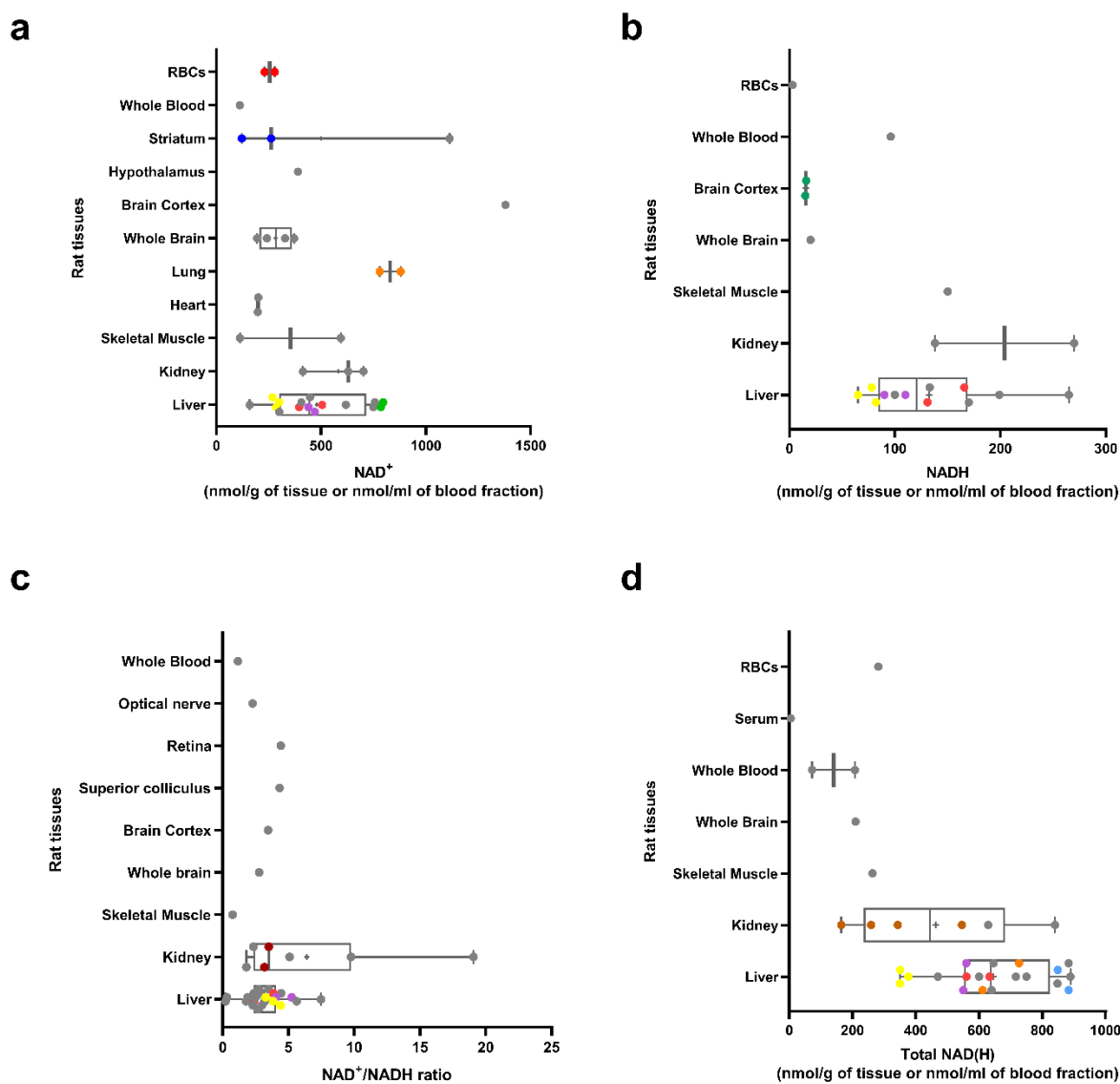
Supplementary figure 3: Systematic search workflow



