SUPPLEMENTARY

PROCEDURES

Randomisation

As patients became eligible for randomization, randomization codes were assigned strictly sequentially to assign each patient to a specific treatment sequence (dapagliflozin:placebo or placebo:dapagliflozin). The randomization schedule assigned sequences using blocks of equal and fixed size, with blocks permuted and sequence randomized. An external partner (PAREXEL Ltd) provided the initial system randomization code, dummy list generation and emergency code break support.

Table S1: Inclusion and exclusion criteria of the trial

Inclusion criteria	
1	Patients were able to provide signed and dated written informed consent prior to any study specific procedures.
2	Women were post-menopausal (defined as at least 1 year post cessation of menses) and aged ≥45 and ≤70 years. Males were aged ≥40 years and ≤70 years. Patients were to have suitable veins for cannulation or repeated venipuncture
3	Patients diagnosed with T2DM for at least the previous 6 months, based on American Diabetes Association 2016 standards
4	Patients were on no other anti-diabetic drug treatment, or on stable maximum 3000 mg daily dose metformin treatment and/or on stable dose of a DPPIV inhibitor treatment for at least the prior 3 months
5	HbA1c levels ≥6.0% (42 mmol/mol) and ≤9.0% (75 mmol/mol).
6	Body mass index (BMI) ≤38 kg/m^2
Exclusion criteria	
1	Involvement in the planning and conduct of the study (applicable to both AstraZeneca staff and staff at third party vendor or at the investigational sites).
2	Previous enrolment in the present study or participation in another clinical study with an investigational product (IP) during the previous 3 months or as judged by the Investigator
3	History of or presence of any clinically significant disease or disorder including a recent (<3 months) cardiovascular event which, in the opinion of the Investigator, may have either put the patient at risk because of participation in the study or influence the results or the patient's ability to participate in the study.

4	Clinical diagnosis of Type 1 diabetes, maturity onset diabetes of the young, secondary diabetes, or diabetes insipidus.
5	Unstable/rapidly progressing renal disease or estimated glomerular filtration rate <60 mL/min (Cockcroft-Gault formula). Males: $\text{Creatinine clearance (mL/min)} = \frac{\text{Weight (kg)} \times (140 - \text{Age})}{\text{Serum creatinine (µmol/L)}} \times 1.23$ Females: $\text{Creatinine clearance (mL/min)} = \frac{\text{Weight (kg)} \times (140 - \text{Age})}{\text{Serum creatinine (µmol/L)}} \times 1.04$
6	Clinically significant out of range values of serum levels of either alanine aminotransferase (ALT), aspartate aminotransferase (AST) or alkaline phosphatase (ALP) in the Investigator's opinion.
7	Contraindications to dapagliflozin according to the local label.
8	Use of antidiabetic drugs other than metformin or DPPIV inhibitor treatment within 3 months prior to screening.
9	Weight gain or loss >5 kg in the previous 3 months, ongoing weight loss diet (hypocaloric diet) or use of weight loss agents
10	History of drug abuse or alcohol abuse in the previous 12 months. Alcohol abuse is defined as >14 drinks per week for women and >21 drinks per week for men (1 drink = 35 cL beer, 14 cL wine, or 4 cL hard liquor), or as judged by the Investigator.
11	Any clinically significant abnormalities in clinical chemistry, hematology, or urinalysis or other condition the Investigator believes would interfere with the patient's ability to provide informed consent, comply with study instructions, or which might confound the interpretation of the study results or put the patient at undue risk.
12	Plasma donation within 1 month of screening or any blood donation/blood loss >500 mL within 3 months prior to screening or during the study.
13	Anemia defined as hemoglobin (Hb) <115 g/L (7.1 mM) in women and <120 g/L (7.5 mM) in men.
14	Use of anti-coagulant treatment such as heparin, warfarin, platelet inhibitors, thrombin, and factor X inhibitors.