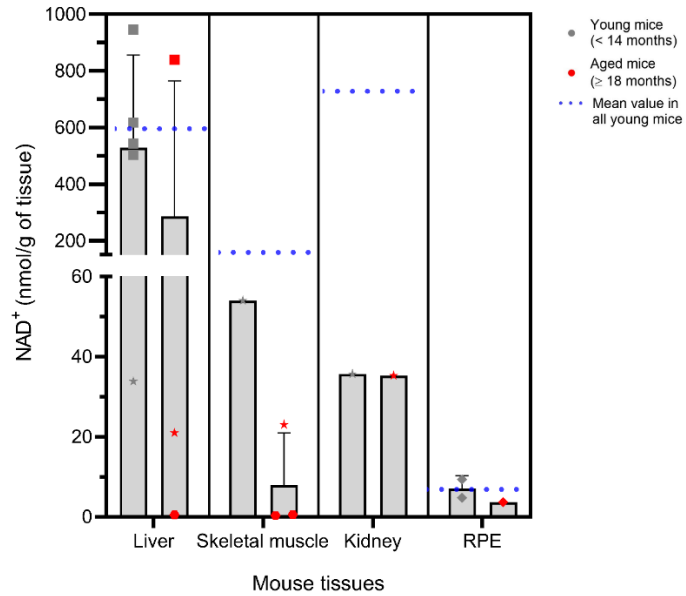
From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009), Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *PLoS Med* 6(7): e1000097. doi:10.1371/journal.pmed1000097
 For more information, visit www.prisma-statement.org.



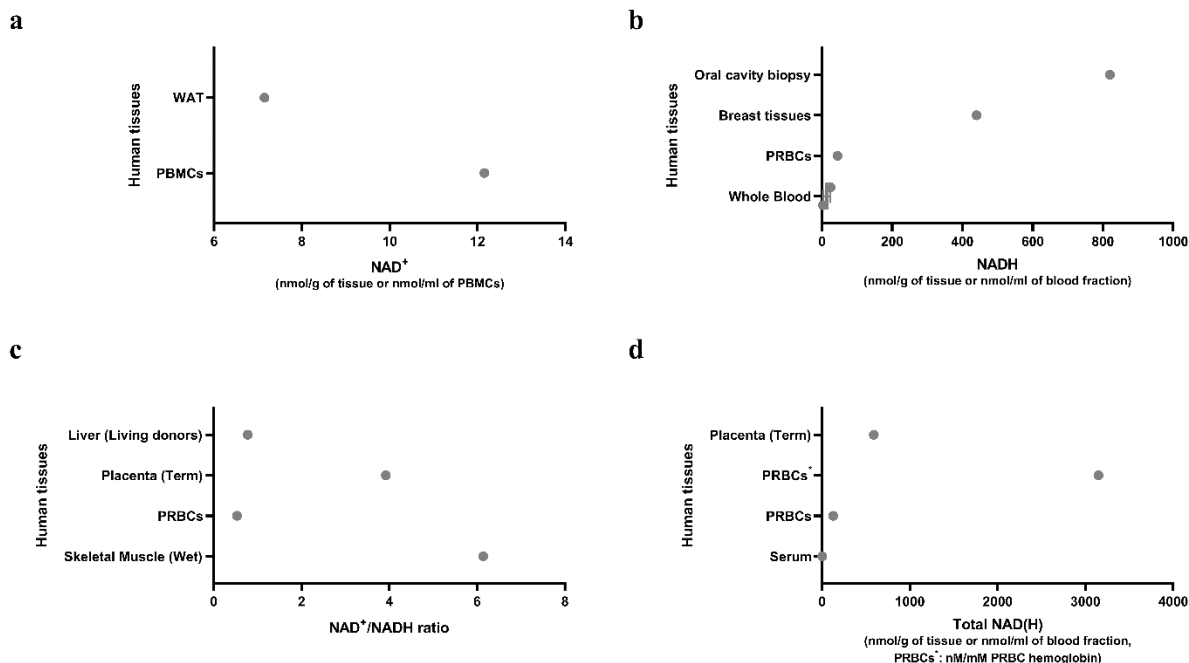
Supplementary figure 4: Reported physiological NAD⁺, NADH, total NAD(H) and NAD⁺/NADH ratio in mouse tissues with n<3. a-d: Reported mean (a) NAD⁺, (b) NADH, (c) NAD⁺/NADH levels and (d) total NAD(H) in various mouse tissues collected from young control mice (<14 months old). BAT: Brown adipose tissue, RBCs: Red blood cells, RPE: Retinal pigmented epithelium.