15	Use of medication such as oral glucocorticoids, anti-estrogens, or other medications that are known to markedly influence insulin sensitivity.
16	Use of loop diuretics.
17	Regular smoking and other regular nicotine use.
18	Any contraindication to magnetic resonance imaging scanning. These contraindications included patients with following devices: – Central nervous system aneurysm clip – Implanted neural stimulator – Implanted cardiac pacemaker or defibrillator – Cochlear implant – Metal containing corpora aliena in the eye or brain
19	Patients who did not want to be informed about unexpected medical findings, or did not wish that their physician be informed about coincidental findings, could not participate in the study.

Blood sampling scheme in respiration chamber

During the stay in the respiration chamber, blood samples were drawn at seven timepoints and used for measuring insulin, glucagon, glucose, NEFA, β-hydroxybutyrate and FGF21. The exact timing of the blood sampling is provided in table S2.

Table S2: Timing of blood draws during the metabolic chamber measurements

Timepoint	
08:30 AM	Immediately before breakfast
10:30 AM	2 hr after breakfast
01:00 PM	Immediately before lunch
03:00 PM	2 hr after lunch
06:00 PM	Immediately before dinner
08:00 PM	2 hr after dinner
10:00 PM	4 hr after dinner

IHL determination

After an overnight fast in the respiration chamber, proton magnetic resonance spectroscopy (MRS) was used to quantify intrahepatic lipid (IHL) content. Measurements were performed on a 3.0-Tesla whole body MR system (Achieva 3Tx; Philips Healthcare), as previously described (1), however with a voxel size of 20x20x20 mm. IHL concentrations were expressed as ratios of the CH2 peak relative to the unsuppressed water resonance (as percentage). T2 relaxation times of 59.10ms for methylene peaks and 26.30ms for water were used.

Biochemical analysis

Lactate (Roche, Basel, Switserland), glycerol (Sigma, Saint Louis, Missouri, USA), NEFA (WAKO, Neuss, Germany) and β-hydroxybutyrate (Stanbio Laboratories) were analyzed enzymatically in serum samples using a Pentra 400 (Horiba). Insulin (Beckman Coulter Inc.) and FGF21 (&D Systems inc.) were measured in EDTA plasma samples using immunoenzymatic assays. Glucagon (Millipore Corporation /LINCO Research) was measured in EDTA plasma samples using a radioimmunoassay. Urinary nitrogen was measured with an enzymatic colorimetric assay. Uric acid was measured in serum using enzymatic analysis using COBAS (Roche diagnostics, Indianapolis, USA).HbA1c was determined by HPLC (Bio-rad, Hercules, CA, USA). High-sensitivity C-reactive protein was measured by immunonephelometry using the Siemens BNII Nephelometer (Siemens Healthcare diagnostics, Deerfield, USA).

Calculations

Cockcroft-Gault formula was used to calculate eGFR, see table S1 for the formula.

Power calculation

The power of the study was based on an anticipated effect of insulin sensitivity measured as rate of glucose disposal (Rd) corrected for glucose losses. Based on Mudaliar et. al. 2014 (2), it was assumed that Rd in subjects treated with dapagliflozin 10 mg is 17.5% greater than in participants treated with placebo (2). Earlier studies in our lab show an average Rd of 18.8 µmol/kg/min and an intra-individual standard variation of 3.53 µmol/kg/min in Rd in patients with T2DM. Inter and intra-individual variability was assumed to be equal. nQuery software calculated an estimated 22 subjects, within a two-group cross-over design, which provides approximately 80% statistical power, when using a treatment difference of 3.29 µmol/kg/min

(17.5% of 18.8 µmol/kg/min) in Rd, with a two-sided alpha level of 0.05. Considering a 15% drop-out rate, a total of 26 subjects (13 subjects per sequence) were randomized. The primary outcome was defined as the difference in Rd between basal and high-rate insulin infusion.

RESULTS

Figure S1: Study charter.

REFERENCES

1. Lindeboom L, Nabuurs CI, Hesselink MK, Wildberger JE, Schrauwen P, Schrauwen-Hinderling VB. Proton magnetic resonance spectroscopy reveals increased hepatic lipid content after a single high-fat meal with no additional modulation by added protein. Am J Clin Nutr. 2015;101(1):65-71.
2. Mudaliar S, Henry RR, Boden G, Smith S, Chalamandaris AG, Duchesne D, et al. Changes in insulin sensitivity and insulin secretion with the sodium glucose cotransporter 2 inhibitor dapagliflozin. Diabetes Technol Ther. 2014;16(3):137-44.

TABLES

Table S3: Subject characteristics

Characteristic	**Total (n=24)**
Age, years (mean ± SD)	64.2 ± 4.6
Sex, n (male/female)	19/5
Body mass index, kg/m^2 (mean ± SD)	28.1 ± 2.4
HbA1c, mmol/mol (mean ± SD) / % (mean ± SD)	51.7 ± 6.8 / 6.9 ± 0.6
eGFR, ml/min (mean ± SD)	141 ± 13.0
Duration of diabetes, years, (median range))	8.0 (1-15)
Metformin use, (%)	71 (17/24)
Any diabetes complications, (n, yes / no)	1/23

Table S4: Results of change in blood pressure measured after 2 weeks (visit 3/6) and end-of-treatment. Parameters are expressed in least square (LS) means and 95% CI.

	Dapagliflozin Lsmean 95% CI		Placebo Lsmean 95% CI		p-value
Systolic blood pressure change from baseline to week 2 of treatment, mmHg	-5.113	(-9.632, -0.594)	1.653	(-2.866, 6.172	P=0.015
Diastolic blood pressure change from baseline to week 2 of treatment, mmHg	-2.681	(-5.317, -0.045)	-0.832	(-3.468, 1.804)	P=0.33
Systolic blood pressure change from baseline to week 5 of treatment, mmHg	-8.319	(-13.152, -3.487)	-4.735	(-9.568, 0.097)	P=0.28
Diastolic blood pressure change from baseline to week 5 of treatment, mmHg	-3.318	(-6.145, -0.491)	-2.667	(-5.494, 0.160)	P=0.74

Table S5: Blood parameters measured during the stay in respiration chamber. AUC= area under curve. Parameters are expressed in least square (LS) means and 95% CI.

	Dapagliflozin LS mean (95% CI)		Placebo LS mean 95% CI		p-value
hsCRP, mg/L	1.389	(0.739, 2.040)	1.166	(0.516, 1.817)	P=0.50
Urate, µmol/L	267.7	(241.4, 294.0)	324.5	(298.2, 350.7)	P<0.0001
Hemoglobin, mmol/L	8.96	(8.74, 9.18)	8.78	(8.55, 9.00)	P=0.029
Erythrocyte fraction, L/L	0.420	(0.410, 0.430)	0.416	(0.406, 0.426)	P=0.43
HbA1c, %	6.89	(6.55, 7.23)	6.96	(6.62, 7.30)	P=0.33
Glucose AUC, mmol/L/hr	114.157	(99.006, 129.308)	130.647	(115.496, 145.798)	P=0.0006
β-hydroxybutyrate AUC, mmol/L/hr	1.870	(1.610, 2.129)	1.580	(1.321, 1.840)	P=0.047
Free fatty acids AUC, mmol/L/hr	5.176	(4.528, 5.824)	3.917	(3.270, 4.565)	P=0.0026
Insulin ultrasensitive AUC, mIU/L/hr	368.931	(212.083, 525.779)	442.126	(285.278, 598.974)	P=0.29
Glucagon AUC, pmol/L/hr	324.973	(273.253, 376.693)	300.098	(248.379, 351.818)	P=0.039
FGF21 AUC, ng/L/hr	3310.415	(2626.919, 3993.911)	3554.716	(2871.220, 4238.212)	P=0.16
	mean	**SD**	**mean**	**SD**	
Fasting glucose levels, mmol/L	7.82	1.39	8.89	1.92	P<0.0001
Fasting NEFA levels, mmol/L	0.69	0.20	0.61	0.33	P=0.22
Fasting β-hydroxybutyrate, mmol/L	0.25	0.26	0.14	0.05	P=0.045