Supplementary figure 5: Observed physiological NAD⁺, NADH, total NAD(H) and NAD⁺/NADH ratio in rat tissues. Reported mean physiological (a) NAD⁺ and (b) NADH levels, (c) NAD⁺/NADH ratios and (d) total NAD(H) in various rat tissues collected from young control rats (<18 months old). The boxes represent the 25th to 75th percentiles with the median represented by the line inside the box. The mean values are shown as “+”. The whiskers cover the minimum to maximum values. For each study including more than one control group, similar colors were assigned to the corresponding datapoints. RBCs: Red blood cells.



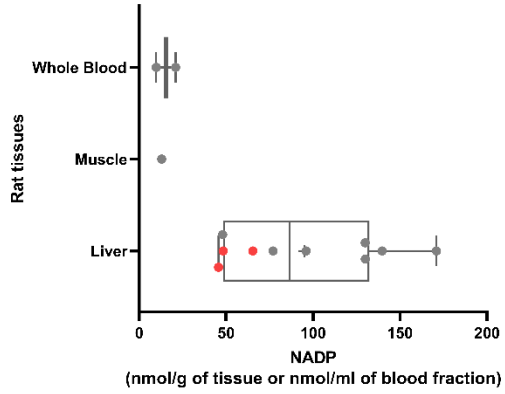
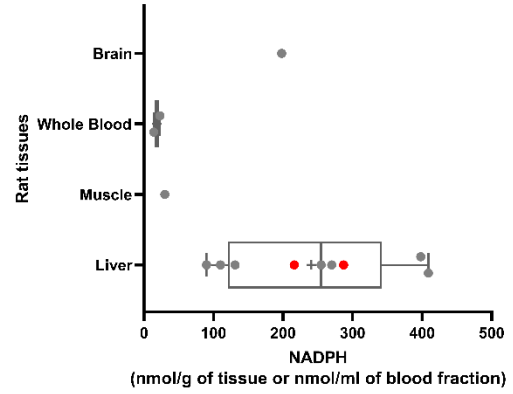
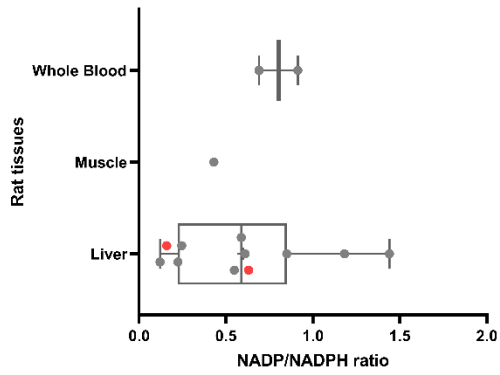
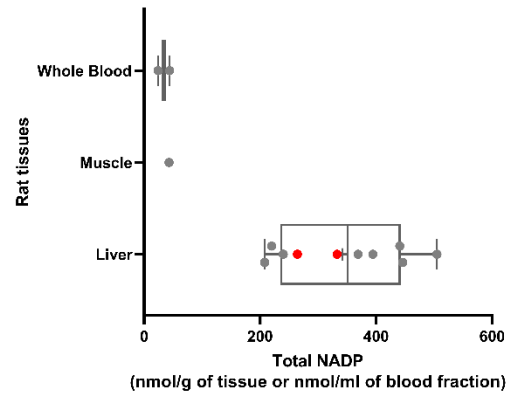
Supplementary figure 6: Mean physiological NAD⁺ levels in young mice (<14 months) compared to the corresponding aged mice (18 months or older) from the same studies. Each study is represented by a specific datapoint shape. The mean physiological NAD⁺, NADH, total NAD, and the NAD⁺/NADH ratio in all young mice data is represented by the dotted lines.