Table S6: Results of specific euglycemic hyperinsulinemic clamp measured parameters. Parameters are expressed in least square (LS) means and 95% CI.

	Dapagliflozin LS mean	95% CI	Placebo LS mean	95% CI	p-value
Glucose rate of disposal $_{\textbf{basal}}$, µmol/kg/min	10.610	(9.589, 11.630)	10.494	(9.474, 11.515)	P=0.85
Glucose rate of disposal $_{\textbf{low insulin}}$, µmol/kg/min	8.845	(8.209, 9.481)	9.392	(8.756, 10.028)	P=0.088
Glucose rate of disposal $_{\textbf{high insulin}}$, µmol/kg/min	19.133	(16.253, 22.014)	20.086	(17.206, 22.967)	P=0.33
Δ Glucose rate of disposal ($_{\textbf{high-basal}}$**)**, µmol/kg/min	8.523	(5.566, 11.481)	9.592	(6.634, 12.549)	P=0.30
Δ Glucose rate of disposal ($_{\textbf{high-basal/SSI}}$**)**	0.018	(0.011, 0.024)	0.020	(0.014, 0.027)	P=0.27
Glucose infusion rate, ml/hr	103.63	(86.57, 120.68)	96.96	(78.82, 115.09)	P=0.21
M-value, µmol/kg/min	18.00	(14.79, 21.20)	18.77	(15.32, 22.22)	P=0.44
Endogenous glucose production $_{\textbf{basal}}$, µmol/kg/min	12.427	(11.548, 13.306)	10.159	(9.280, 11.038)	P<0.0001
Endogenous glucose production $_{\textbf{low}}$, µmol/kg/min	7.771	(6.914, 8.629)	7.206	(6.349, 8.063)	P=0.25
Endogenous glucose production $_{\textbf{high}}$, µmol/kg/min	1.625	(1.079, 2.171)	1.647	(1.101, 2.193)	P=0.93
Δ Endogenous glucose production ($_{\textbf{low-basal}}$**)**, µmol/kg/min	-4.656	(-5.494, -3.817)	-2.951	(-3.790, -2.112	P=0.0036
Δ Endogenous glucose production ($_{\textbf{high-basal}}$**)**, µmol/kg/min	-10.803	(-11.726, -9.880)	-8.512	(-9.435, -7.589)	P<0.0001
Nonoxidative glucose disposal $_{\textbf{basal}}$, µmol/kg/min	6.836	(5.464, 8.209)	4.990	(3.617, 6.362)	P=0.012