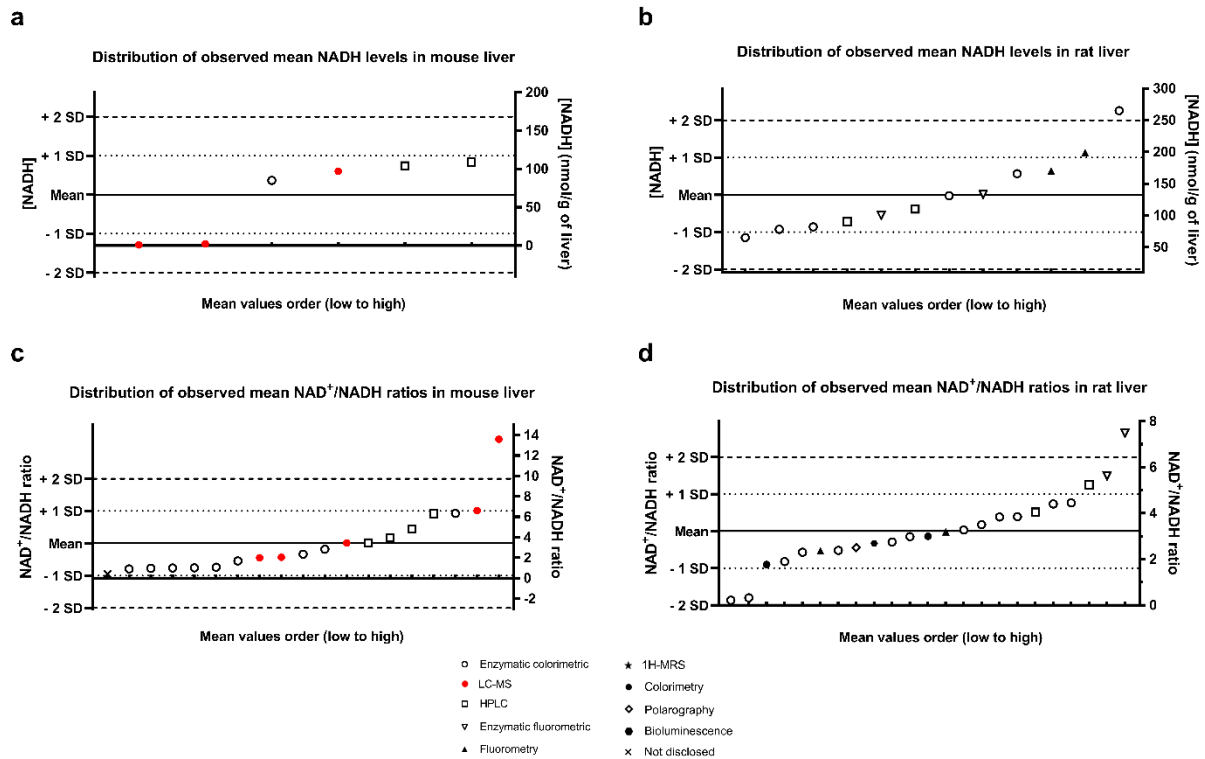
Supplementary figure 7: Reported physiological NAD⁺, NADH, NAD⁺/NADH ratio, and total NAD(H) in human tissues with n<3. a-d: Reported mean (a) NAD⁺, (b) NADH, (c) NAD⁺/NADH and (d) total NAD(H) levels in various human tissues. PBMCs: Peripheral blood mononuclear cells, PRBCs: Packed red blood cells, RBCs: Red blood cells.

Supplementary table 3: Mean physiological NAD(H) levels measured in human samples. All results are expressed as mean +/- S.D. n = number of control groups.