Nonoxidative glucose disposal $_{low}$, µmol/kg/min	2.919	(1.666, 4.171)	1.503	(0.250, 2.755)	P=0.10
Nonoxidative glucose disposal $_{high}$, µmol/kg/min	8.592	(6.334, 10.850)	8.430	(6.172, 10.688)	P=0.87
Δ Nonoxidative glucose disposal ($_{high-basal}$**)**, µmol/kg/min	1.726	(-0.722, 4.174)	3.488	(1.039, 5.936)	P=0.13
Glucose excretion rate, µmol/kg/min	2.465	(2.191, 2.729)	0.004	(-0.270, 0.278)	P<0.0001
Respiratory exchange ratio $_{basal}$	0.756	(0.742, 0.769)	0.782	(0.768, 0.795)	P=0.0018
Respiratory exchange ratio $_{low}$	0.787	(0.769, 0.804)	0.816	(0.799, 0.834)	P=0.031
Respiratory exchange ratio $_{high}$	0.857	(0.839, 0.874)	0.874	(0.857, 0.891)	P=0.049
Δ Respiratory exchange ratio ($_{high-basal}$**)**	0.101	(0.080, 0.122)	0.089	(0.068, 0.110)	P=0.18
Carbohydrate oxidation$_{basal}$, µmol/kg/min	3.774	(2.843, 4.704)	5.504	(4.574, 6.435)	P=0.0016
Carbohydrate oxidation$_{low}$, µmol/kg/min	5.859	(4.616, 7.101)	7.888	(6.645, 9.131)	P=0.030
Carbohydrate oxidation$_{high}$, µmol/kg/min	10.687	(9.424, 11.950)	11.797	(10.534, 13.060)	P=0.071
Δ Carbohydrate oxidation$_{high-basal}$, µmol/kg/min	6.910	(5.472, 8.349)	6.139	(4.700, 7.577)	P=0.17
Fat oxidation$_{basal}$, µmol/kg/min	4.064	(3.807, 4.322)	3.535	(3.277, 3.792)	P=0.0022
Fat oxidation$_{low}$, µmol/kg/min	3.454	(3.169, 3.739)	2.957	(2.672, 3.242)	P=0.015
Fat oxidation$_{high}$, µmol/kg/min	2.309	(2.018, 2.599)	2.011	(1.720, 2.301)	P=0.055
Δfat oxidation$_{high-basal}$, µmol/kg/min	-1.783	(-2.148, -1.418)	-1.501	(-1.866, -1.137)	P=0.13

Insulin levels basal state, pmol/L	43.12	(34.73, 51.51)	61.29	(52.90, 69.68)	P<0.0001
Insulin levels low-insulin state, pmol/L	124.71	(107.91, 141.51)	145.43	(128.64, 162.23)	P=0.019
Insulin levels high-insulin state, pmol/L	507.93	(469.31, 546.54)	517.65	(479.04, 556.27)	P=0.46
NEFA levels basal, µmol/L	799.82	(667.75, 931.88)	590.94	(455.29, 726.59)	P=0.011
NEFA levels low-insulin state, µmol/L	421.16	(352.11, 490.20)	368.96	(299.91, 438.00)	P=0.076
NEFA levels high-insulin state, µmol/L	223.85	(148.82, 298.87)	277.06	(202.03, 352.08)	P=0.031
Suppression of NEFA low-insulin state, % suppression from basal	-39.67	(-58.92, -20.43)	-27.91	(-47.41, -8.41)	P=0.12
Suppression of NEFA high-insulin state, % suppression from basal	-70.84	(-85.80, -55.88)	-48.91	(-64.28, -33.55)	P=0.016
Glycerol levels basal, mmol/L	0.067	(0.054, 0.081)	0.046	(0.032, 0.059)	P=0.013
Glycerol levels low-insulin state, mmol/L	0.027	(0.019, 0.035)	0.036	(0.028, 0.044)	P=0.10
Glycerol levels high-insulin state, mmol/L	0.032	(0.025, 0.040)	0.039	(0.031, 0.047)	P=0.16
$\Delta glycerol_{low-basal}$, mmol/L	-0.040	(-0.056, -0.025)	-0.010	(-0.026, 0.006)	P=0.010
$\Delta glycerol_{high-basal}$, mmol/L	-0.036	(-0.050, -0.021)	-0.007	(-0.021, 0.008)	P=0.009
Lactate levels basal state, mmol/L	2.00	(1.80, 2.20)	2.13	(1.93, 2.33)	P=0.26
Lactate levels low-insulin state, mmol/L	1.70	(1.54, 1.87)	1.90	(1.73, 2.06)	P=0.032
Lactate levels high-insulin state, mmol/L	1.73	(1.61, 1.86)	1.84	(1.71, 1.96)	P=0.092