Human samples	NAD ⁺ (nmol/g of tissue or nmol/ml of blood)	NADH (nmol/g of tissue or nmol/ml of blood)	Total NAD(H) (nmol/g of tissue or nmol/ml of blood)	NAD ⁺ /NADH ratio
Whole blood	44.62 +/- 18.69 (n=23)	13.49 +/- 15.29 (n=2)	28.94 +/- 16.16 (n=6)	9.066 +/- 9.405 (n=3)
Plasma	0.3718 +/- 0.392 (n=8)	0.387 +/- 0.429 (n=8)	60.23 +/- 178.1 (n=9)	1.570 +/- 1.031 (n=7)
RBCs	46.96 +/- 9.947 (n=7)	1.75 +/- 0.957 (n=7)	49.66 +/- 9.88 (n=9)	23.65 +/- 12.94 (n=8)
PRBCs	79.80 +/- 34.14 (n=3)	44.4 (n=1)	127.8 nmol/ml PRBCs (n=1), 3149 nmol/mmol PRBCs hemoglobin)	0.53 (n=1)
PBMCs	12.16 (n=1)	/		/
Serum	/	/	2.327 (n=1)	/
Skeletal Muscle (Wet)	191.9 +/- 18.62 (n=4)	121.8 +/- 65.51 (n=4)	620.8 +/- 388.5 (n=4)	6.137 (n=1)
Skeletal Muscle (Freeze-Dried)	1713 +/- 150.4 (n=3)	136.8 +/- 42.89 (n=6)	1853 +/- 180.2 (n=3)	12.7 +/- 2.338 (n=3)
Placenta (term)	/	/	591 (n=1)	3.92 (n=1)
WAT	7.15 (n=1)		/	/
Breast tissues	/	440 (n=1)	/	/
Oral cavity biopsy	/	820 (n=1)	/	/
Liver (from living healthy donors)	/	/	/	0.775 (n=1)

a**b****c****d**

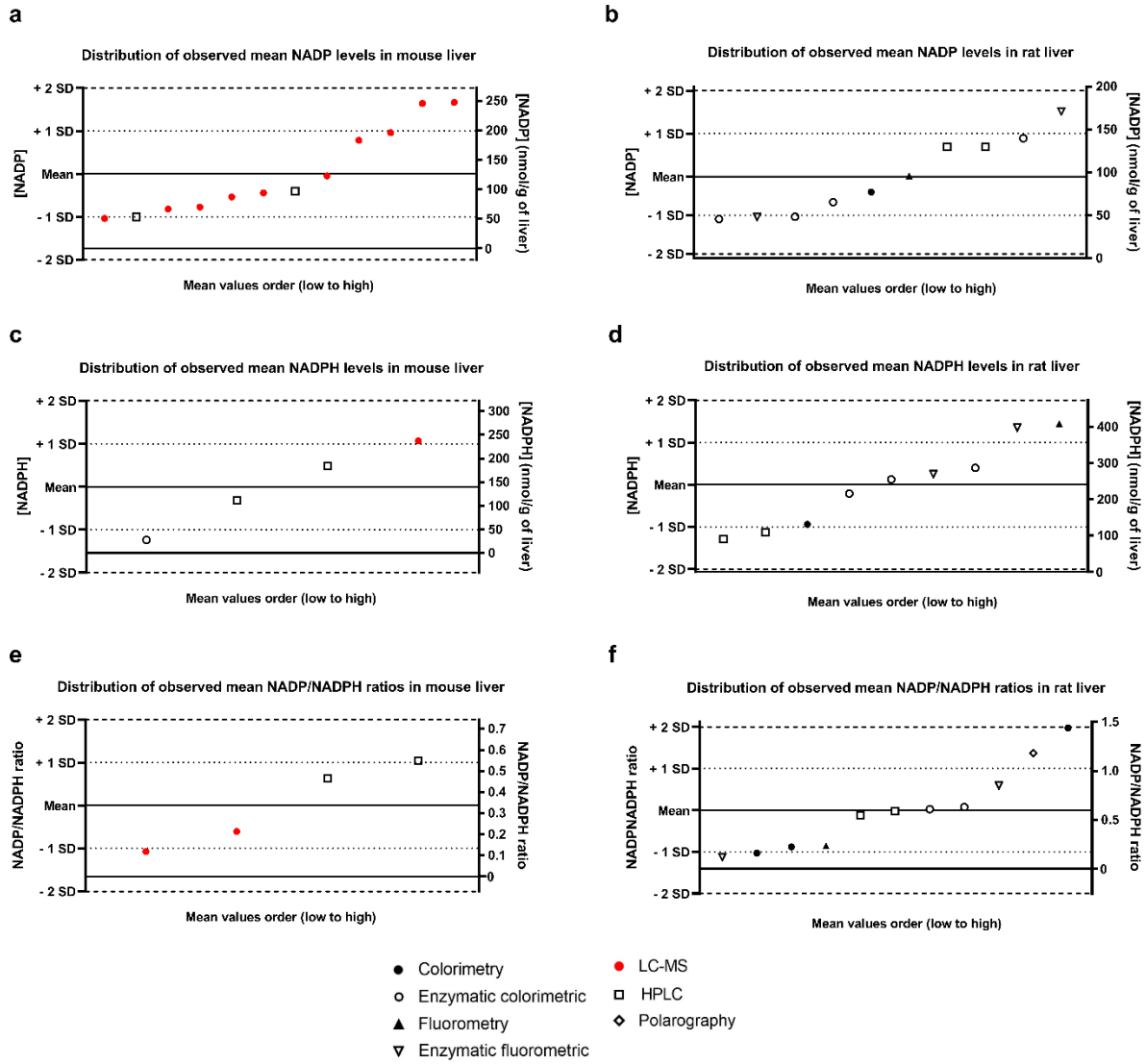
Supplementary figure 8: Reported physiological (a) NADP and (b) NADPH levels, (c) NADP/NADPH ratio, and (d) total NADP(H) in various rat tissues. Data represents results obtained from young control rats (<18 months old). The boxes represent the 25th to 75th percentiles with the median represented by the line inside the box. The whiskers cover the minimum to maximum values. For each study including more than one control group, similar colors were assigned to the corresponding datapoints.