Table S7: Results of parameters measured during the stay in the respiration chamber. Parameters are expressed in least square (LS) means and 95% CI.

	dapagliflozin		Placebo		p-value
	LS mean	95% CI	LS mean	95% CI	
24h energy expenditure, MJ/day	9.519	(9.017, 10.020)	9.628	(9.126, 10.130)	P=0.11
Sleeping metabolic rate, MJ/day	6.571	(6.251, 6.891)	6.621	(6.302, 6.941)	P=0.36
Diet induced thermogenesis, MJ/day	1.279	(1.102, 1.456)	1.317	(1.140, 1.493)	P=0.64
24h respiratory exchange ratio	0.812	(0.803, 0.821)	0.835	(0.826, 0.844)	P=0.0001
Respiratory exchange ratio day time	0.817	(0.807, 0.827)	0.841	(0.831, 0.851)	P=0.0001
Respiratory exchange ratio night time	0.797	(0.786, 0.807)	0.830	(0.819, 0.840)	P<0.0001
Δrespiratory exchange ratio (day-night)	0.021	(0.013, 0.028)	0.011	(0.003, 0.019)	P=0.016
Protein oxidation 24h, g/day	75.096	(68.448, 81.720)	76.008	(69.360, 82.632)	p=0.77
Protein oxidation day time, g/day	82.272	(74.880, 89.664)	84.264	(76.872, 91.656)	P=0.55
Protein oxidation night time, g/day	61.464	(53.592, 69.312)	61.632	(53.784, 69.504)	P=0.96
Carbohydrate oxidation 24h, g/day	167.12	(1478.58, 185.65)	216.51	(197.98, 235.04)	P<0.0001
Carbohydrate oxidation daytime, g/day	204.09	(180.61, 228.56)	257.44	(233.97, 280.91)	P<0.0001

Carbohydrate oxidation nighttime, g/day	105.39	(90.01, 120.78)	153.08	(137.70, 168.47)	P<0.0001
Fat oxidation 24h, g/day	130.50	(119.08, 141.93)	110.80	(99.38, 122.23)	P<0.0001
Fat oxidation day time, g/day	142.09	(128.51, 155.68)	121.91	(108.32, 135.49)	P<0.0001
Fat oxidation night time, g/day	107.34	(97.77, 116.90)	85.29	(75.73, 94.86)	P<0.0001
Glucose excretion 24h, mmol/day	510.7	(434.8, 586.7)	40.7	(-35.2, 116.7)	P<0.0001
Glucose excretion daytime, mmol/hr	23.98	(20.41, 27.56)	2.15	(-1.42, 5.72)	P<0.0001
Glucose excretion nighttime, mmol/hr	10.99	(8.80, 13.19)	0.30	(-1.89, 2.50)	P<0.0001
Energy balance, MJ/day	0.47	(0.23, 0.71)	0.38	(0.13, 0.62)	P=0.34
Energy balance corrected for urinary glucose loss, MJ/day	-1.10	(-1.41, -0.78)	0.26	(-0.05, 0.57)	P<0.0001
Protein intake, g/day	86.02	(80.67, 91.38)	87.03	(81.59, 92.46)	N.A.
Carbohydrate intake, g/day	277.44	(258.10, 296.78)	275.74	(257.91, 293.57)	N.A.
Fat intake, g/day	95.26	(88.26, 102.26)	96.07	89.16, 102.99)	N.A.
Protein balance, g/day	10.92	(6.27, 15.57)	10.61	(5.96, 15.26)	P=0.91
Carbohydrate balance corrected for urinary glucose loss, g/day	21.35	(6.04, 36.66)	52.68	(37.37, 67.99)	P=0.0003

Fat balance, g/day	-35.24	(-42.77, -27.72)	-14.73	(-22.26, -7.21)	P<0.0001
Activity levels, counts/min	199.48	(179.03, 219.94)	193.15	(172.69, 213.61)	P=0.42