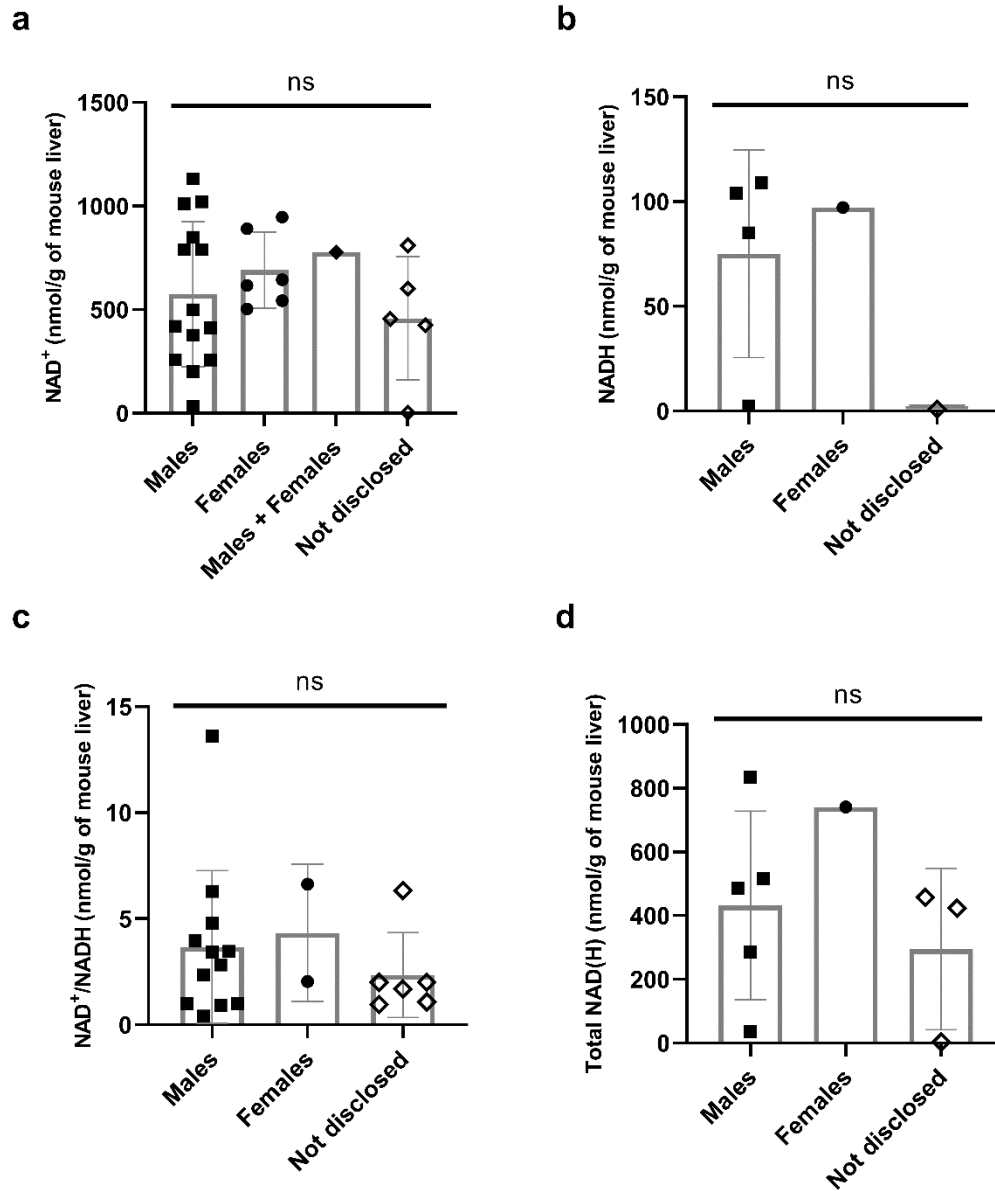
Supplementary figure 9: a,b: Distribution of mean NADH values measured using different quantification methods in (a) mouse liver with mean \pm S.D = 66.4 \pm 50.8 nmol/g of tissue (CV=76.5%) and (b) rat liver with mean \pm S.D = 132.4 \pm 58.7 nmol/g of tissue (CV=44.4%). c,d: Distribution of mean NAD⁺/NADH ratios measured using different quantification methods in (c) mouse liver with mean \pm S.D = 3.41 \pm 3.16 (CV=92.7%) and (d) rat liver with mean \pm S.D = 3.219 \pm 1.612 (CV=50.1%). Tissues were collected from young control animals: <14 months for mice and <18 months for rats.

Supplementary table 4: NAD(H) levels (nmol/g of wet tissue weight) observed in control rats and mice liver samples. All results are expressed as mean +/- S.D. n = number of studied groups. Results from old animals (Older than 14 months for mice and 18 months for rats) and from animals receiving high fat diets were excluded.

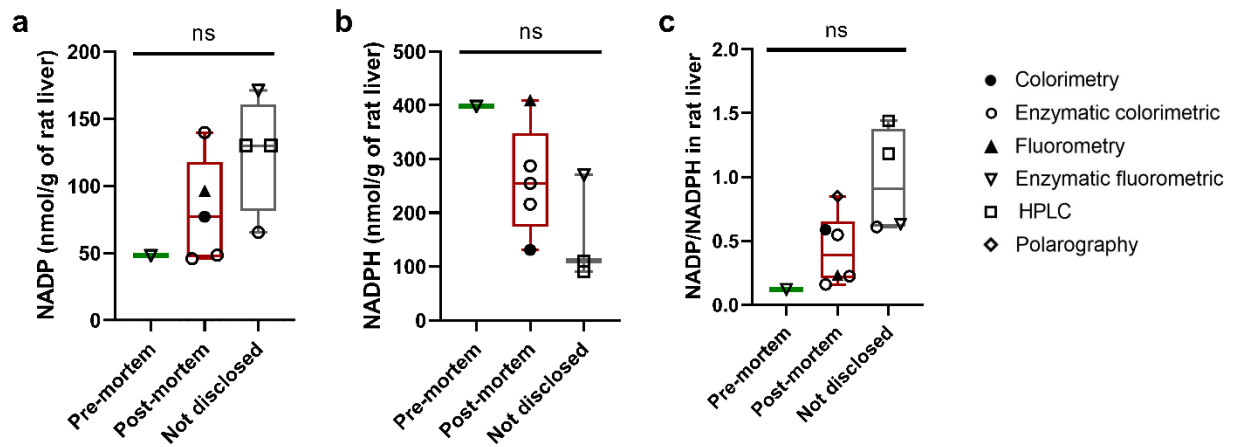
Species	Rat				Mouse			
Quantification method	NAD ⁺	NADH	Total NAD(H)	NAD ⁺ /NADH ratio	NAD ⁺	NADH	Total NAD(H)	NAD ⁺ /NADH ratio
Enzymatic colorimetric	396.7 +/- 128.5 (n=7)	131.1 +/- 75.8 (n=6)	627.7 +/- 214.9 (n=10)	2.779 +/- 1.363 (n=13)	690.9 +/- 330.8 (n=9)	85.09 (n=1)	389.1 +/- 91.33 (n=3)	2.016 +/- 1.758 (n=9)
LC-MS	/	/	/	/	556.9 +/- 342.9 (n=12)	33.5 +/- 55.17 (n=3)	389.3 +/- 427.5 (n=4)	5.54 +/- 4.88 (n=5)
HPLC	622.7 +/-194.1 (n=4)	100.0 +/- 14.1 (n=2)	612.0 +/- 80.8 (n=4)	4.64 +/- 0.8 (n=2)	394.5 +/- 24.75 (n=2)	106.5 +/- 3.536 (n=2)	612.3 +/- 193.4 (n=3)	4.630 +/- 1.243 (n=4)
Enzymatic fluorimetric	752.0 +/- 5.6 (n=2)	116.5 +/- 23.3 (n=2)	868.5 +/- 29.0 (n=2)	6.55 +/- 1.3 (n=2)	849.8 (n=1)	/	/	/
Colorimetry	300.0 (n=1)	170.0 (n=1)	470.0 (n=1)	2.38 +/- 0.86 (n=2)	/	/	/	/
Fluorimetry	447.0 (n=1)	199.0 (n=1)	363.5 +/- 19.1 (n=2)	2.78 +/- 0.58 (n=2)	/	/	/	/
Bioluminescence	/	/	/	2.690 (n=1)	499.3 (n=1)	/	/	/
Polarography	/	/	/	2.500 (n=1)	/	/	/	/
1H-MRS	/	/	/	/	456.0 (n=1)	/	/	/
Not disclosed	159.0 (n=1)	/	600.0 (n=1)	/	/	/	/	0.41 (n=1)
All methods	479.9 +/- 204.4 (n=16)	132.4 +/- 58.7 (n=12)	644.7 +/- 174.9 (n=20)	3.219 +/- 1.612 (n=23)	596.0 +/- 312.6 (n=26)	66.43 +/- 50.82 (n=6)	456.1 +/- 287.6 (n=10)	3.41 +/- 3.16 (n=19)



Supplementary figure 10: Distribution histogram of reported physiological NADP(H) levels in mouse and rat liver. Mean NADP (a,b), NADPH (c,d), and NADP/NADPH (e,f) values measured in mouse and rat liver respectively using different quantification methods. Values are sorted from lowest to highest value (nmol/g of tissue weight). Tissues were collected from young control animals: <14 months for mice and <18 months old for rats.



Supplementary figure 11: Sex differences in NAD(H) metabolites levels in mouse liver. (a) NAD⁺, (b) NADH, (c) NAD⁺/NADH ratio, and (d) total NAD(H). Results from mice older than 14 months were excluded. Statistical significance was determined by one-way ANOVA with multiple comparisons using Tukey's test as post-hoc test. Error bars represent standard deviation.



Supplementary figure 12: Effects of pre- versus post-mortem tissue collection on NADP(H) levels in rat liver. a-c: Reported (a) NADP, (b) NADPH, (c) NADP/NADPH ratio in young (<18 months old) control rat liver samples harvested at different timepoints relative to sacrifice. Boxes represent 25th and 75th percentile with median line. Whiskers show min. to max. values. Statistical significance was determined by one-way ANOVA with multiple comparisons using Tukey's test as post-hoc test.